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# TROPICAL AGRICULTURE

The Journal of The Faculty of Agriculture (Imperial College of Tropical Agriculture),  
The University of The West Indies

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### Submission of Papers

TROPICAL AGRICULTURE is glad to receive and consider for publication manuscripts in English on topics concerning tropical agriculture with particular emphasis on subjects such as agronomy, crops and pastures, biology and physiology of economic crops, biomass for energy (production and conversion), crop breeding, ecology of the tropics and land-and-water-use capability, economics and sociology of agriculture, farming systems (analysis and development), horticulture, livestock breeding, management and nutrition, plantation crops, post-harvest technology, protection against pests, diseases and weeds and soil science and management. Authors wishing to submit review articles should first consult with the Editor-in-Chief. TROPICAL AGRICULTURE is indexed in Current Contents/Agriculture, Biology and Environmental Science, CABS, Chemical Abstracts, Biological Abstracts, Environmental Periodicals Bibliography (EPB).



## Foreword

This year The University of the West Indies (UWI) celebrates its 50th Anniversary having started with the Medical Faculty on the Mona Campus in 1948. The establishment of the University was pre-dated by the founding of the Imperial College of Tropical Agriculture (ICTA) in 1922. The ICTA which had a strong tradition of teaching in tropical agriculture, was succeeded by the Faculty of Agriculture which was established in 1962.

The Journal, *Tropical Agriculture* was first published in 1924 and has been published every year since then. During the 74 years of its existence, it was published from 1954 to 1992 on our behalf in the United Kingdom. It returned to the St Augustine Campus in 1992 and has been published solely by the UWI since then. The Journal has made, and continues to make, a significant contribution to tropical agriculture through the publication of research results on the many constraints to its development. Many of these findings have been implemented in the tropics and the Journal continues to be a valuable source of information to researchers in tropical, sub-tropical, and temperate regions. It remains the premier journal in tropical agriculture. Although the main focus of the journal is on technological constraints, it recognizes that the technology must be relevant to the social context in which the technology must be relevant to the social context in which the technology is to be applied, if it is to be successfully adopted and sustained. It has therefore welcomed articles on agricultural extension and adoption, and agricultural economics. The challenge for tropical agriculture in the 21st century is to devise policies and technologies for sustainable agricultural development. For sustainability, agriculture must be productive and profitable while protecting the environment. The Journal will play its role through the publishing of articles that address this theme.

Over the last three decades, the Journal has published the proceedings of three conferences which it has convened, Tropical Soils in 1973, Clay Soils Management in 1980, and Biological Nitrogen Fixation in 1981. These conferences were aimed at promoting the agro-ecological approach to soil productivity and farm in the tropics. This year the Journal has taken the decision to publish the Proceedings of the 11th Symposium of the International Society of Tropical Root Crops (ISTRC) as Issues 1 and 2 of Volume 75 (1998), i.e., as regular issues of the Journal. The Journal publishes very infrequently, the proceedings of conferences and symposia because its policy is to publish articles that satisfy our strict publication criteria. In accordance with our policy, articles must be reviewed and recommended for publication by at least two international scientists of high standing in the scientific community. In the event of contradictory recommendations, the Editor-in-Chief arbitrates or seeks the views of a referee. After an article is accepted for publication, it is thoroughly edited for technical accuracy, style, and format. In the case of proceedings of conferences and symposia, there is normally a requirement for all papers to be published.

The articles of the ISTRC proceedings have not been subjected to our normal review process. The articles have been edited for technical accuracy, removal of contradictions and improving style, readability, and conciseness. The articles that are published in Abstract form only are mainly articles that were not available at all, or not available in a suitably amended form by the closing date. The decision was taken to publish the Proceedings of the ISTRC Symposium to mark the 30th anniversary of the founding of the Society in Trinidad. The staff of the UWI played a major role in the founding of the Society. The return of the Symposium to Trinidad after 30 years was considered worthwhile for inclusion in the celebration of the 50th Anniversary of the University. The publication of the ISTRC Symposium also meant that some disciplinary areas which are not normally accepted for publication in the Journal, for example, food science have been included.

The Journal is happy to be associated with the publication of the ISTRC Symposium. Due recognition is paid to the organizations that have co-sponsored the publication of the proceedings. Finally, the Journal joins with the University in celebrating the many achievements of the University during the 50 years of its existence and recognizes the occasion in the four Issues of the Journal in 1998.

**F.A. Gumbs**  
*Editor-in-Chief*

## Address of the Honourable Minister of Agriculture, Land and Marine Resources

Mr Chairman, Dr. Sang Ki Hahn, President of the International Society for Tropical Roots (ISTRC), Mrs Tota Maharaj, Parliamentary Secretary, Dr. Nigel Poulter, Secretary of the ISTRC, Professor Compton Bourne, Principal, St. Augustine Campus, The University of the West Indies, Dr. Vincent Moe, Permanent Secretary, Ministry of Agriculture, Land and Marine Resources, Distinguished Ladies and Gentlemen. It gives me great pleasure to welcome you to Trinidad and Tobago on the occasion of the 11th International Symposium on Tropical Root Crops. I am informed that the first symposium was held in Trinidad in 1967, so we are specially honoured to welcome you again to our shores.

I wish to commend the International Society for Tropical Root Crops for continuing these Symposia every 3 years for the last 30 years. This is a notable achievement and I would like to congratulate the Society on its 30th anniversary.

Mr Chairman, the chosen theme of your Symposium "*Tropical Root Crops: Staples for Sustainable Food Security into the Next Millennium*" is extremely instructive at this time, since the regional grouping — CARICOM — is particularly concerned with the developments in the international trade arena and the implications for our small and fragile economies. These developments, Mr Chairman, are occurring at a time when we are faced with the global problem of poverty, the extent of which is so widespread and devastating. Mr Chairman, I am of the view that it is incumbent on organizations such as your society, and particularly this Symposium to consider and recommend actions to be taken to alleviate this 20th century scourge on humanity, in an age of unprecedented advances in science, technology, and prosperity. These actions must seek to ensure sustainable food production and security for all of our peoples, if we are to improve their standard of living. I am also of the view, that the eradication of poverty must be accomplished, if we are to achieve our overall goals and objectives.

In 1996, during the United Nations International Year for the Eradication of Poverty, some of the chilling facts of global poverty were identified. It was reported, for example, that approximately 1.5 billion people or 25% of the world population, mainly in developing countries, are acutely poor and the number is increasing by some 25 million every year. More significantly, every minute of every day, an additional 50 babies are born into poverty worldwide. The consequences of poverty on children are devastating, particularly in developing countries, where some 95 million children under the age of 15 are estimated to be working to help their poverty-plagued families and another 100 million are estimated to be homeless and destitute. They are referred to as "*street children*". Mr Chairman, what is even more disturbing is that this devastating problem of poverty co-exists with affluence.

In the half century since the creation of the United Nations in 1945, the world has witnessed an unprecedented growth in prosperity, with global GNP increasing sevenfold and per capita income more than tripling. Developing countries' growth rates, averaging 4.5 per cent in 1995, are expected to continue outstripping those of industrialized countries at approximately 2.5 per cent per annum. Two of the world's most populous countries are enjoying buoyant economic expansion. China's economy has been growing at an annual rate of about 12 per cent since the late 1980s, while India has recorded nearly 5 per cent annual economic growth over the past decade. These gains however, have not been equally distributed. Even amid plenty the number of poor people is increasing. Today, one in every five people suffers from debilitating poverty. The gap between rich and poor (developed countries and developing countries) is widening, especially the least developed countries (LDCs).

Mr Chairman, despite the impressive successes in improved living conditions in many Caribbean countries, poverty still persists throughout the region. In some of our CARICOM Member States, poverty levels are estimated to be higher than 40%. With few exceptions, general living standards have declined since the late 1970s and early 1980s due to low economic growth.

Estimates of poverty levels in the Caribbean region average roughly 38% of the total population, ranging from a high of 65% in Haiti to a low of 5% in the Bahamas. Excluding Haiti, approximately 25% of the total CARICOM population is poor. These estimates place the Caribbean close to the world aggregate average of poverty in developing countries. Macroeconomic shocks, inappropriate policy prescriptions and responses, deficiencies in the labour market, and a deterioration in the overall quality of social services no doubt have contributed to this decline. In addition, crime and violence, retrenchment of workers, and changes in family structures and values have also contributed to the declining living conditions being experienced in the region.

Mr Chairman, poor households in the Caribbean tend to generate their incomes through the "*informal*" sector in the urban areas, small scale farming or as agricultural wage earners in the rural areas. The majority of the employed poor, work in these informal sectors. The contribution of unskilled workers and small scale farmers as defined by the World Bank to the economy of the CARICOM region is particularly significant. The population of the English-speaking CARICOM Region is approximately 6.28 million. Assuming the retail cost of food consumed per person to be conservatively estimated at U.S. \$2.00 per day, then total annual consumption will cost U.S. \$4.58 billion (population  $\times$  365  $\times$  U.S. \$2.00).

If this consumption figure is used to crudely calculate the value of agricultural production, using the formula [Production = Consumption plus Exports minus Imports]. The value so estimated is U.S. \$3.13 billion. This calculation amazingly reveals, that CARICOM food exports at U.S. \$950 million are only 30% of total pro-

duction and CARICOM food imports at U.S. \$1.18 billion only 38% of total production. In other words, ladies and gentlemen, the official statistics based on the 'formal' sectors of agriculture, (commodities such as sugar, banana, rice, cocoa, coffee, poultry meat, etc.) seldom take into account that the majority of the food in the region is produced in the 'informal' sector on small farms and operated by part-time farmers. Moreover Mr Chairman, the vast majority of this food includes the tropical root and tuber crops (cassava, yam, sweetpotato, tannia, dasheen, and eddoe).

This is the contribution of the so-called 'informal' groups which the World Bank defines as *Unskilled Workers and Small-scale Farmers*. These are the people for whom work is irregular, wages are low and often inadequate for supporting their families, and there is no form of social insurance.

Economic growth is fundamental to poverty eradication. Economic growth reduces poverty through rising employment, increased labour productivity, and higher real wages. Those countries in the Caribbean which have sustained positive growth rates and invested heavily in human development have experienced relatively low levels of poverty.

It is ironic however, that the countries of the Caribbean which the world Bank has championed as models of economic growth, are those that have invested heavily in the Hotel Industry. These countries, in fact, have experienced the lowest rates of poverty. However, the jobs created were at much greater costs than that required for agriculture. Some comparative costings would reveal that in the region's Hotel Industry, the cost is U.S. \$50 000 per job; in Manufacturing it is U.S. \$15 000 per job; in the 'Formal' Agriculture sector, it is U.S. \$2500 per job; and in 'Informal' Agriculture it is only U.S. \$1000 per job.

The irony of this situation is that for relatively small investments in agriculture, including research, training, and extension, a much larger percentage of the food for the rapidly expanding Hotel Industry, estimated at U.S. \$800 million per annum, could be produced by CARICOM farmers. Greater employment opportunities would also be created.

Mr Chairman, my Ministry has initiated the reversal of this non-recognition of what is called the *informal* agricultural sector by using primary produce from this sector in our School Nutrition Programme. I am anxiously looking forward to the results and recommendations emanating from this Symposium to enhance the contribution of the so called 'informal' agricultural sector to the formal economy of the Republic of Trinidad and Tobago.

Mr Chairman, in concluding, I wish to point out, that the formalization of the inter-island trade in food crops, including the tropical tubers requires a regional recognition of the importance of these crops, and of their real contribution to the national economies of CARICOM Member States. It also requires a greater penetration of the tubers into the regional supermarkets, in order to increase trade in these food crops in the formal marketplace. It must be fully recognized, that such penetration will demand improvements in the marketability, and utilization of tubers through the evenness of size and shape; the eating and handling quality and shelf life.

The question we may ask ourselves is how can the increased production and trade of tropical tubers assist with the alleviation of the consequences of poverty and thereby attain sustainable food security? I am of the opinion, that there are several ways in which this can be done, but each country must develop systems suited to the peculiarities of its own national circumstance.

In Trinidad and Tobago, my Government, with the assistance of my Ministry, has decided on the strategy of extensive use of tubers as a reasonably priced source of locally-produced carbohydrates in our School Nutrition Programme. This strategy, we expect, will recreate the taste for locally-produced tropical tubers among our children, a desirable trait in the development of a Total Quality Nation to which we aspire; a trait which is rapidly disappearing with the onslaught of the foreign fast-food chains.

It is for this reason Mr Chairman, that I am impressed with the range and balance of topics planned for the sessions of this Symposium. I am particularly impressed by the number of papers in the sessions on Utilization and Utility, an area of investigation hitherto neglected not only in tropical root crops, but also in most tropical food crops as well as in the traditional export crops.

Mr Chairman, the eradication of poverty and hunger, the achievement of greater equity of income distribution, and overall human resource development in the region, remain major challenges for us all. Sustainable food security into the next millennium would greatly assist in the achievement of this noble goal.

In conclusion, I sincerely wish to thank you for the opportunity to address this prestigious gathering of your Society. Please again accept my heartiest congratulations on your 30th Anniversary, as well as the best wishes of my Ministry and the Government of the Republic of Trinidad and Tobago. I feel assured that my confident expectations for a most successful Symposium will be satisfied. May I also invite you and your colleagues to enjoy the warmth and hospitality of the peoples of Trinidad and Tobago.

May God bless you all.

I thank you.

**Reeza Mohammed**  
The Honourable Minister of Agriculture,  
Land and Marine Resources  
Abridged version

# Tropical Tuber Crops: Staples for a New Green Revolution into the Next Millennium

The privilege which has been given me in making this address I have already assumed, in suggesting the change in the theme of my address to: **Tropical Tuber Crops: Staples for a New Green Revolution into the Next Millennium**. I suggest this change, "First, because of my firm conviction that all tuberous organs should be called "tubers" in view of their common structure and function, irrespective of their origin in hypocotyl, stolon, rhizome, modified main or lateral stems or root; notwithstanding the controversy which the proposal might elicit; and, second, because nothing short of a new Green Revolution based on these tuberous crops could realize the Sustainable Food Security desired into the Next Millennium."

Let me first introduce the theme of the 11th Symposium — **Tropical Tuber Crops: Staples for Sustainable Food Security into the Next Millennium**, with brief references to cereal and tuber staples, world food security, and notions of sustainability.

The first Symposium in 1967 was held during the halcyon days of Ford and Rockefeller Foundation-supported international agricultural research programmes on wheat, maize, and rice, in CIMMYT in Mexico and IRRI in the Philippines, in the 1960s. These initiatives led to the formation of a consortium of funding agencies named the CGIAR in 1971. It also resulted in the addition of tropical tuber crops to the international agricultural research agenda, with the establishment of CIAT in Colombia and IITA in Nigeria. The substantial grant given by the Rockefeller Foundation to The UWI Faculty of Agriculture for the first Symposium, may be indicative of the role of the symposium in the introduction of tuber crops into the CGIAR research agenda.

After the first successes of the Green Revolution in cereal production, which resulted from international agricultural research on wheat, maize, and rice, it soon became obvious that, far from solving all the problems of food shortages and hunger in the world, the Green Revolution might itself have created a few problems of its own, e.g., monoculture, chemical pollution, and lopsided development. Accordingly, after global reviews of escalating hunger and poverty, the 1974 World Food Conference, the 1984 World Food Security Compact, and the Declaration of Barcelona all reaffirmed the notion of **World Food Security**, in which, "every man, woman and child has the inalienable right to be free from hunger and malnutrition in order to develop fully and maintain their physical and mental faculties".

The FAO Committee on World Food Security defined such Food Security as: "the economic and physical access to food for all people at all times." It led to the slogan **Food for All**.

Alas, the green Revolution had not and still has not solved this intractable problem of human society, as the end of the 20th millennium approaches. In the meantime, however, industrial, agro-ecological, and socio-economic problems of the global environment began to emerge, and, in 1983, the UN established the World Commission on Environment and Development, which culminated with the 1992 UN Conference on Environment and Development.

The Commission drew attention to the relationship between environmental conservation, production sustainability, and economic development and the Conference ratified the now well known inter-generational definition of sustainable development as: "Development that meets the needs of the present without compromising the ability of future generations to meet their own needs."

Since the 1992 UN Conference, considerable progress has been made in the definition of sustainable development in operational terms. Particularly interesting, is the notion of dividing the stock of accumulated goods or **Total Capital** to be maintained or enhanced within and between generations, into four separate components: Natural Capital; Human Capital; Institutional Capital; and Social Capital.

This allows, it is claimed, the balancing of trade-offs between the component capitals, in the quest for national development by enhancing the **Total Capital**.

Notwithstanding, the comprehensive concerns in these conceptualizations of sustainable development, as a crop scientist, I am more impressed with the more limited, but more precisely measurable parameters, in the definition of sustainable cropping systems or farming systems. Accordingly, such systems were defined by Lynam and Herdt in 1990 as: "Systems with a non-negative trend in total factor productivity."

Total factor productivity being defined as: "The ratio of the total value of all outputs produced by the system over one cycle to the total value of all inputs used by the system over the same cycle."

Together, these definitions of sustainability at universal and farm levels, posed the challenging question as to whether the Green Revolution had taken into account both of the proposed constancy of the stock of natural capital in the Biospheric World View and the need to meet the trend of increasing societal needs with development. For example, are the sources of energy and agricultural chemicals needed for cereal production using Green Revolution technology sustainable?

The World Commission on Environment and Development also raised the question of the progressive loss of biodiversity, both at the species level by destruction of tropical forests at an alarming rate, and at the varietal level, by genetic erosion caused by use of a few elite varieties to the exclusion and possible loss of myriads of land races as in rice. Moreover, attention has been drawn to the fact that out of a total of some 40,000 wild and locally cultivated species, only 25 species have been domesticated, selected, and cultivated worldwide. Four species, wheat, maize, rice, and sorghum supply most of the world's staple foods.

Despite the dominance of cereal staples in World food supply, the definition of a staple as "a commodity which has widespread use or appeal and for which there is constant demand" necessitates the inclusion of tubers in the listing of world staple commodities. Thus, cassava in Africa and tropical South America; yams in Africa and the Caribbean; sweetpotato in Asia and Latin America; and the edible aroids in Africa and the South Pacific, are important staples for at least one billion people worldwide.

It will be argued that neglect of research and development on these tuber crops poses a serious threat to World Food Security, both from the point of view of limited prospects for further increases in the productivity and production of cereal crops, as well as from that of the largely untapped potential for increases in productivity, production, and utilization of the tropical tuber crops, as staples for which there is unquestionable demand in many tropical countries.

## The Paradox of World Food Security

Notwithstanding current concerns for World Food Security, the technological conceptualization of adequate, global **Food supply** was realized with the Green Revolution in cereal production of the 1960s and 1970s. Enough wheat and maize in temperate and sub-tropical ecosystems and rice in tropical ecosystems was produced to feed the world and indeed many silos of surplus were stored. The global per capita supply of food increased from 2300 calories per day in the early 1960s to 2700 calories in the 1990s, despite an increase of 2.4 billion people in the world's population. Through the efforts of FAO and international and national communities, enough food is now produced on earth to feed everyone of the 5.8 billion men, women, and children on the planet. In other words, **QUANTITATIVE, GLOBAL FOOD SECURITY HAS BEEN THEORETICALLY ACHIEVED.**

The bad news is, that although more food has been reaching a greater number of people, food is by no means reaching everyone. We are experiencing a 20th century **PARADOX OF PLENTY** whereby food surpluses, widespread obesity and hunger, as well as affluence and poverty still co-exist. Although there is enough food to feed the entire planet, *at global level*, 1.5 billion people in the world are desperately poor and 800 million of these are chronically undernourished (an increase of some 350 million people since 1978); among the undernourished, 200 million children under the age of five suffer from acute protein and energy deficiencies.

*At national level*, FAO determined that 82 countries fell into the category of Low Income Food Deficit Countries, as at March 1996 (6 in the Near East and North Africa; 7 in Latin America and the Caribbean; 9 in Europe and the Commonwealth of Independent States; 19 in Asia and the Pacific; and 41 in sub-Saharan Africa).

Poverty and hunger are for the most part rural problems in this 20th Century Paradox of Plenty; and low income countries in Africa, for example, Malawi, Zaire, Tanzania, Rwanda; in Asia for example, Bangladesh, India, China, and in Latin America and the Caribbean, for example, Bolivia, Nicaragua, Haiti and the Dominican Republic are prominent examples of the Paradox. In most of these countries, the plight of the poor and the hungry could be dramatically improved by increased productivity, production, and utilization of tropical tuber crops.

At the 6th International Symposium on Tropical Root Crops held at CIP in Peru in February 1983, developing market economies with calorie intake from tuber crops greater than 10% were identified. Nineteen of these countries were also in the 1996 FAO list of 82 Low Income Food Deficit Countries (LIFDC) — 15 in Africa (or 37% of the 41 LIFDC); 3 in Oceania (or 50% of the 6 LIFDC); and 1 in Latin America (or 14% of the 7 LIFDC).

If we accept the fundamental principle of the right to food, we must all assume some responsibility for its denial in so many countries and among so many people, in a world plenteous with food. In other words, we must assume responsibility for the **PARADOX OF WORLD FOOD SECURITY**, not only today, but into the 21st millennium, when in 2010, the world population is predicted to be 7.0 billion people and in 2025, 8.3 billion, an increase of 43% over the 1996 population. The role of tubers in alleviating the paradox must be clearly defined.

## Prospects for a New Green Revolution

Commenting on food security in Africa at the fourth IFPRI Distinguished Lecture, Series in 1996, Professor George Benneh of Ghana stated the following: "*As the realization grows in the developed world that there is something to be said for depending on nature, there has been a shift toward looking at the kinds of small holder agricultural systems, that have been practised in Africa all along — mixed crops, agroforestry, chemical free agriculture.*"

These mixed cropping systems are firmly grounded in tropical tuber crop species, cassava, yams, taro, and sweetpotato. But Professor Benneh went on to lament "*African farmers are at the end of their wisdom. They do not know whether to bring in innovation or to keep on doing what they have done in the past. Food production is declining by 2% and population is growing by 2.8%, resulting in 100 million food insecure people. At the same time, the natural resource base is deteriorating rapidly, with FAO estimating that 3.7 million hectares of forest are lost every year and erosion is accelerating.*"

Professor Benneh's African problem differs only in the degree of its severity in certain Asian states, in tropical Latin America, and the Caribbean and South Pacific Island states. It is also emerging in those Asian states in which environmental, technical, and socio-economic limits of productive rice production is approached, in the Green Revolution countries.

I submit, that the wisdom of traditional farmers, particularly that of the tuber crop farmers, based on centuries of experience, is not at the end; it is only at the cross roads of choice of systems, most appropriate to satisfy the increasing social needs for sustainable food production, into the 21st millennium.

This submission is based on the observation that each society has the capacity to develop technology (like language) peculiar to the particularities of its environment and culture, through the **EVERYDAY THINKING** necessary to solve practical problems. And there are some 20 000 languages worldwide.

Therefore, in the application of the principles of modern science to the particularities of improving the technology of traditional farmers, it must be remembered that indigenous technology is just as legitimate as indigenous language. Indeed, it has been suggested by Paul Richards, that, by analogy with language, the particularities of indigenous technology reveal the **UNIVERSALS OF SCIENCE**, embedded at the level of **DEEP STRUCTURE** (*sensu* Chomsky) in the human mind. In other words, technology has often preceded the science which explains it, e.g., Einstein asserted, "*the whole of science is nothing more than the refinement of every day thinking*" and Bronowski wrote, "*settled agriculture creates a technology from which all physics, all science takes off.*"

Consequently, it is not surprising that the wisdom of traditional farmers has led international agricultural research scientists to proclaim a set of guidelines for a **NEW GREEN REVOLUTION**, at the FAO — mounted World Food Summit of Heads of State and Government, held in Rome, in November 1996. Thus, in a document entitled: *Lessons from the Green Revolution — Towards a New Green Revolution* the guidelines listed included:

## Addresses

1. Intensive soil and water management for soil conservation and increased, by combating soil erosion, high acidity, phosphorus, nitrogen, and organic matter deficiencies and deficiencies in the availability and quality of water.
2. Integrated Pest Management to minimize use of chemical inputs.
3. Use of appropriate animal power and easily constructed farm mechanical equipment to improve labour productivity.
4. Mixed cropping systems, based on genetically improved species, with particular reference to: optimization of system productivity and sustainability; resistance to physical stress, e.g. drought and biological stress, e.g., pests and disease invasion and chemical stress, e.g., high soil acidity; inclusion of tree species to optimize agro-forestry benefits, e.g., nutrient recycling, soil conservation; and *Mycorrhiza*-assisted P-uptake and micro-organism assisted N-fixation to relieve nutrient deficiencies.

These guidelines are derived from technologies originated and perpetuated by the wisdom of traditional farmers. They must now be refined by the research and development inputs of scientists. But we cannot wait for 6000 years for the scientific refinement of the technologies as was the case of the invention of the plough in the Middle East around 6000 BC and explanation of the principle of the lever to the Greeks by Archimedes.

The principles emphasize the need to conserve and to enhance the stock of NATURAL CAPITAL through support of the research and development component of the INSTITUTIONAL CAPITAL as well as the labour productivity component of the HUMAN CAPITAL. The FAO document concludes that *"The Concept of the New Green Revolution attempts to ensure that all four components of the TOTAL CAPITAL are strengthened, and that the lessons learnt from the (old) Green Revolution of lopsided development are not repeated."*

In this paradigm for a New Green Revolution, tropical tuber crop species have many advantages over their cereal counterparts. These include high and unexploited yield potential with low levels of inputs.

The practical wisdom of the farmers awaits the expertise of the scientists for the refinement and optimization of these attributes of tuber species into viable and sustainable mixed cropping systems for food security, into the next Millennium.

One of the problems of the Old Green Revolution was that for the most part, it excluded research for improvement of utilization technologies from its international agenda, on grounds that such research was more appropriate for the private sector. But the private sector in many developing countries has, so far, been concerned more with importation and profitable distribution of cereal products from developed countries, than with the commercialization of tuber staples. As a result, the necessary support for such research has not materialized. Unfortunately, such research is critical for two components of the TOTAL CAPITAL to be maintained or enhanced within and between generations. These are the HUMAN CAPITAL which requires that *"every man, woman, and child be free from hunger and malnutrition in order to develop fully and maintain their physical and mental faculties"* and the SOCIAL CAPITAL which requires acquisition by rural people, particularly in LIFDC, not only of improved production technology, but also of advanced utilization technology, towards a more equitable distribution of income both within and between generations.

We must ensure that the NEW GREEN REVOLUTION remains committed to all the components of TOTAL CAPITAL ENHANCEMENT, and in so doing, avoids the acknowledged lopsided development of the OLD GREEN REVOLUTION.

## Agenda for the 21st Millennium

I close with an update on the global nutrition situation in 1996. New estimates of trends of malnutrition show some improvement worldwide, but at a substantially slower rate in the last few years, than in the 1980s. This slowdown is cause for concern, because it indicates that the problem of food insecurity will still be with us well into the 21st millennium. The percentage of underweight children in the 1-5 years old cohort provides the commonest indicator of malnutrition. These percentages show South Asia (59% children underweight), South East Asia (32%), sub-Saharan Africa (30%), and Developing Countries (29%) or a total of 160 million underweight children, with some 85 million in South Asia. The U.N. Sub-committee on Nutrition drew attention to the intergenerational consequences of such malnutrition in these words *"Being underweight — even mildly — increases risk of death and inhibit cognitive development in children, leading to less fit and productive adults; moreover, it perpetuates the problem from one generation to the next through malnourished women having low birth weight babies"*.

The participants of this 11th International Symposium on Tropical Tuber Crops in Trinidad and Tobago are called upon to respond to the question. *"How can sustainable production and utilization of tropical tuber staples be realized in time, to contribute to the avoidance of this impending intergenerational disaster in human nutrition."*

Your responses to this question will be the agenda for research and development on tropical tuber crop production and productivity for a NEW GREEN REVOLUTION as well as on tuber utilization and utility to relieve the paradox of WORLD FOOD SECURITY into the next millennium.

L.A. Wilson  
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# Sponsors' Statements

The official statements of the four organizations which contributed to the 11th ISTRC Symposium are presented hereunder.

## **The International Society for Tropical Root Crops (ISTRC)**

The objectives of the Society are to foster, stimulate, and support any type of activity leading to the general improvement of tropical root and tuber crop production and utilization. The Society is concerned with the following crops: cassava, yam, sweetpotato, potato, aroids, and minor tuberous crops of the tropics. To achieve its objectives the Society:

- (a) sponsors international and regional meetings, workshops, and training courses; an international symposium of the Society is held every three years at a place and date determined by the General Meeting during the preceding symposium;
- (b) encourages, sponsors, and supports the establishment of regional branches of the Society;
- (c) sponsors study groups on subjects of importance to the goals of the Society;
- (d) strengthens cross-linkages between national, regional, and international research centres and organizations, including universities, through involvement in jointly-planned research and training programmes;
- (e) publishes appropriate informative communications, such as the Newsletter, summaries of the status of particular crops, lists of research workers and their area of specialization, proceedings of meetings, and other appropriate publications;
- (f) facilitates the exchange of personnel and germplasm materials;
- (g) provides financial assistance, if possible, or seeks assistance of donors who provide such assistance to members of the Society from developing countries, to attend Society's meetings;
- (h) awards Society prizes for outstanding publications or any achievement for the improvement of tropical root and tuber crops.

### Membership of ISTRC

Membership of ISTRC is open to any person or organization interested in the objectives of the Society. For additional information concerning the Society and its activities, and to become a member please contact: Dr L. Wickham, Secretary/Treasurer ISTRC, Faculty of Agriculture and Natural Sciences, The University of the West Indies, St Augustine, Trinidad, West Indies. Telephone: 1-868-645-3232, Ext. 2110; Facsimile: 1-868-663-9686; Email: [istrc@carib-link.net](mailto:istrc@carib-link.net).

## **Technical Centre for Agricultural and Rural Cooperation (ACP-EU)**

The Technical Centre for Agricultural and Rural Cooperation (CTA) was established in 1983 under the Lomé Convention between the African, Caribbean, and Pacific (ACP) States and the European Union Member States.

The CTA's tasks are to develop and provide services that improve access to information for agricultural and rural development, and to strengthen the capacity of ACP countries to produce, acquire, exchange, and utilise information in these areas. The CTA's programmes are organized around three principal themes: strengthening facilities at ACP information centres, promoting contact and exchange of experience among CTA's partners, and providing information on demand.

Address: CTA, Postbus 380, 6700 AJ Wageningen, The Netherlands.

### **The International Potato Center (CIP)**

The International Potato Center (CIP) is a scientific, non-profit institution dedicated to the increased and more sustainable use of potato, sweetpotato, and other roots and tubers in the developing world, and to the improved management of agricultural resources in the Andes and other mountain areas. The CIP is part of the global agricultural research network known as the Consultative Group on International Agricultural Research (CGIAR).

### **Department for International Development (DFID)**

The goals of the Renewable Natural Resources Research Strategy (RNRRS) of the Department for International Development (DFID) of the Government of the United Kingdom are poverty elimination, the promotion of economic growth and of economic reform, and the mitigation of environmental problems. The Crop Post-harvest Programme, which has contributed to this international symposium, is one of twelve research programmes supported through the RNRRS. The programme aims to improve the productivity and productive potential of post-harvest crop systems through the reduction of losses and the development of storage, processing, and marketing innovations. The programme is interdisciplinary and participative and is designed to help people who are involved in post-harvest commodity systems by providing them with better opportunities for employment, higher incomes, and more food. For further details about the RNRRS and the Crop Post-harvest Programme please contact: CPHP Programme Manager, Natural Resources International Ltd, Central Avenue, Chatham, Maritime, Kent ME4 4TB, United Kingdom. Telephone: +44 (0) 1634 883572; Facsimile: +44 (0) 1634 883937; Email: [nigel.poulter@nri.org](mailto:nigel.poulter@nri.org). Further information may be found on the Internet: <http://www.nrinternational.co.uk>.

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- (i) Commonwealth Foundation
- (ii) European Commission (DG XII)
- (iii) Technical Centre for Agricultural and Rural Cooperation (CTA)
- (iv) Department for International Development (Central funds)
- (v) Department for International Development (RNRRS Crop Post-Harvest Research Programme)
- (vi) International Foundation for Science (IFS)

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# Root biomass and nutrient uptake of taro in the lowlands of Papua New Guinea

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Data are presented on nutrient uptake of taro [*Colocasia esculenta* (L.) Schott] roots in relation to corm yield and above-ground biomass on a Typic Tropofluvents in the humid lowlands of Papua New Guinea. Fertilized (100:50:100 kg NPK ha<sup>-1</sup>) and unfertilized plants ( $n = 4$  each) were harvested 126 days after planting (DAP) (mid-season) and 231 DAP (harvest). Rooting depth at both sampling times was <0.2 m and a unit soil area equivalent to the planting distance (0.5 m × 0.8 m) was removed from the field whereafter roots were washed on a 0.5-mm sieve. Root biomass at 126 DAP was 0.26 Mg ha<sup>-1</sup> (15% of total biomass) in the unfertilized plots and 0.52 Mg ha<sup>-1</sup> (13% of total) in the fertilized plots, but at 231 DAP root biomass was similar (0.50 Mg ha<sup>-1</sup>). Root nutrient concentration at 126 DAP was similar in both plots but N, Ca, and S significantly declined in the unfertilized plots at 231 DAP whereas B increased with 18 mg kg<sup>-1</sup> ( $P < 0.01$ ). In the fertilized plot, P, K, Mg, Mn, and Cu had decreased at 231 DAP whereas Zn had significantly ( $P < 0.01$ ) increased. Nutrients in the root biomass as a fraction of the total nutrient uptake were similar at 126 DAP for both treatments. At 231 DAP, however, the fraction of nutrients in the root biomass was considerably lower in the fertilized plots. There was a high uptake of Mg by taro roots in the unfertilized plots at 126 DAP (38% of total) and at 231 DAP (36% of total). This study has shown that the amount of nutrients taken up by roots of fertilized and unfertilized taro was similar at harvest, but that a much larger proportion of plant nutrients is allocated to the roots under unfertilized conditions.

Keywords: Taro; Roots; Nutrient uptake; Nutrient concentration; Fertilizer

In most field studies with food crops, root biomass production and nutrient uptake receive little attention, because the root system is hidden from direct observation and the quantification of roots is tedious and difficult because of problems in extracting roots from the soil. It is also complex because of the spatial and temporal variability of roots in the soil matrix. Despite these problems, various destructive and advanced non-destructive methods have been developed to study roots of field crops (Taylor *et al.*, 1991) in addition to sampling schemes for their quantification (Noordwijk *et al.*, 1985). Much of the research on roots is conducted in the temperate regions and information on root biomass and its nutrient content of tropical crops is limited. This is particularly the case for tropical root and tuber crops (Jacobs and Clarke, 1993; Goenaga and Chardon, 1995).

Root and tuber crops are the major sources of dietary energy for many people in the Pacific Islands countries (de la Peña, 1996). In Papua New Guinea sweetpotato [*Ipomoea batatas* (L.)] is the main staple crop (Allen *et al.*, 1995), although taro [*Colocasia esculenta* (L.) Schott] is usually the first crop to be

planted after the forest or fallow vegetation is cleared (Moles *et al.*, 1984). It is grown under upland conditions and no irrigation or fertilizers are applied. Most small-scale farmers in Papua New Guinea grow taro for one season only, because pests and disease, weed infestation, and (or) the depletion of soil nutrients usually result in low yields in successive seasons. To sustain and improve taro yields, inorganic fertilizers are a viable option and taro responds well to fertilizers (De Geus, 1972; Kabeerathumma, 1992). Small-scale farmers use little inorganic fertilizers because of low nutrient-use efficiency (Noordwijk and Willigen, 1991) with the associated risk that investments in fertilizers are not profitable (McIntire, 1986). An essential step to increase the efficiency of fertilizers in order to improve yields is an understanding of the nutrient uptake and allocation within the taro plant during a growing season.

An experiment was therefore conducted which aimed to quantify root biomass production and nutrient concentrations and total uptake of fertilized and unfertilized taro. In order to make an accurate estimation of root dry weight, destructive measurements were made whereby whole taro plants were harvested.

## Materials and Methods

### The site

The research was conducted on the experimental farm (6°41' S, 146°98' E) of the University of Technology in Lae, Papua New Guinea, located in the humid lowlands with mean rainfall of about 4400 mm yr<sup>-1</sup> which is fairly well distributed throughout the year. Average daily temperatures are 26.3°C with a daily minimum of 22.9°C and a maximum of 29.7°C. Annual evaporation (U.S. Class A pan) is 2139 mm, and rainfall exceeds evaporation in each month (McAlpine *et al.*, 1975). The climate classifies as Af (Köppen) i.e., a tropical rainy climate with the driest month having over 60 mm rain. During the experiment (23 March to 13 November 1996) 2605 mm of rain was recorded.

The farm is located at an alluvial plain with an altitude of about 65 m above sea level (asl). The soil at the farm is well drained and classified as a sandy, mixed, isohyperthermic Typic Tropofluvents (USDA Soil Taxonomy) or Eutric Fluvisol (FAO-Unesco). Air-dried and sieved (2 mm) topsoil (0–0.23 m) had the following properties: pH (1:5 soil:water suspension), 5.9; organic C, 23.8 g kg<sup>-1</sup>; Olsen P, 12 mg kg<sup>-1</sup>; total N, 2.0 g kg<sup>-1</sup>; cation exchange capacity (CEC) (1M NH<sub>4</sub>OAc, pH 7), 126 mmol<sub>c</sub> kg<sup>-1</sup>; sand, 790 g kg<sup>-1</sup>; and clay, 80 g kg<sup>-1</sup>;  $r_b = 1.10 \text{ Mg m}^{-3}$ .

### Experimental setup and management

The site at which the experiment was conducted had been under pasture for eight years and was ploughed in January 1996. Two plots (5.6 m × 9.5 m) of taro [*C. esculenta* (L.) Schott. var. *esculenta*] local cultivar Nomkoi were planted on 23 March 1996 at a spacing of 0.5 m × 0.8 m (25 000 plants ha<sup>-1</sup>). Planting material consisted of corm apical portions from main plants from which the petioles were cut 0.25–0.30 m above the corm to remove the leaf lamina. One plot was fertilized with 100 kg N ha<sup>-1</sup> (sulphate of ammonia) given in split applications at 49 and 79 days after planting (DAP), and 50 kg P ha<sup>-1</sup> (triple superphosphate) and 100 kg K ha<sup>-1</sup> (muriate of potash) were given as a basal dressing at planting. The N fertilizers were broadcast over the plot and slightly incorporated into the topsoil. The other plot was not fertilized. Weeding was done manually at regular intervals and weeds were not removed from the plots. Biocides were used to control hawkmoth (*Hippotion celerio* L.) and taro leaf blight (*Phytophthora colocasiae*).

### Sampling and nutrient analysis

In the mid-season (126 DAP) and at harvest (231 DAP) four taro plants were selected in both the fertilized and unfertilized plots to determine total biomass production and nutrient up-

take. The plants were harvested and divided in corms and leaves (including petioles). No distinction was made between main plants and sucker plants and for each plant, corms or leaves of the main plants and suckers were combined into one sample. The samples were washed with distilled water and oven-dried at 70°C for 72 h after which dry weight was recorded. The whole plant part (i.e., corms and leaves) was ground (mesh 0.2 mm) for nutrient analysis.

For the root biomass, an area equal to the spacing (0.5 m × 0.8 m) was pegged out around each taro plant which is called the 'unit soil area' by Noordwijk *et al.* (1985). Pits were dug to observe the rooting depth of the taro, and in the mid-season and at the harvest the taro had not rooted deeper than 0.15–0.18 m. All soil to a depth of 0.2 m (0.08 m<sup>3</sup>) was collected in plastic bags and taken to the laboratory. The roots were washed from the soil with pressurized water on a 0.5-mm sieve. The sieved root and organic debris material were put in plastic trays filled with water after which the floating roots were handpicked the same day. After washing the roots with distilled water, they were immediately oven-dried to avoid loss of nutrients (Misra, 1994).

Nutrient analysis on roots, corm, and leaf biomass samples was conducted at the laboratories of the Department of Agriculture of the University of Queensland. One subsample was digested in 5:1 nitric:perchloric acids and analysed for P, K, Ca, Mg, S, B, Mn, Zn, and Cu using ICP AES (Spectro Model P). A second subsample was digested according to the Kjeldahl procedure and analysed for N on an Alpkem Rapid Flow Analyser Series 300.

## Results

### Biomass

Fertilized taro had significantly ( $P < 0.05$ ) more total biomass than unfertilized taro at both sampling times which in the mid-season (126 DAP) was due to the larger root and leaf biomass (Table 1). There had been little corm development in the mid-season and differences in the corm weight of fertilized and unfertilized taro were not significant. At harvest (231 DAP), however, the difference in total biomass was due to the greater corm and leaf biomass in the fertilized taro. The root biomass was similar for both fertilized and unfertilized plants at harvest. In fertilized taro, maximum root biomass was achieved by the mid-season (52 g m<sup>-2</sup>), whereas, root development was still occurring in the mid-season unfertilized taro (26 g m<sup>-2</sup>). At 126 DAP, root biomass was 15 and 13% of the total biomass in the unfertilized and fertilized taro, respectively. At harvest, the proportion of roots of the total biomass was 10% in the unfertilized taro and 4% in the fertilized

**Table 1** Biomass production and dry matter content of unfertilized and fertilized taro

	Plant part	Mid-season (126 DAP)		At harvest (231 DAP)	
		Unfertilized	Fertilized	Unfertilized	Fertilized
Dry weight (Mg ha <sup>-1</sup> )	Roots	0.26	0.52***	0.51	0.50
	Corms	0.82	1.21	2.53	6.99*
	Leaves <sup>1</sup>	0.67	2.13*	2.00	3.64*
	Total	1.75	3.86*	5.04	11.13*
Dry matter content (%)	Roots	4	5***	12	11
	Corms	21	19	30	30
	Leaves <sup>1</sup>	8	7	16	16

<sup>1</sup>Leaf biomass includes petioles

\*, \*\*\*, indicates significant difference between fertilized and unfertilized taro at  $P < 0.05$  and  $P < 0.001$ , respectively

taro. The coefficient of variation of dry root weight was between 6 and 24% at 126 DAP and between 1 and 14% at 231 DAP. The variation in root measurements was larger in fertilized taro.

The dry matter (DM) content of all plant parts increased from the mid-season to the end of the season, and was unaffected by fertilizer except for mid-season sampling when the roots of fertilized taro had a slight but significantly higher DM content (Table 1).

## Nutrients

Nutrient analysis showed that the Ca concentration was significantly lower in taro roots ( $P < 0.001$ ) and corms ( $P < 0.05$ ) at the end of the season compared to the mid-season for both unfertilized and fertilized taro (Table 2). At harvest, B and Zn concentrations had significantly ( $P < 0.01$ ) increased in unfertilized and fertilized taro roots, respectively. Potassium concentration in the roots were similar at 126 DAP and 231

DAP and not affected by fertilizer. The K, Ca, Mg, Mn, and Cu concentration in the taro corms were all lower at the end of the season than in the mid-season for unfertilized and fertilized taro. The concentration of N and S decreased significantly in the corms of fertilized taro only. Overall, it appeared that fertilizers had little effect on the nutrient concentration in taro roots, whereas, in the corms of fertilized taro, the concentration of most nutrients was slightly lower.

Roots of fertilized taro at 126 DAP had taken up significantly higher ( $P < 0.01$ ) amounts of all major nutrients (Table 3) which may be due to the greater biomass (Table 1). The total N uptake in the corm was larger in fertilized taro (14 kg ha<sup>-1</sup>) than in unfertilized taro (5 kg ha<sup>-1</sup>). Overall, in the mid-season, fertilized taro had taken up significantly more N, Ca, Mg, and S. The fraction of nutrients taken up by the roots from the total uptake was, however, not different between fertilized and unfertilized taro at 126 DAP. The uptake of N and P in roots was about 7 to 12% of the total uptake whereas 13 to 22% of the total K, Ca, and S uptake had occurred in the roots at 126 DAP. The Mg uptake in the roots accounted for 36 to 38% of the total Mg uptake in both treatments.

At the end of the season, there were no differences in the amount of nutrients taken up by the roots of fertilized and unfertilized taro. On a whole-plant basis, however, fertilized taro took up significantly ( $P < 0.05$ ) more Ca, Mg, and S than unfertilized taro. The proportion of nutrients taken up by the roots were similar for unfertilized taro at 126 and 231 DAP. However, nutrient uptake in the roots as a proportion of the total uptake decreased between 126 and 231 DAP for both fertilized and unfertilized taro, especially for Ca (from 18 to 7%) and Mg (from 36 to 20%).

**Table 2** Nutrient concentration<sup>1</sup> in unfertilized and fertilized taro roots and corms

Nutrient	Unfertilized				Fertilized			
	Roots		Corms		Roots		Corms	
	126 DAP	231 DAP	126 DAP	231 DAP	126 DAP	231 DAP	126 DAP	231 DAP
N	13.0	11.4*	8.3	5.8	14.1	10.6	14.5	4.5**
P	2.1	2.2	2.6	2.1**	1.8	2.2*	1.9	1.8
K	52.7	48.9	20.9	17.0*	47.7	46.3	19.0	13.0**
Ca	14.0	10.7***	5.8	3.6*	14.4	11.0***	4.9	3.1*
Mg	5.9	5.9	1.4	0.8**	7.1	6.0*	1.3	0.8**
S	1.2	1.5*	0.5	0.4	2.3	2.0	0.8	0.4***
B	12.0	30.0**	4.0	16.0**	25.0	25.0	13.0	17.0
Mn	77.0	84.0	40.0	24.0*	120.0	94.0*	43.0	21.0*
Zn	91.0	105.0	68.0	37.0**	63.0	114.0**	28.0	27.0
Cu	42.0	39.0	18.0	11.0*	62.0	29.0**	19.0	9.0***

<sup>1</sup>N, P, K, Ca, Mg, and S in g kg<sup>-1</sup>, other nutrients in mg kg<sup>-1</sup>

\*, \*\*, \*\*\*, indicates significant difference at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  between mid-season (126 DAP) and at harvest (231 DAP)

**Table 3** Nutrient uptake ( $\text{kg ha}^{-1}$ ) in roots, corms, and leaves of unfertilized and fertilized taro

Sampling period	Plant part	Unfertilized taro					Fertilized taro						
		N	P	K	Ca	Mg	S	N	P	K	Ca	Mg	S
Mid-season (126 DAP)	Roots	3	<1	13	4	2	<1	8**	1***	25**	8***	4***	1**
	Corms	5	2	17	4	1	<1	14**	2	22	6	2	1
	Leaves <sup>1</sup>	19	5	46	8	1	1	63	9	119	29*	5*	4
	Total	27	9	76	16	4	2	85*	13	166	42*	10*	5*
At harvest (231 DAP)	Roots	6	1	25	5	3	1	5	1	23	5	3	1
	Corms	13	5	42	8	2	1	31*	12*	86*	23	5*	3**
	Leaves <sup>1</sup>	34	10	80	21	3	2	55	18	106	46	6	4
	Total	53	16	147	35	8	4	91	31	215	74*	15*	8*

<sup>1</sup>Leaf biomass includes petioles\*, \*\*, \*\*\*, indicates significant difference at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  between mid-season (126 DAP) and at harvest (231 DAP)

## Discussion

Fertilized taro produced twice the biomass of unfertilized taro. Differences were already pronounced in the mid-season when fertilized taro had three times more leaf biomass and two times more root biomass. At harvest, however, root biomass was not different and was about  $0.50 \text{ Mg ha}^{-1}$ . This root biomass was much larger than that recorded by Goenaga and Chardon, (1995) who found between  $0.14$  to  $0.31 \text{ Mg ha}^{-1}$  for fertilized and drip-irrigated taro in Puerto Rico. Goenaga and Chardon (1995) also found that root biomass developed within 120 DAP but did not change thereafter. The present research suggested the same for fertilized taro but showed that unfertilized taro had not fully developed its root system by 126 DAP. The advantages of the rapidly developed root system are obvious and can be simplified as the more roots, the better shoot growth (Noordwijk and Willigen, 1991) which the present data confirmed. As whole plants were dug up, variation in root biomass measurements were relatively low ( $1 < \text{CV}\% < 24$ ) compared to other destructive sampling techniques like core samples and pinboards (Noordwijk *et al.*, 1985; Taylor *et al.*, 1991).

In the present experiment, it was found that large amounts of nutrients are taken up by the roots and little differences were found between fertilized and unfertilized taro at harvest. Some caution is, however, needed in the interpretation of the nutrient data of the roots as traces of soil may have adhered to the roots and nutrients may be washed from the roots with separation (Misra, 1994). Nitrogen in the roots at harvest was  $8 \text{ kg ha}^{-1}$  (9% of total uptake) and  $5 \text{ kg ha}^{-1}$  (6%) for fertilized and unfertilized taro, respectively. This is much higher than that reported by Gliessman (1982) who found only  $0.5 \text{ kg N ha}^{-1}$  in the taro roots at harvest which was 2% of the total uptake. The difference is large and may be partially explained by differences in taro cultivars (Jacobs and Clarke,

1993; Goenaga and Chardon, 1995) and the growing conditions. Very little P was found to be taken up in the roots ( $\leq 1 \text{ kg ha}^{-1}$ ) and the majority of the P uptake was in the leaves (including petioles). Potassium was found in large quantities in taro roots and up to  $25 \text{ kg ha}^{-1}$  was recorded. This may be an underestimation as it is easily lost from roots with washing. Misra (1994) found that wet separation (washing) of *Eucalyptus* roots resulted in a K loss of 24%. Although Mg was not taken up in large quantities in taro roots under unfertilized conditions ( $3 \text{ kg ha}^{-1}$  at harvest), it accounted for about 36% of the total uptake, whereas Mg uptake in roots of fertilized taro was 20% of the total uptake. In the mid-season, the proportion of Mg in taro roots was even higher (36–38% of total uptake). These data are much higher than those found by Kabeerathumma *et al.* (1985) who found only 5% of the total Mg to be taken up by taro roots.

## Conclusions

Root biomass in fertilized taro was fully developed at the mid-season whereas only half of the root biomass was formed in unfertilized taro. At harvest, root biomass of fertilized and unfertilized taro was  $0.50 \text{ Mg ha}^{-1}$ . Nutrient uptake by roots of fertilized and unfertilized taro was similar at harvest and about  $5 \text{ kg N}$ ,  $1 \text{ kg P}$ ,  $25 \text{ kg K}$ ,  $5 \text{ kg Ca}$ , and  $3 \text{ kg Mg ha}^{-1}$  were taken up.

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# Better nutrition for the improvement of sweetpotato and taro yields in the South Pacific

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Poor crop nutrition contributes to the low yields of root crops in the South Pacific region where symptoms of nutritional disorders are frequently observed. This study was therefore undertaken to assess the effect of inorganic fertilizers on sweetpotato [*Ipomoea batatas* (L.)] and taro [*Colocasia esculenta* (L.) Schott] yields. It indicates the importance of the correct diagnosis of the nutritional problems at each site for improved nutrition management and describes procedures used to identify and correct nutritional limitations to growth. Nutrient deficiencies were detected using nutrient omission pot trials and the amounts of fertilizer nutrients required to correct deficiencies were determined in a series of pot and field trials. Placement of P fertilizers and number and timing of N applications were found to increase fertilizer efficiencies. Critical leaf concentrations of N and P were determined. Leaf concentrations sampled during early vegetative growth were found to be good indicators of the effect of N or P deficiency on yield. Economic analysis of fertilizer applications demonstrated a high level of profitability.

Keywords: Sweetpotato; Taro; Nutrient deficiency; Soil fertility; Nitrogen; Phosphorus; Potassium

Root crops provide the main staple food for most Pacific Island communities. Sweetpotato [*Ipomoea batatas* (L.)] is the dominant species in upland areas of the high islands of Melanesia, while taro [*Colocasia esculenta* (L.) Schott] and other aroids are usually preferred in the lowlands and coral islands. Traditionally, they are grown in mixed crop gardens which may be cultivated for 1 to 3 years before fallowing for 5 to 30 years. Due to population growth and the expansion of cash cropping, many areas are experiencing land shortages, with the result that cropping periods are extended and fallow periods are reduced (Rangai, 1982). Yield declines are commonly associated with this process. The food security of these communities depends on their ability to develop more intensive cropping practices which do not degrade the fertility of the soil.

The experience of the Green Revolution in cereal production has demonstrated that yields can be improved and sustained under more intensive management. While genetic improvement and better management of weeds and pests and diseases have contributed to yield increases, the ability to sustain these yields over time has been largely attributed to nutrient inputs. Borlaug and Dowswell (1994) estimated that half of the global increase in crop yields achieved during this century is due to the adoption of inorganic fertilizers. They demonstrated a

strong correlation between the success of nations in increasing food production per capita over the past 20 years and their increase in fertilizer use over the same period.

Root crops have shared little in the Green Revolution. For example, in most developing countries, yields of sweetpotato have remained static since the early 1960s (Horton *et al.*, 1989), and many countries have recorded yield declines over recent years (FAO, 1996). The exception to this trend is China, where sweetpotato yields have been doubled since 1965 (Horton *et al.*, 1989), largely due to the high priority given to fertilizers in agricultural development policy in China (Borlaug and Dowswell, 1994).

Symptoms of nutritional disorders are frequently observed on root crops in the Pacific region, indicating that nutrient availability is often a considerable constraint to yield. Fertilizers are commonly used on cash crops in South Pacific countries, but are rarely applied to root crops. As in many other developing countries, there is a perception that fertilizers are not necessary for traditional crops, and that purchasing inputs for subsistence crops does not make economic sense.

Extensionists and agricultural policy makers may have been discouraged from promoting fertilizer use by the variable and often poor responses obtained in fertilizer trials. These trials

have frequently been conducted on poorly characterized sites, and it is likely that other constraints, including deficiencies of other nutrients, have limited the responses to nutrients applied. As part of the current project, comprehensive diagnostic information for nutritional disorders of sweetpotato and taro was established for the first time (O'Sullivan *et al.*, 1996a, 1997, 1998). Visible symptoms and tissue nutrient concentrations were used in reconnaissance surveys in 1992 and 1993, to identify sites in Tonga, Papua New Guinea, and Western Samoa where nutritional disorders were evident.

The aims of the work presented in this paper were to determine whether continuous or extended cropping could be sustained through appropriate nutrient inputs, and whether the use of inorganic fertilizers is likely to be profitable for South Pacific root crop growers.

## Materials and Methods

The research was undertaken over the period 1993–96. In Tonga, glasshouse experiments were conducted at the Ministry of Agriculture and Forestry's Vaini Research Station, and in Western Samoa at The University of the South Pacific, School of Agriculture, Alafua Campus. The Papua New Guinea University of Technology and the Keravat Station of the Papua New Guinea Department of Agriculture and Livestock also contributed to the work presented.

Sites were selected on the basis that symptoms of nutritional disorders were seen on sweetpotato or taro crops, that the soil type was typical of a root-crop-producing district, and that the site would be available for field trials with adequate supervision and security. In Tonga, 22 sites were selected for characterization, representative of the main soil types on all main islands in the archipelago. Of these, 14 sites were selected for field trials. In Western Samoa, four locations were selected representing main agro-ecological zones on the island of Upolu, and at each location, heavily cropped and newly cleared sites were compared.

The procedure for determination of the nutrient requirements at each site consisted of four experimental stages:

- (1) Nutrient omission pot trials were conducted using top soil collected from the site to identify which nutrients were likely to be insufficient for optimum crop growth. In this technique, short-term growth of a test plant is used as a bioassay for nutrient availability in the soil;
- (2) Single-nutrient-rate pot trials were used to approximate the optimum rate of application of deficient nutrients;
- (3) Single-nutrient-rate field trials were conducted to determine optimum rates for actual yield

of the crop of interest, under field conditions. Other nutrients found to be insufficient were applied at an estimated optimum rate, based on pot trial results. Where several nutrients are deficient at a site, single-nutrient trials allow each nutrient to be tested at several levels while keeping the total number of treatments small, whereas a factorial arrangement at this stage would be very large and costly; and

- (4) If more than one nutrient was deficient at the site, factorial field trials were conducted, combining the most promising rates of each deficient nutrient to optimise fertilizer inputs.

The procedure requires little specialised equipment, namely a greenhouse or polycarbonate rain shelter with adequate light penetration, and a source of high-quality water, comprising a rainwater collection system and a small demineralising unit. The few months required for pot trials greatly increases the efficiency of the field trials, so that smaller areas and fewer cycles are necessary to reach a sound conclusion.

Approximately 200 kg of soil were collected from each site to a depth of 15 cm and transferred to the experiment station. Nutrient omission pot trials were conducted using maize (*Zea mays* L.) as the test species (Asher and Grundon, 1991). These trials identify which nutrients are deficient for plant growth by comparing growth in pots in which all mineral nutrients have been supplemented, to growth in treatments receiving all, minus one nutrient. If omission of a nutrient does not cause a significant reduction in growth, then it is concluded that the soil supply of that nutrient is adequate. Any nutrient whose omission results in a significantly lower yield than the 'All' treatment is considered to be deficient.

Nutrient-rate pot trials were conducted on each soil, for each nutrient found to be deficient. By converting nutrient rates per pot to equivalent kilogram per hectare rates, the results were used to optimise the range of treatments applied in the field. Maize was the usual test plant, but the procedure has been successfully adapted for use with sweetpotato tip cuttings (Dowling *et al.*, 1995).

Field trials were conducted using local farmers' practices, with respect to land preparation, plant spacing, and weed control. Either sweetpotato or taro was grown, depending on the local prevalence. Chemical analysis of leaf tissue collected during the trials was used to establish critical nutrient concentrations under field conditions, and to compare these with glasshouse-derived critical nutrient concentrations. Analyses of soil samples collected during the trials were used to calibrate soil tests against crop performance and response to fertilizers, for each of the soil types investigated.

## Results and Discussion

### Occurrence of nutrient deficiencies

A wide range of mineral nutrient deficiencies was identified throughout the project area, both by observation of visible symptoms supported by leaf tissue analyses and by nutrient-omission pot trials (Table 1). Visible symptoms usually only reveal severe deficiencies. For most plant nutrients, crop growth and yield are adversely affected at milder levels of deficiency than are required to induce symptoms on the foliage. Visible symptoms also usually indicate only the single most limiting nutritional disorder, while several other nutrients may also have less than optimal availability. Nutrient-omission pot trials are able to detect multiple deficiencies, as the test plants are required to depend on soil supply of only one nutrient per treatment. A typical omission trial results for two contrasting soils with multiple nutrient deficiencies is illustrated in Figure 1. The Fahefa soil is a volcanic ash soil from Tonga, and the Madang

soil is a calcareous soil formed on a raised coral platform in Papua New Guinea.

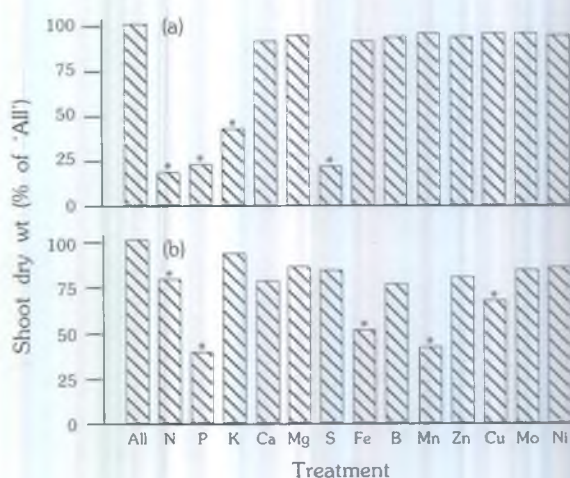
The most common deficiency diagnosed was that of P. The volcanic ash and basaltic soils, common throughout the region, have high P-fixing capacity, and the availability of P to crops is largely dependent on the organic fraction in the soil. Most volcanic soils in the region are neutral to slightly acid, but in more highly weathered soils pH may be below 5.2, and solubilised aluminium may further reduce P availability. On calcareous soils, deficiencies of P and various micronutrients, particularly Fe, Zn, and Mn, are frequently observed due to precipitation of these nutrients at high pH.

Sulphur deficiency was often observed in pot trials using top soil. Most of the volcanic soils in the region are highly porous and are subjected to heavy rainfall conditions under which S is readily leached. However, on Tongan sites indicating severe S deficiency in pot experiments, no response to S was obtained in the field. Crops appear to obtain sufficient S from the subsoil, which may show high accumulations of S (Cowie *et al.*, 1991), and from rainfall acces-

**Table 1** Incidence of nutrient deficiencies diagnosed in root crops in Tonga, Western Samoa, and Papua New Guinea

Method of identification	No. of crops/ soils examined	No. of crops/soils testing deficient in										
		N	P	K	S	Mg	Fe	B	Mn	Zn	Cu	Mo
Symptoms diagnosed	22	4	9	9	0	6	2	2	3	4	0	0
Nutrient-omission pot trials	48	31	39	10	10	3	2	0	3	5	2	2

In each recorded incident of crops diagnosed, the observation of visible symptoms was supported by nutrient analyses of leaf tissue; Deficiencies recorded in nutrient omission pot trials were those which caused a significant ( $P < 0.05$ ) reduction in growth of maize



**Figure 1** Results of nutrient-omission pot trials on (a) a volcanic ash soil from Fahefa, Tonga, using maize as the test plant, and (b) a calcareous soil from Madang, Papua New Guinea, using sweetpotato as the test plant. Treatments marked with an asterisk produced significantly less yields than the 'All' treatment ( $P < 0.05$ )

sions, which in Tonga have been estimated to contribute  $6 \text{ kg S ha}^{-1} \text{ yr}^{-1}$ , approximately equal to S removal rates by crops (Manu, 1992).

### Fertility indicators in heavily cropped soils

Among the four locations studied in Western Samoa, nutrient-omission pot trials revealed a higher incidence of deficient nutrients and greater severity of deficiency in soils that had been continuously cropped for several years, compared with adjacent soils that had been under bush fallow for several years (Table 2). Deficiencies of N and P were invariably increased following continuous cropping. Among the locations tested, K deficiency was observed at only one location, but was more severe on the cropped soil. Soil tests revealed that cropped sites contained, on average, less than half the organic carbon of newly-cleared sites. The reduction in organic matter was associated with reduced cation exchange capacity and available water capacity.



**Table 2** A comparison of top soil from continuously cropped and newly cleared sites at four locations on Upolu Island, Western Samoa, using nutrient-omission pot trials and soil analyses

	Fallowed soils		Cropped soils	
Soil analyses				
Organic carbon (%)		8.2±1.50 <sup>1</sup>		3.9±0.20
Total N (%)		0.64±0.10		0.27±0.05
pH (H <sub>2</sub> O)		5.5±0.22		5.3±0.12
CEC (meq 100 g <sup>-1</sup> )		24.4±3.80		18.0±3.20
Available water (%)		15±1.80		12±0.25
Nutrient-omission pot trials				
Deficiency of	Incidence <sup>2</sup> (n = 4)	Mean yield reduction (%) <sup>3</sup>	Incidence (n = 4)	Mean yield reduction (%)
N	1	7	4	17
P	2	14	4	26
K	1	9	1	13
Fe	0	1	1	9
Mn	0	3	1	9
Zn	0	6	1	8
Mo	0	0	1	7

<sup>1</sup>The mean values ± standard deviation of soils from four locations are given

<sup>2</sup>No. of soils to which addition of the named nutrient significantly ( $P < 0.05$ ) increased plant growth in nutrient-omission pot trials

<sup>3</sup>Difference between shoot weight of maize grown in treatments receiving additions of all nutrients ('All') and those from which the named nutrient was omitted, as per cent of 'All', giving an indication of the relative severity of nutrient deficiency

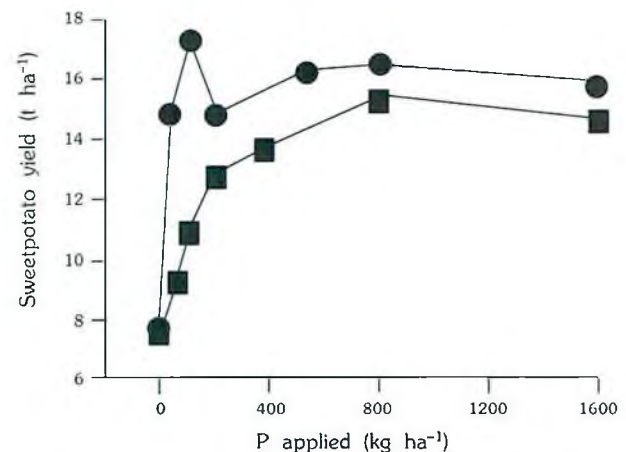
In field trials, taro responded strongly to P fertilizer on both cropped and new sites. However, P fertilizer alone did not restore fertility of cropped sites relative to new sites, as the response was always greater on the new site, indicating that factors other than P deficiency were more severely limiting on the cropped site. By combining P fertilizer and fresh mulch of foliage from *Erythrina subumbrans*, a leguminous tree which may be intercropped with taro, similar yields were obtained on the cropped and new sites (Eaqub *et al.*, 1994). The *Erythrina* mulch provided N and K in excess of the crop's requirements, as well as improving water retention in the soil. The results suggest that fertility of soil depleted by cropping may be restored with appropriate inputs.

#### Correction of nutritional disorders using inorganic fertilizers

The Fahefa site in Tonga is typical of the soils on which multiple nutrient deficiencies were detected. Pot experiments indicated deficiencies of N, P, K, and S (Figure 1a), and suggested optimum rates of 250, 800, 200, and 60 kg ha<sup>-1</sup>, respectively, for correction. Single-nutrient-rate field trials did not obtain a significant response to S additions. For the other deficient nutrients, fertilizer rates giving maximum sweetpotato yield were 70 kg N, 400 kg K, and 800 kg P ha<sup>-1</sup>, when each nutrient was broadcast before planting. The very high rates of P required were due to the high P fixation by the soil (approximately 60%). Due to the high cost of fertilizers, such rates were found to be uneconomical, despite increasing the yield from 8 to 15 t ha<sup>-1</sup>. To overcome this, in a parallel rate trial, P fer-

tilizer was placed close to each plant and incorporated in the soil. This technique gave similar yields at 100 kg P ha<sup>-1</sup> to those obtained at 800 kg ha<sup>-1</sup> of broadcast P (Figure 2).

Increased fertilizer efficiency was also obtained for N, by spreading applications over the first half of the growing season. In a trial on Vaini soil in Tonga, urea was applied to sweetpotato and taro either as a single application at planting, or in split doses. For sweetpotato, two and four splits were applied (0 and 2 months, and 0, 1, 2, and 4 months, respectively). For taro, up to eight applications were made (0 and 3 months, 0, 2, 3, and 4 months, and 0, 1.5,



**Figure 2** The response of sweetpotato to fertilization with P (triple superphosphate) on a Fahefa soil in Tonga; Two methods of application were compared: (■), broadcast, and (●), placed close to each plant

2, 2.5, 3, 3.5, and 4 months). Considerably greater responses to N were obtained with split applications compared with a single application (data not shown). The particularly poor response of taro to single N applications at planting is probably attributable to the slow establishment rate of this crop. While responses increased up to the highest number of splits tested, the greatest improvement was obtained in the first split. Nitrogen recoveries were calculated from the weight and N content of plant tops and storage roots or corms at harvest (data not shown). The apparent recovery of applied N was in the order of 20% when applied in a single application at planting, but through split applications, recoveries over 80% were obtained at lower rates of application, and approximately 50–60% for the application rates giving maximum yield.

Having determined the optimum rates and methods of application for each deficient nutrient, factorial arrangement field trials were used to determine the best combination of rates of each of the deficient nutrients. At the Fahefa site, the trial contained 27 treatments, using the best two rates from single nutrient trials plus zero to give three levels of each nutrient. The inclusion of zero rates, even when positive responses have been clearly demonstrated, is important for the economic assessment of outcomes. Responses to each nutrient, and interactions between them, were statistically significant.

The comparison clearly illustrates that fertilizer trials which do not address all deficiencies present at the site can be misleading, underestimating the benefit of inputs.

### Economic analysis of fertilizer application

At prevailing fertilizer costs, the economic benefit of the nutrient inputs was clearly demonstrated. The greatest gross margin was obtained with N(75): P(100): K(400), which was also the treatment which gave greatest yield (Table 3). However, the greatest efficiency of marginal ex-

penditure was obtained at N(75): P(100): K(200).

The efficiency of each nutrient input was greatly improved if near-optimal levels of other nutrients were present (data not shown). Therefore, if funds are limiting, it may be better to apply adequate nutrients to only part of the cropped area, rather than to spread available inputs over the whole area. In this case, the appropriate level of inputs would be those maximizing the marginal return. However, if sufficient funds are available, the best strategy would be to maximize gross margin by using the higher input level.

### Field verification of critical nutrient concentrations

In order to utilise these results for fertilizer recommendations on other sites, diagnostic or prognostic tests are needed to identify the nutrient limitations present at the site, and anticipate their likely effect on crop yield. Soil tests provide an indication of nutrient availability, but must be calibrated for each soil type used. Soil samples taken from field trials throughout Tonga are currently being used to calibrate soil tests for each of the main agricultural soil types in the nation. This work is not presented here.

Plant tissue analysis also provides a diagnostic tool for assessing the nutritional status of a crop. The main disadvantage of tissue testing compared with soil testing, is that the crop must be established before samples are taken. However, if analyses are done early in the season, corrective nutrient applications may be possible. An advantage of tissue testing is that it is not dependent on soil type.

In earlier work by the authors, critical leaf concentrations were determined in sweetpotato and taro for all nutritional disorders likely to be encountered in the field (O'Sullivan *et al.*, 1996b, c). Plants were grown in solution culture under glasshouse conditions. While this sys-

**Table 3** Economic analysis of fertilizer applications to sweetpotato on a Fahefa soil in Tonga. Fertilizer combinations presented were selected from the factorial NPK fertilizer trial as those offering the best return on fertilizer expenditure

Treatment (kg ha <sup>-1</sup> )			Yield (t ha <sup>-1</sup> )	Costs			Revenue		
N	P	K		Fertilizer	Other costs <sup>1</sup>	Total	Total	Gross margin	Marginal return
				----- T\$ ha <sup>-1</sup> -----			----- T\$ ha <sup>-1</sup> -----		
0	0	0	8.0	0	1300	1300	2800	1500	—
75	0	0	9.5	65	1345	1410	3325	1915	4.77
75	0	200	15.1	265	1430	1695	5285	3590	6.88
75	100	200	21.5	390	1495	1885	7525	5640	11.79
75	100	400	25.0	590	1530	2120	8750	6630	5.21

<sup>1</sup>Includes cost of cultivation, manual application of fertilizer, planting, weeding, and harvesting

tem allows accurate control of nutrient availability to the plants, it provides an environment different from that of field-grown plants. Also, the glasshouse-derived concentrations were calibrated against the vegetative growth of the plants up to the time of sampling. The relationship between early vegetative growth and economic yield was not known.

The field trials offered an opportunity to compare critical concentrations derived from solution culture with those in the field. Index leaves of sweetpotato (7th to 9th youngest open leaf blades) were collected at two months after planting. Vine length was measured at this time, to provide a relative assessment of vegetative growth, which is directly comparable with the solution-culture data. The relationship between leaf concentration of either N or P, and vegetative growth was almost identical for plants grown in solution culture and those grown in the field (data not shown). Very similar relationships are obtained when N or P concentration at two months is related to the final yield of storage roots demonstrating that early vegetative growth is strongly correlated with yield.

## Conclusions

The findings verify that nutrient deficiencies frequently limit yields of root crops in the South Pacific region. While P and N deficiencies are most common, a wide range of disorders occur, and these must be diagnosed and addressed for maximum yield improvements to be obtained. Nutrient inputs can greatly increase yields on soils which have been subjected to sustained cropping. When supplied as inorganic fertilizers, these inputs are likely to be economically beneficial to farmers. Where alternative sources of nutrients are locally available, these may further reduce the costs of nutrient inputs. Related studies are examining the use of plant mulches for the correction of N and K deficiencies. Green manure of sufficient quantity and quality is often not available in conventional farming systems, but may be provided through alternative cropping systems, particularly those involving legume species as intercrops or improved fallows. It is likely that optimum solutions will combine the use of both organic and inorganic nutrient sources, as the latter are more efficient for the correction of some disorders, such as P deficiency on soils with high P retention.

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# The growth performance of taro (*Colocasia esculenta*) grown from true seed

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Taro [*Colocasia esculenta* (L.) Schott] seedlings derived from two naturally pollinated inflorescences (Ph15 and Ph17) were grown to maturity. The growth performance of the seedlings was found to be equal to, or better than, the vegetatively-propagated parent plants. The seedlings had greater leaf lamina length and width, leaf area, petiole length, and had more leaves and suckers than the parent plants. The corm yields of all the seedlings as a group were also similar to, or greater than, the parent plants. However, the corm yield of the taro seedlings that showed resistance to taro leaf blight was significantly greater than the parents and the seedlings that were susceptible to taro leaf blight. The seedlings were also a source of considerable variation in parameters such as leaf and petiole colour, type of suckers, and the incidence of infections of taro leaf blight. This variation within the seedlings illustrate the importance of the production of sexually derived seedlings in breeding and selection programmes.

Keywords: Taro; Growth performance; True seed; Vegetative propagation; Seedlings

Taro leaf blight (TLB) (*Phytophthora colocasiae*) is the major cause for the decline in taro [*Colocasia esculenta* (L.) Schott var. *esculenta*] cultivation and production in parts of the Pacific (Jackson 1977; Jackson and Gollifer, 1977). The spread of this disease throughout the Pacific Islands has led to an increased interest in taro breeding and selection programmes (Ivancic *et al.*, 1990; Sivan, 1991). Sivan and Tavaqia (1984) also concluded that there is considerable potential for improving taro yields through breeding for parameters besides pest and disease resistance.

Taro is rarely grown from true seed and there are few reports in the literature on the growth of taro seeds, but most of these deal only with seed germination and early growth (Shaw 1975; Jackson *et al.*, 1977; Strauss *et al.*, 1979; Pardales, 1981). Information on the growth and development of taro seedlings in the field is important for taro breeding programmes. This study reports on growth of taro seedlings from TLB-resistant parents compared to that of their vegetatively-propagated parents.

## Materials and Methods

The study was conducted during 1994–95. The seeds were planted in February 1994 and the plants in the field were harvested in February

1995. Taro seeds were obtained from open pollinated inflorescences of two mother plants Ph15 and Ph17 (Bubia Agricultural Research Centre accession, PNG numbers). Their seedlings were designated S15 and S17, respectively.

Seeds were germinated in saturated subsoil. Eight weeks after sowing, they were transplanted into 50-mm diameter pots filled with potting mix (3 parts top soil:2 parts sand:2 parts peat) and kept under flooded conditions. Ten weeks after sowing, the seedlings were transplanted into 200-mm diameter pots and grown in well-watered but not flooded conditions. From germination to 18 weeks after sowing, the seedlings were maintained under 75% shade, then hardened in full sunlight for two weeks before planting in the field at 20 weeks after sowing. A total of 158 seedlings were planted in the field (127 of S15 and 31 of S17).

Planting setts obtained from suckers of the female parent plants (Ph15 and Ph17) and from two potential pollinators, Ph21 and Col A-1 genotypes (Bubia Agricultural Research Centre accession numbers) were planted for comparison with the seedlings. The setts were from suckers (approximately 400–500 mm tall), from which the corm was removed at 5 mm below the petiole base of the oldest living leaf. The petioles were cut 250–300 mm from the corm to remove the leaf lamina and the setts were approximately 50–60 mm in diameter at the cut surface of the corm. The number of setts of each parent planted, depended on its availability and was 59 (Ph15), 68 (Ph17), 30 (Ph21), and 16 (Col A-1). Seedlings and setts were randomly planted at a depth of 150–200 mm at a spacing of 1 m<sup>2</sup>.

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Leaf measurements were made on the second youngest fully expanded leaf of the main plant and included leaf lamina length, width area, and petiole length. The number of leaves and suckers per plant was also recorded. Leaf measurements were carried out on a sub-sample of 20 randomly chosen plants per cultivar.

At 8, 10, 12, and 14 weeks after planting in the field (40, 42, 44, and 46 weeks after sowing) a random sub-sample of 20 plants per treatment was selected and measurements of petiole length and lamina width, length, and area from the youngest fully expanded leaf of the main plant, the number of leaves, and suckers and (or) stolons per plant were recorded. Plants were harvested 33 weeks after planting in the field and the number of suckers, the main corm, and sucker fresh weights were recorded.

Taro leaf blight infection was scored visually on a scale of one to nine, one showing no sign of infection and nine being most severe.

Data were analysed using the General Linear Model analysis of variance (MINITAB). The proportion of plants with stolons was analysed using Chi-square (MINITAB)

## Results

Plants of Ph15 (the mother plants of seedlings S15) were light green in colour, and all the suckers were borne on stolons and regarded as a 'semi-wild type'. Those of Ph17 (the mother plants of seedlings S17) had purple petiole and purple blotches on dark green leaf laminae and a high proportion (65%) of plants also had suckers that were borne on stolons and were also regarded as a 'semi-wild type'.

Both the Ph15 and Ph17 mother plants which flowered at the same time, were likely sources of pollen for each other. Other possible sources of pollen were Ph21 and Col A-1 plants. The Ph21 plants had purple petioles with dark green leaf laminae without purple blotches and had suckers close to the mother plant. The Col A-1 plants had yellow-brown leaf laminae and petioles, and had suckers close to the mother plant. Both Ph21 and Col A-1 plants had suckers without stolons. Plants of Ph15, Ph17, and Ph21 were considered to be resistant to TLB (Anton Ivancic, pers. commun.).

Both S15 and S17 plants showed petiole and leaf lamina colours ranging from light green through pink to purple and dark green. The seedling plants had both suckers that arose close to the mother plant or stolons. The proportion of plants with stolons (30 and 35%, respectively) were not significantly different by the Chi-square test.

At the first sampling (eight weeks after planting) S15 and S17 seedlings and sucker-derived plants were of similar size (Figures 1-6). At other sampling dates, petiole length (Figure 1), leaf lamina length (Figure 2), leaf lamina width (Figure 3), and leaf area (Figure 4) of both S15

and S17 seedlings, were significantly greater than Col A-1 plants. The seedlings had more leaves per plant but the differences were not significant (Figure 5). The seedlings produced significantly more suckers than the parent plants

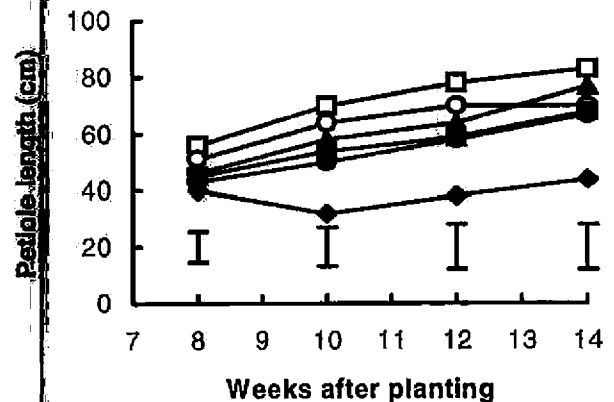


Figure 1 The change in petiole length of (□), S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$

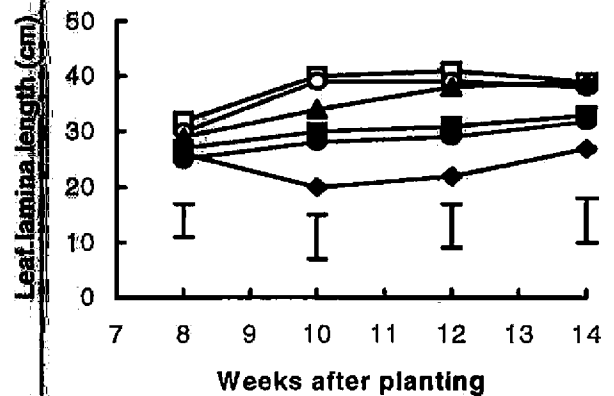


Figure 2 The change in leaf lamina length of (□) S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$

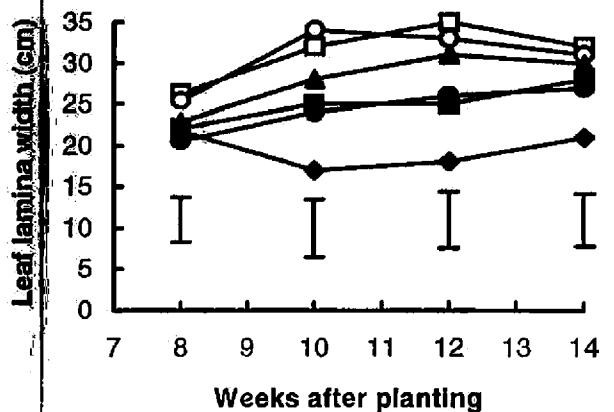
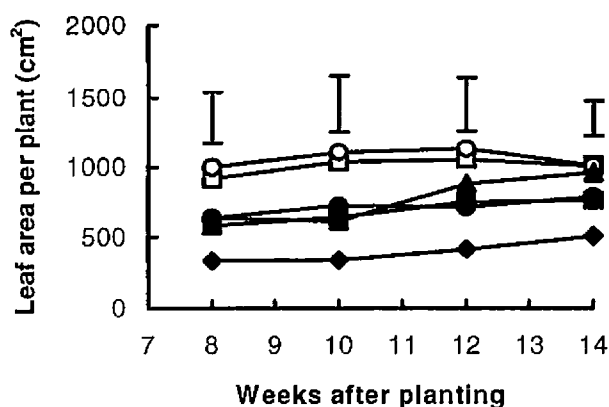
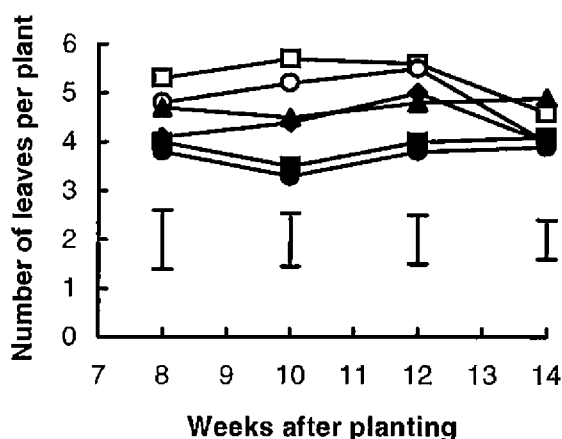


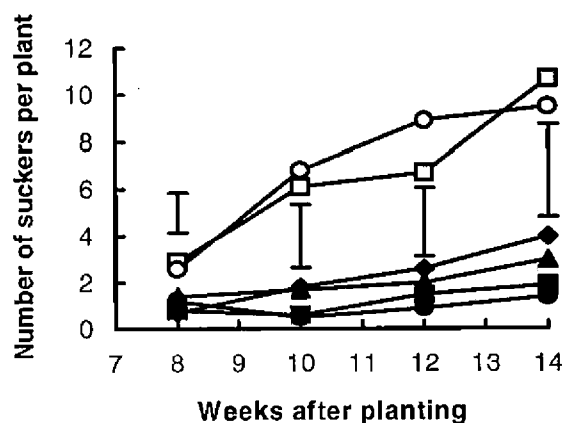
Figure 3 The change in leaf lamina width of (□), S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$



**Figure 4** The change in leaf area of (□), S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$



**Figure 5** Change in the number of leaves per plant of (□), S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$



**Figure 6** The number of suckers per plant produced by (□), S15 and (○), S17 seedlings and their vegetatively-propagated parent plants, (■), Ph15, (●), Ph17, (▲), Ph21, and (◆), Col A-1, over time; Vertical bars represent  $LSD_{0.05}$

(Figure 6). At 14 weeks after planting, the seedlings had an average of 10–12 suckers whereas the parent plants had an average of 1–4 suckers. Although the number of suckers increased until harvest, the seedlings still had significantly more suckers than the parent plants (Table 1).

Flower production in the seedlings started about 30 weeks after sowing indicating that the seedlings were physiologically mature by this age.

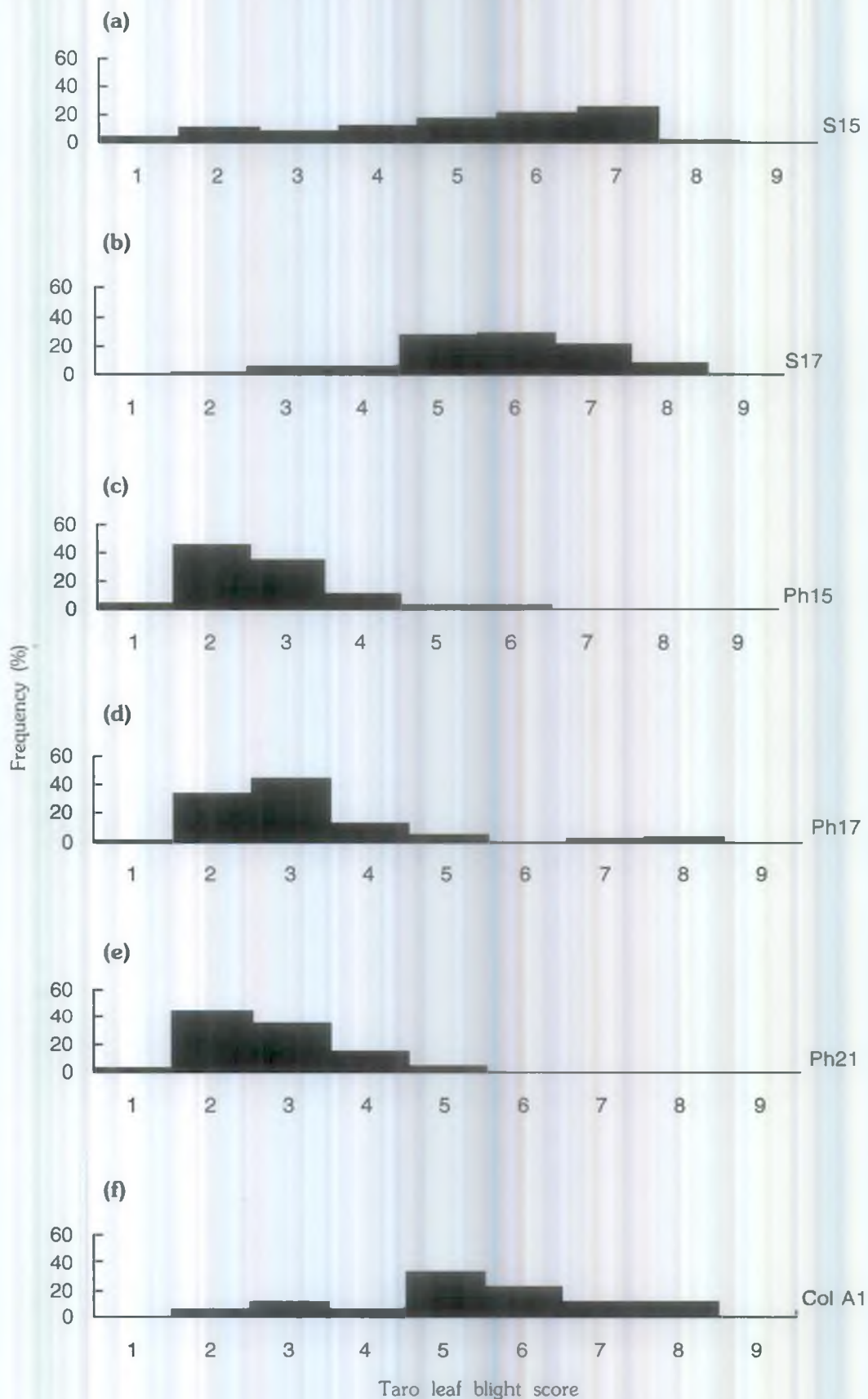
Taro leaf blight resistance was estimated by scoring the degree of infection. The parent plants Ph15, Ph17, and Ph21 all appeared to have some degree of resistance to TLB (Figure 7). However, the parent Col A-1 was susceptible. This may account for the poor performance of its growth parameters examined. The S15 and S17 seedlings showed a range of TLB scores which would indicate that within the seedlings, there may be a range of susceptibility to TLB. Some seedlings had a good level of resistance to TLB, especially some of the S15 seedlings.

The main corm yield of the seedlings S15 and S17 was significantly greater ( $P < 0.05$ ) than the Ph15 and Ph17 parents but was similar to the Ph21 and Col A-1 parents (Table 1). The main corm, sucker corms, and total corm yield of the seedlings that were TLB-resistant was significantly greater ( $P < 0.05$ ) than all the parents (Table 1). The weights of sucker corms per plant of the seedlings and the parent plants were not significantly different (Table 1). The total corm yield of the S15 seedlings was significantly greater ( $P < 0.05$ ) than the parents Ph15 and Ph17 but not significantly different from the Ph21 and Col A-1 parents (Table 1). The total corm yield of the S17 seedlings was not significantly different from the parents. Overall, the seedlings performed as well as, or better than, their vegetatively-propagated parents.

**Table 1** Mean number of suckers at harvest and corm fresh weight yields of the seedlings and the vegetatively-propagated parent plants

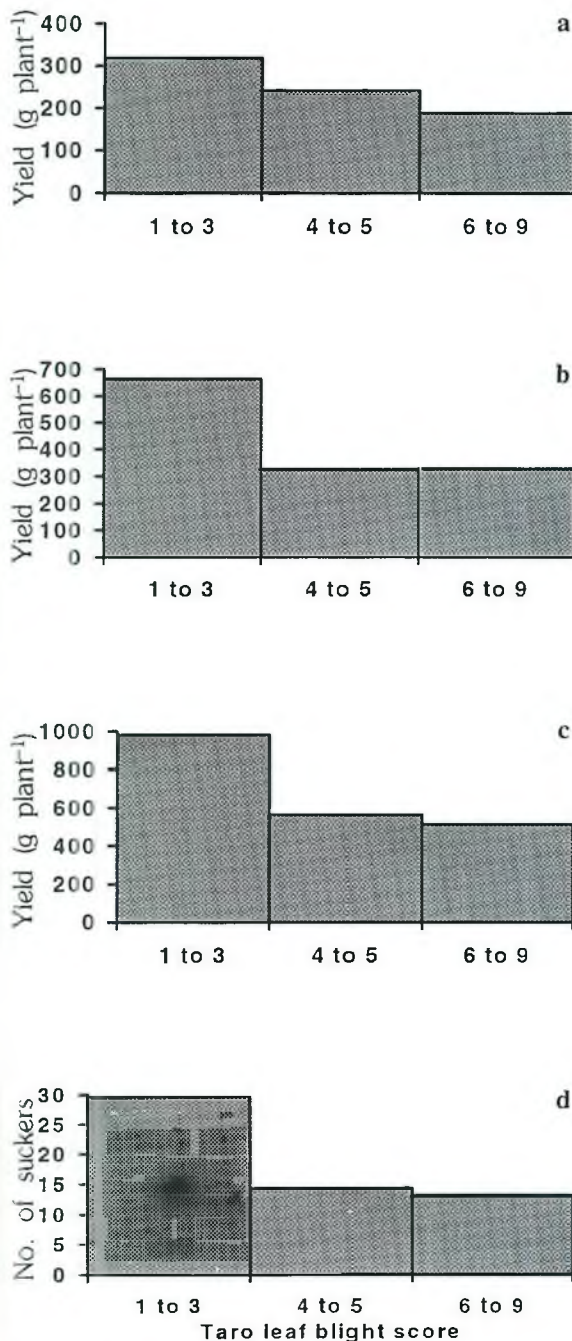
Plant type	No. of suckers per plant	Main corm weight (g plant <sup>-1</sup> )	Sucker corm weight (g plant <sup>-1</sup> )	Total corm weight (g plant <sup>-1</sup> )
<b>Parents</b>				
Ph15	9.4 (2.2)	122 (10)	269 (62)	391 (81)
Ph17	7.1 (1.0)	148 (50)	179 (78)	327 (23)
Ph21	16.3 (1.4)	234 (19)	457 (43)	691 (54)
Col A-1	10.1 (1.5)	191 (28)	319 (70)	510 (93)
<b>Seedlings</b>				
S15	19.3 (1.9)	219 (13)	454 (38)	706 (39)
S17	16.1 (1.9)	221 (2)	328 (49)	567 (67)
S-TLB	29.6 (4.0)	318 (20)	663 (69)	981 (77)

S-TLB represents seedlings that are resistant to TLB, i.e., with resistant scores of 1, 2, or 3  
Values in parentheses are standard error of the mean (SEM)



**Figure 7** The taro leaf blight score for seedlings (a) S15 and (b) S17 and the vegetatively-propagated parent plants, (c) Ph15, (d) Ph17, (e) Ph21, and (f) Col A-1; Plants with a score of one show no visible signs of taro leaf blight and a score of nine is most severe





**Figure 8** (a) The main corm yield; (b) sucker corm yield; (c) total corm yield; and (d) number of suckers of seedlings grouped according to taro leaf blight score

The seedlings were separated into three groups based on their TLB-resistant scores as follows: scores of 1, 2, and 3, TLB resistant; scores of 4 and 5, slightly resistant; and scores of 6, 7, 8, and 9, susceptible.

Among the seedlings, the main corm yield was greatly reduced by the incidence of TLB (Figure 8a). The average main corm yield of the TLB resistant group (318 g) was significantly higher ( $P < 0.05$ ) than the slightly resistant group which was significantly higher ( $P < 0.05$ ) than the susceptible group. The seedling main corm yields of the slightly resist-

ant and susceptible TLB groups were approximately 59 and 76%, respectively, lower than the resistant group. The sucker corm yield of the TLB-resistant seedlings was also significantly greater ( $P < 0.05$ ) than the other seedlings (Figure 8b). The sucker corm yields of the slightly resistant and susceptible seedlings was approximately 50% lower than the resistant seedlings. The total corm yield followed similar trends to the main corm and sucker yields (Figure 8c). The TLB-susceptible and slightly resistant seedlings produced only 52 and 58% as much total corm, respectively, as the resistant seedlings.

The TLB-resistant seedlings produced twice as many suckers as the other seedlings (Figure 8d) which would account for the difference in the sucker corm yield as the mean sucker weight of approximately 23.5 g, was similar for all seedlings. The increase in the number of suckers produced by the TLB-resistant seedlings is most likely due to the increased vigour of these resistant plants.

The above results suggest that the semi-wild type, TLB-resistant parents Ph15, Ph17, and Ph21 could be useful for breeding with the high-yielding cultivars that are not resistant to TLB.

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# Integration of cassava tuber and forage legume seed production for sustained soil fertility

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*Centro* (*Centrosema pubescens*) was intercropped with cassava (*Manihot esculenta*) to determine whether the latter can provide vining support to the forage legume for increased seed yields, and improve soil conditions for subsequent croppings without significantly reducing tuber yield. Results showed that legume seed yield from the cassava-centro intercrop was 552 kg ha<sup>-1</sup> whereas 1000 kg ha<sup>-1</sup> was harvested from the centro sole crop that was staked. The cassava varieties (Tereka, Bukalasa, and Bao) that provided appropriate support for the legume succumbed to African Cassava Mosaic Virus (ACMV) disease. Similar cassava root yields of ACMV-resistant cultivars intercropped with centro planted at the same time, one, five, and six months later implied that the legume did not have a significant effect on tuber yield. After harvesting, the soil from the cassava-centro intercrop had a higher soil N and organic matter (OM) content than the sole crop of cassava. It was concluded that intercropping cassava and centro could provide tubers and forage legume seed and also improve soil N and OM for subsequent crops.

Keywords: Nitrogen; Organic matter; Phosphorus; Staggered planting

*Centro* (*Centrosema pubescens*) is a climbing forage legume that is recommended for improved pastures in Uganda (Stobbs, 1969). Lack of seed is one of the major problems that has hindered its incorporation into planted pastures. Cassava (*Manihot esculenta*) is the second most important staple crop in Uganda. It is known for its ability to produce yields on soils depleted of nutrients as a result of continuous cropping. Cassava has erect growth habits and the stems could provide necessary support for increasing seed yields of centro (Lusembo *et al.*, 1993). The N-fixing ability of the companion forage legume could aid the regeneration of the soil under cassava. Cassava-forage legume intercropping, therefore, is a strategy for production of food for humans, produce forage legume seed for pasture improvement, and regenerate the soil. Because of varietal differences in plant architecture, there is need for evaluation of cassava varieties for compatibility in cassava-forage legume intercropping. The objective of the study was, therefore, to identify cultivars of cassava suitable for intercropping with forage legumes to produce roots for human consumption and seed for pasture improvement and also to determine the effect of the intercrop on soil fertility and productivity for subsequent crops.

## Materials and Methods

The trial was conducted at NAARI (0°32' N, 32°35' E; 1150 m above sea level). Site soils are ferralitic sandy clay loams, low in available P (4 µg g<sup>-1</sup> P) with pH (H<sub>2</sub>O) of 5.4–6.0. The site lies in a sub-humid zone of Uganda with a mean annual rainfall of about 1100 mm, which is bimodally distributed with peaks in March–May and September–November. Mean annual temperature is 20°C with mean maximum and minimum temperatures of 29°C and 10°C, respectively. The site had been under a *Chloris gayana* seed crop for the past three years before it was ploughed. A fine seed bed was later prepared using a hand hoe. Soil samples were taken to determine the soil nutrient status of the experimental site prior to laying down the experiment. The soil contained: total N of 0.15 and 0.10 for 0–15 cm and 15–30 cm depth, respectively; whereas, available P (Bray II) was 11.20 and 4.41 ppm, respectively. Organic matter (OM) was 3.65 and 2.90%, respectively. Single superphosphate (SSP) was applied to the soil at a rate equivalent to 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> to correct for the inherently low P in Uganda soils (Stephens, 1970). The trial was conducted as a split-plot design with main plots in randomized complete blocks (RCBD) and four replications. Plot size was 8 m × 8 m. Local cultivars of

cassava (Bukalasa, Bao, and Tereka) and two newly evaluated and promising cultivars, TMS 60142 and TMS (4) 1425 were the main plots, while time of planting centro relative to cassava planting were the subplots. The cassava cuttings were 15 m long and were planted at a spacing of 1 m × 1 m. Centro seeds were scarified with concentrated (1M) sulphuric acid for 15 min to reduce hard-seededness. Seed was planted at the same time ( $M_1$ ) as cassava, at one month ( $M_2$ ), five months ( $M_3$ ), and six months ( $M_4$ ) thereafter. Planting was done in March and April and also in August and September, 1995, during the first months of the two rainy seasons. This was intended to ensure adequate soil moisture conditions for germination and seedling development of the legume. Centro seeds were planted in clusters 30 cm from the side of each planted cutting. Insect pests of centro were controlled by foliar applications of Ambush CY (permethrin 500 g L<sup>-1</sup> at a rate of 500 g a.i. ha<sup>-1</sup>). The harvest plot was 6 m × 6 m. Cassava tubers of the TMS cultivars were harvested piecemeal at 12 months after planting (MAP). Pods of centro were harvested whenever they matured and yield (kg ha<sup>-1</sup>) determined. Cassava tubers of the TMS cultivars were sorted into marketable (>2 cm diameter) and unmarketable (<2 cm diameter) and total weight determined.

At 18 months after laying down the trial, the site was slashed and the trash left for two months to allow for partial decomposition and mineralization of the vegetative material. Soil samples were taken at the beginning of the rainy season in September 1996, and analysis carried out for determination of percentage N, OM, and P. Statistical analyses were carried out using the MSTATC software (MSTAT, 1988).

## Results and Discussion

Bukalasa, Tereka, and Bao cultivars succumbed to African Cassava Mosaic Virus (ACMV) at 4 MAP. The disease caused general chlorosis and deformation of the leaves and subsequent dieback of the primary stems (Otim-Nape *et al.*, 1994). TMS cultivars were resistant and development of the companion forage legume crops appeared constrained. This was attributed to competition between crops for nutrients and light. The legumes in treatments with ACMV-susceptible cultivars grew more vigorously. Flowering and seed set were more profuse than in intercrops with the TMS series. The effect was attributed to low demand for environmental growth factors (hence low competition) caused by the disease in the two varieties. Vigorous growth, profuse flowering, and seed set in ACMV-intercrops supports the contention. Seed yields in ACMV-tolerant intercrops were suppressed and were too low to be meaningfully measured.

Total seed yield of centro under the three

**Table 1** Effect of support system and time of planting *Centrosema pubescens* at the same time ( $M_1$ ), one month ( $M_2$ ), five months ( $M_3$ ), and six months ( $M_4$ ) after cassava on the seed yield (kg ha<sup>-1</sup>) of centro

Planting time	Support system			
	Cassava	Staked	Unstaked	Means
$M_1$	552	1000	250	601
$M_2$	548	995	252	598
$M_3$	210	500	100	370
$M_4$	190	450	110	317
Means	475	761	178	472
S.E. Time means			±20.1***	
S.E. Support system means			±18.3**	
S.E. Time × support system means			±12.4 ns	

ns, Not significant

\*\*, \*\*\*, significant at  $P < 0.01$  and  $P < 0.001$ , respectively

S.E., standard error

support systems is given in Table 1. Provision of support increased seed yield by more than twofold. Similar findings were observed in earlier studies (Lusembo *et al.*, 1993). Staggering planting of the legume in the intercrop significantly ( $P < 0.01$ ) reduced seed yield by 1, 62, and 66% for  $M_2$ ,  $M_3$ , and  $M_4$ , respectively. In the staked crop delayed planting caused 1.5, 50, and 45% reduction, respectively. In the unstaked legume crop 3, 60, and 56% reductions in seed yield were observed for  $M_2$ ,  $M_3$ , and  $M_4$  treatments, respectively, and the reductions were significantly ( $P < 0.01$ ) different. Reduced yield associated with staggered planting is attributable to relatively shorter harvesting periods. Differences between staked and intercrops suggest losses due to competition for soil nutrients and other environmental factors. Root yields of Bukalasa, Tereka, and Bao were too low for meaningful records. The data were discarded in the analysis.

The TMS series established dense canopies at 5 and 6 MAP. Thereafter, defoliation reduced shading and some centro plants were able to develop and produce some seed. These TMS

**Table 2** Total tuber yield (t ha<sup>-1</sup>) of cassava intercropped with *Centrosema pubescens* planted at the same time ( $M_1$ ), one month ( $M_2$ ), five months ( $M_3$ ), and six months ( $M_4$ ) later

Cassava cultivar	Time of planting centro				
	$M_1$	$M_2$	$M_3$	$M_4$	Means
Means					
TMS 60142	28 a	30 a	30 a	30 a	30
TMS (2) 1425	26 a	28 a	28 a	29 a	28
Means	27	29	29	30	
S.E. Time means		±3.2 ns			
S.E. Cultivar means		±4.3 ns			
S.E. Time × cultivar means		±2.9 ns			

ns, Not significant

S.E., standard error

**Table 3** Soil N and organic matter (0–15 cm) under different support/cropping systems of *Centrosema pubescens* after 18 months of planting

Support system	Total N (%)	Organic matter (%)
Bukalasa	0.18	3.97
Tereka	0.19	3.95
Bao	0.20	3.96
TMS 60142	0.11	3.77
TMS (4) 1425	0.12	3.76
Staked	0.22	4.02
Unsupported	0.19	3.98

cultivars developed tubers (Table 2) and appeared to be resistant to ACMV as reported by Otim-Nape *et al.* (1994). Root yields did not differ between varieties and time of planting the legume. Intercropping did not affect root yields of either cultivar suggesting that cassava had competitive advantage over the legume. Much of the competitive advantage of cassava over the centro was attributed to canopy cover and light interception.

Despite the generalization that most soils in Uganda are low in available P, analysis showed that this element was relatively high at the experimental site. This was attributed to an accumulation of P due to repeated application by research scientists over the years. At the end of the trial most of the plots has similar high levels of P. Single superphosphate is a slow-release fertilizer and may remain in the soil long after application. In the present study, there was no detectable difference in P uptake from the soil by cassava and centro. Studies in Ethiopia showed that in the first year of application, plants use only about 25% of all the P applied as single superphosphate (Haque *et al.*, 1986). Among the soil fertility components measured, only N and OM showed marked differences among the different cropping systems of centro (Table 3). The high soil N and OM in cassava plots that succumbed to ACMV and allowed centro to flourish was attributed to high biomass of centro and its ability to fix N. At the end of the experiment, soil N was found to be higher in the staked than in the unstaked crop. This was attributed to the greater vegetative biomass in the staked crop than in the unstaked crop. The two months that elapsed after the vegetation had been cut seem to have

facilitated partial decomposition and mineralization of the herbage. Normally, cassava is planted as the last crop after continuous cropping has exhausted the soil of most nutrients. The above findings may imply that centro intercropped with cassava may have a positive effect on the N and OM content of the soil. This could reduce the length of the fallow period that may be required for resumption of normal cropping.

## Conclusion

It was concluded that a cassava-centro intercrop for root and legume seed production and recovery of soil fertility requires cassava varieties that are resistant to ACMV, are erect, and have light foliage.

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# Evaluation of nitrification inhibitor to improve fertilizer N use efficiency in potato crop

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Two field experiments were conducted (a spring and an autumn) during 1996-97 to study the effect of application of two nitrification inhibitors (nitrapyrin and wax-coated  $\text{CaC}_2$ ) at two N rates (100 and 250 kg N  $\text{ha}^{-1}$ ) on fertilizer use efficiency (FUE), N recovery, yield of potato (*Solanum tuberosum* L.), and to compare it with split application of N. The results revealed that the treatment receiving inhibitors had more mineral N accumulation than the treatment receiving no inhibitors in the active root zone (0-30 cm) up to 65 days after sowing (DAS). Ammonium was a major fraction of mineral N in the treatments receiving inhibitors. At 95 DAS, the soil at 0-30 cm depth did not show major differences in the mineral-N status of treated and untreated plots. However, at 60-90 cm and 90-120 cm depths, the treatment receiving no inhibitors contained higher  $\text{NO}_3\text{-N}$  indicating greater  $\text{NO}_3$  leaching from the active growth zone. Maximum tuber yield and N concentration was obtained at low N level treated with nitrification inhibitors followed by two split applications of 250 kg N  $\text{ha}^{-1}$ .

Keywords: Potato; Nitrification inhibitors; Nitrapyrin; Calcium carbide; Plant response

Potato (*Solanum tuberosum* L.) is an important vegetable crop of Pakistan. Three crops are raised in a year; a spring and an autumn crop in the plains and a summer crop in hilly areas at high altitudes. The crop has a high N demand.

Ammonium and nitrate are the N species that are taken up by most crop plants. Being soluble in water, the nitrate is highly mobile in the soil and, thus, subject to leaching and denitrification, posing serious problems to water quality and health (Allison, 1966; Tyler and Thomas, 1977; Chichester and Smith, 1987). The amounts of N recovered by plants and in soil are about 60% or less, the remainder being lost from the soil-plant system (Smith *et al.*, 1989; Smith and Whitefield, 1990; Iqbal *et al.*, 1995).

One of the reasons that causes incomplete plant recovery of applied N is rapid nitrification (Shah and Shah, 1991). Many laboratory, greenhouse, and field experiments have shown that nitrification inhibitors under certain conditions can reduce N losses by improving fertilizer use efficiency (FUE) and thus increase crop yields through suppression of nitrification (Huber *et al.*, 1977; Frye *et al.*, 1981; Walters and Malzer, 1990a, b). Results from a field experiment on  $^{15}\text{N}$  labelled ammonium sulphate applied to a potato crop at this institute (NIFA, unpubl. data) revealed that up to 20% of the applied N was utilized by the crop; the remainder was lost from the rhizosphere. Field experiments were therefore conducted to evaluate the effect of nitrification inhibitors (nitrapyrin and wax-coated  $\text{CaC}_2$ ) on FUE in terms of total N uptake and crop yield.

## Materials and Methods

Two field experiments, a spring and an autumn, were conducted with potato cv. Cardinal at the experimental farm of NIFA, Peshawar, Pakistan during 1996-97. The soil of the experimental site was a silty clay having pH in the range of 8.1 to 8.4, electrical conductivity (EC) in the range of 1.8 to 2.0 dS  $\text{m}^{-1}$ ,  $\text{CaCO}_3$  from 15-16%, and organic matter from 0.4-0.9%. The experimental site is representative of the irrigated area of Peshawar valley used for potato growing. The effect of two nitrification inhibitors [nitrapyrin at 1.12 kg  $\text{ha}^{-1}$  and  $\text{CaC}_2$  (wax-coated) at 30 kg  $\text{ha}^{-1}$ ] and two N rates (100 and 250 kg N  $\text{ha}^{-1}$ ) on yield, mineral-N status, and FUE was assessed in 4 m  $\times$  5 m plots in a randomized complete block design with four replicates. Basal doses of P at 100 kg  $\text{P}_2\text{O}_5$   $\text{ha}^{-1}$  in the form of single superphosphate and K at 250 kg  $\text{K}_2\text{O}$   $\text{ha}^{-1}$  in the form of sulphate of potash were applied to all the plots. The treatments were as follows: Control (no N); 100 kg N  $\text{ha}^{-1}$  all at sowing time and worked into soil; 100 kg N  $\text{ha}^{-1}$  + nitrapyrin worked into soil at sowing time; 100 kg N  $\text{ha}^{-1}$  +  $\text{CaC}_2$  worked into soil at sowing time; 250 kg N  $\text{ha}^{-1}$  all at sowing time and worked into soil; 250 kg N + nitrapyrin worked into soil at sowing time; 250 kg N +  $\text{CaC}_2$  worked into soil at sowing time; 50 kg N at sowing time + 50 kg N at earthing up; and 125 kg N at sowing time + 125 kg N at earthing up.

The wax-coated  $\text{CaC}_2$  was prepared in the laboratory according to the procedure of Freney

et al. (1992). Potato was sown on ridges which were 30 cm high and 80 cm apart. The seed potatoes pre-treated with Dithane-M, to control against attack of wilt and other fungal diseases, were sown at the depth of 15 cm in the ridges. Common cultural practices were followed throughout the growing season. During the autumn experiment, a total of 109 mm rainfall, and during the spring experiment, a total of 152.4 mm of rainfall, was received. In the autumn experiment, 8 irrigations of 3.5 cm each (28 cm water) and in the spring experiment, 10 irrigations of 6.0 cm each (60 cm water) were applied. Soil samples were collected 30, 65, and 95 days after sowing (DAS) from the central three rows (six rows in each plot) from 0–30, 30–60, and 90–120 cm depth. The samples from each depth of different cores were bulked and thoroughly mixed. Total mineral N in the soil was determined by the steam distillation method of Keeney and Nelson (1982). Plant samples (tuber and straw) were taken from each plot at maturity. The plant material was dried at 60°C in an oven. The dried material was ground to pass through 0.42-mm sieve and analysed for total N according to the Kjeldahl method (AOAC, 1975).

## Results and Discussion

### Effect of inhibitors on mineral N

Application of N in the form of urea to the soil increased the mineral N concentration considerably in the active root zone (0–30 cm). The mineral N remained higher up to 30 DAS especially in the case of higher rate of N application (data not shown). Higher  $\text{NH}_4\text{-N}$  concentration was maintained in the 0–30 cm depth in the treatment receiving inhibitors in both experiments up to 65 DAS (data not shown). After this time, the  $\text{NH}_4\text{-N}$  level in all the treat-

ments decreased, however, the nitrate level was still lower in all the treatments receiving urea plus the inhibitors and the difference persisted even up to 95 DAS compared to non-inhibitor treatments. The lower  $\text{NO}_3\text{-N}$  concentration in inhibitor treatments compared to non-inhibitor treatments showed that the inhibitors were effective in suppressing oxidation of  $\text{NH}_4$ .

The  $\text{NO}_3$  content in the soil profile increased in response to urea addition but in the case of treatment receiving 100 kg N + the inhibitors, there was little increase up to 65 DAS (data not shown) and  $\text{NH}_4\text{-N}$  remained dominant. Soil samples 95 DAS did not show major differences in the  $\text{NH}_4$  content in 0–30 cm depth. In general, the  $\text{NO}_3$  content at lower depths was increased in the treatments receiving 250 kg N  $\text{ha}^{-1}$  without inhibitor, indicating leaching of  $\text{NO}_3$  from the upper layers. The data suggest that both inhibitors were effective in blocking the nitrification up to 65 DAS and, hence, could be used to control  $\text{NO}_3$  production during this period. Furthermore, both inhibitors were more effective in inhibiting nitrification at the lower level of N compared to the higher level. Among the inhibitors, nitrapyrin (N-serve) was found to be more effective in controlling nitrification compared to  $\text{CaC}_2$ . Other workers (Huber et al., 1977; Frye et al., 1981; Freney et al., 1992) also found nitrification inhibitors (nitrapyrin and  $\text{CaC}_2$ ) to be effective in controlling the nitrification process.

### Plant response

#### Tuber yield

The tuber yield in the N-fertilized treatments was significantly greater than the control treatment (Table 1). Maximum tuber yield in both experiments was obtained in the treatment receiving 100 kg N + inhibitors followed by split application of 250 kg N  $\text{ha}^{-1}$ . However, the inhibitors at higher N level (250 kg N  $\text{ha}^{-1}$ ) did

**Table 1** Tuber and straw (above-ground biomass) yield of potato as influenced by nitrapyrin and wax-coated  $\text{CaC}_2$  added to urea fertilizer at two N rates

S. No.	Treatments (N kg $\text{ha}^{-1}$ )	Tuber yield (t $\text{ha}^{-1}$ )		Straw yield (t $\text{ha}^{-1}$ )	
		Spring experiment	Autumn experiment	Spring experiment	Autumn experiment
T1	0	1.75 e	4.15 c	1.31 e	0.71 e
T2	100	4.60 cd	7.25 b	1.77 ab	1.33 d
T3	100 + nitrapyrin	6.81 a	8.60 a	1.75 ab	1.71 a
T4	100 + $\text{CaC}_2$	6.06 ab	9.20 a	1.77 ab	1.62 ab
T5	250	4.27 d	6.60 b	1.39 de	1.62 ab
T6	250 + nitrapyrin	5.26 bcd	6.90 b	1.58 bcd	1.45 cd
T7	250 + $\text{CaC}_2$	5.10 bcd	7.10 b	1.69 abc	1.50 bc
T8	50 kg at sowing + 50 kg at earthing up	5.52 bc	7.30 b	1.62 cde	1.16 e
T9	125 kg at sowing + 125 kg at earthing up	5.90 ab	8.50 a	1.86 a	1.75 a

not result in any significant increase in yield compared to non-inhibitor treatments. The rate of application of 250 kg N ha<sup>-1</sup> as a single dose at sowing time, decreased the yield compared to its application in two splits despite the fact that it maintained sufficient mineral N in the rooting zone throughout the growing season. The reason for decrease in yield due to single application at sowing may be fertilizer salt injury. Its adverse effect was visible on germination of the crop. These results indicate that N-serve and wax-coated CaC<sub>2</sub> were equally effective, at low dose of N (100 kg N ha<sup>-1</sup>), in maintaining sufficient mineral N (NH<sub>4</sub>-N) in the root zone throughout the growing season for obtaining maximum yield. These results are in agreement with the findings of McCormick *et al.* (1984) who reported similar results with some other crops.

### Straw yield

Straw yield (above-ground biomass) was significantly increased by N application (Table 1). Addition of nitrification inhibitors at 100 kg N ha<sup>-1</sup> level improved the straw yield further. However, at 250 kg N ha<sup>-1</sup> level, the inhibitors did not influence the yield significantly. The effect of inhibitors on the straw yield of potato, in general, was not pronounced during either growing season.

### N content of tuber and straw

Application of 100 kg N ha<sup>-1</sup> + inhibitors resulted in higher N accumulation in tubers in both experiments compared to non-inhibitor or split treatments (Table 2). Maximum N was found in treatments receiving 250 kg N ha<sup>-1</sup> as split application in the autumn experiment. However, split application of 250 kg N ha<sup>-1</sup> did not increase the N content significantly in the spring experiment compared to the other

treatment receiving 250 kg N ha<sup>-1</sup> alone or with inhibitors. Addition of inhibitors at 250 kg N ha<sup>-1</sup> level did not cause any significant increase in N content of tuber in either experiment.

### Growing season

The N content of tuber in the spring experiment was higher than the autumn experiment but the tuber yield was lower in the spring experiment which may have resulted in higher N accumulation. Also, the growth duration of the spring experiment was relatively longer which may have led to greater N uptake than the autumn experiment. Straw N content was highest in both the experiments receiving 250 N ha<sup>-1</sup> in a single dose at sowing time followed by 100 kg N ha<sup>-1</sup> + inhibitor treatments.

### Nitrogen recovery

Maximum N recovery of 63.2% in the autumn experiment and 61.2% in the spring experiment was recorded in the treatment receiving 100 kg N + nitrapyrin followed by the treatment receiving 100 kg N ha<sup>-1</sup> + CaC<sub>2</sub> (56.9 and 54.2%, respectively). Split application of N did not result in higher N recovery than single application at sowing time. Also, higher N recovery was noted in the low N level treatments compared to higher N level treatments. The uptake data showed that N-uptake by straw (above-ground parts) of potato was almost double than that by tubers. From the foregoing discussion, it can be concluded that both inhibitors were effective in increasing the N use efficiency only at low N level (100 kg N ha<sup>-1</sup>).

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**Table 2** Nitrogen content of tuber and straw (above-ground biomass) as influenced by nitrapyrin and wax-coated CaC<sub>2</sub> added to urea fertilizer at two N rates

S. No.	Treatments (N kg ha <sup>-1</sup> )	N contents (%)			
		Tuber		Straw	
		Spring experiment	Autumn experiment	Spring experiment	Autumn experiment
T1	0	1.46 b	0.77 b	0.49 d	0.40 c
T2	100	1.67 ab	1.17 ab	2.33 bc	2.17 b
T3	100 + nitrapyrin	2.01 a	1.33 a	2.90 ab	2.83 ab
T4	100 + CaC <sub>2</sub>	2.04 a	1.28 a	2.60 abc	2.41 b
T5	250	1.87 ab	1.45 a	3.26 a	3.34 a
T6	250 + nitrapyrin	2.03 a	1.22 ab	2.93 a	2.32 b
T7	250 + CaC <sub>2</sub>	1.78 ab	1.31 a	2.70 abc	2.39 b
T8	50 kg at sowing + 50 kg at earthing up	1.75 ab	1.15 ab	2.13 c	1.97 b
T9	125 kg at sowing + 125 kg at earthing up	1.89 ab	1.50 a	2.3b c	2.32 b

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# Performance of sweetpotato in different agro-ecologies in Ghana

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Five exotic sweetpotato [*Ipomoea batatas* (L.)] clones (TIS 8266, TIS 86/0350, TIS 84/0320, TIS 3017, and Sauti) and three locally collected clones (Local Red, Dugbadza, and Cape Coast) were selected from several accessions and evaluated in four agro-ecological zones in Ghana in order to recommend suitable genotypes for release to farmers. The agro-ecologies were coastal savannah, deciduous forest, forest-savannah transition, and guinea savannah with mean annual rainfall of 800 mm, 1500 mm, 1300 mm, and 1100 mm, respectively. Harvesting was done four months after planting (MAP). In the coastal savannah, the best four clones were TIS 86/0350, TIS 3017, TIS 8266, and TIS 84/0320 with mean tuber yields ranging from 10.6 to 12.9 t ha<sup>-1</sup>. In the forest zone, the best four (TIS 8266, TIS 84/0320, TIS 3017, and Dugbadza) produced yields ranging from 18.9 to 24.4 t ha<sup>-1</sup>. In the Forest-Savannah transition, the best four were TIS 8266, TIS 3017, Sauti, and Local Red with mean tuber yields between 12.0 and 14.9 t ha<sup>-1</sup>. In the guinea savannah, the best performing clones were TIS 86/0350, Dugbadza, TIS 84/0320, and TIS 8266 with mean yields ranging from 16.9 to 18.5 t ha<sup>-1</sup>. Clone TIS 8266 was among the top four clones in all agro-ecologies.

Keywords: Sweetpotato; Accessions; Agro-ecologies; Farmers; Ghana

In Ghana, sweetpotato [*Ipomoea batatas* (L.)] cultivation and utilization are very prominent particularly in the savannah agro-ecologies. There exists a potential for increased production and utilization both as food and animal feed in other agro-ecologies. Although yields of up to 40 t ha<sup>-1</sup> have been reported (IITA, 1976), yields realized by farmers tend to be low and the quality reduced due to low genetic potential of varieties, diseases (fungal and viral), and pests (*Cylas* sp. and *Alcidodes* sp.). A paucity of information on appropriate agronomic practices also contributes to the low yields at farm level.

To overcome these problems, a number of exotic sweetpotato lines and some local genotypes were assembled and screened for years at the coastal savannah and the forest agro-ecological zones of Ghana (Missah *et al.*, 1995, 1996). Clones which proved promising were identified. Sweetpotato varieties imported from East Africa (high altitude environment) performed well in Ghana (low altitude) (Otoo *et al.*, 1995). The objective of this study, therefore, was to assess the performance of the promising genotypes in more and varied agro-ecological zones in Ghana in order to select, multiply, and release to farmers, high-yielding clones which satisfy utilization requirements in the different regions of Ghana.

## Materials and Methods

Field experiments were conducted during the major growing seasons in 1995 and 1996. In 1995, the experimental locations were Cape Coast and Pokuase (coastal savannah), Fum-esua (Forest), and Akomadan and Wenchi (Forest to Savannah transition). In 1996, two sites, Nyankpala and Wa (Guinea savannah) were added to the locations. The soils and their properties are given in Table 1. Soils in these locations are generally loams and sandy loams. Rainfall distribution is bimodal in the forest, transitional, and coastal ecozones with a mean annual rainfall of 1500 mm, 1300 mm, and 800 mm, respectively. The guinea savannah ecozone receives 1100 mm in a unimodal distribution (PPMED, 1991).

The test clones selected for evaluation multi-locally were TIS 8266, TIS 84/0320, TIS 86/0350, TIS 3017, and Sauti [exotic clones obtained from the International Institute of Tropical Agriculture, (IITA), Nigeria and Malawi], while the local clones were Local Red, Dugbadza, and Cape Coast. These were planted in a randomized complete block design, and each treatment was replicated four times. Each plot consisted of two ridges 10 m long and 1 m apart. Vine cuttings measuring 30 cm were used to plant each treatment at a spacing of 30 cm on the ridges. The resulting plant population, therefore, was 33 000 plants ha<sup>-1</sup>. Local farming implements were used in the preparation of ridges, weeding, and harvesting. No fertilizer was applied. Tuber yield data were collected four months after planting (MAP) followed by statistical analysis. Where dry matter

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**Table 1** Some chemical and physical properties of the soils

Property	Locations and soil type							
	Wenchi (Plinthic Kandiustalfs)	Akomadan (Typic Kandiustalfs)	Ohawu (Typic Haplustalf)	Pokuase (Typic Haplustalf)	Cape Coast (Kandiustalfs)	Fumesua (Plinthustalfs)	Wa (Plinthustalfs)	Nyankpala (Plinthustalfs)
Sand (%)	72.90	30.20	71.60	97.20	61.50	53.00	49.00	70.30
Silt (%)	19.10	30.20	20.10	1.20	6.50	2.40	45.40	21.00
Clay (%)	8.00	39.60	8.30	1.60	22.00	44.60	5.60	8.70
pH	5.36	4.70	5.90	7.30	5.10	6.40	6.30	6.20
Total N (%)	0.04	—	0.017	0.08	0.79	0.753	—	—
Organic matter (%)	1.30	2.03	0.71	0.99	1.19	6.40	0.65	1.07
CEC (meq 100 g <sup>-1</sup> )	3.76	12.43	2.93	4.48	4.99	31.37	2.44	6.26
Exchangeable Mg (meq 100 g <sup>-1</sup> )	0.70	0.33	0.75	0.94	1.40	3.38	0.57	1.35
Exchangeable Ca (meq 100 g <sup>-1</sup> )	2.20	0.81	1.10	4.15	0.56	28.38	1.18	4.31
Base Saturation (%)	85.00	13.0	74.1	—	—	—	—	—
Available P (ppm)	7.60	6.2	4.0	20.0	5.6	15.0	2.4	2.0
Available K (ppm)	80	120	75	140	110	150	90	78

(DM) content was determined, sub-samples of chipped tubers were oven-dried at 80°C for 48 h. The dry weight was then expressed as a per cent of the wet weight. *Cylas* infestation was determined by visually examining 50 randomly selected tubers from each plot for *Cylas* feeding damage and the numbers expressed as percentage. The top 20 cm of the soils were sampled for analysis. The value of pH was measured using a 1:1 soil:water ratio on a Jenway 3010 pH meter. Available P was extracted by the Bray P solution and determined calorimetrically (Bray and Kurtz, 1945), organic matter was determined by the wet combustion method (Walkley and Black, 1934), and total N by the Kjeldahl method, according to Chapman and Pratt (1961). Potassium was extracted by using neutral ammonium acetate extract and determined by EDTA titration (Chapman and Pratt, 1961), and soil particle size distribution was determined by the hydrometer method of Bouyoucos (1926) modified slightly by Day (1956).

## Results and Discussion

Fresh tuber yield and per cent DM of the sweetpotato clones in 1995 are presented in Table 2. Yields were highest in the Forest (Fumesua), followed by Forest-Savannah transition (Akomadan) and coastal savannah (Pokuase and Cape Coast). Whereas the lowest fresh

tuber yield recorded in the forest was 18.0 t ha<sup>-1</sup> (Local Red), the highest in the Forest-Savannah transition and coastal savannah ecologies were 14.4 t ha<sup>-1</sup> (Sauti) and 12.5 t ha<sup>-1</sup> (TIS 8266), respectively. Mean yield across

**Table 2** Fresh tuber yield (t ha<sup>-1</sup>) and dry matter content (%) of sweetpotato clones in three agro-ecological zones in Ghana (1995)

Clone	Locations				Mean
	Coastal savannah		Forest	Forest-Savannah transition	
	Pokuase	Cape Coast	Fumesua	Akomadan	
TIS 8266	8.5	12.5	27.4 (32.1) <sup>1</sup>	13.1	15.4
TIS 84/0320	8.9	7.2	25.9 (29.6)	11.3	13.3
TIS 86/0350	9.4	11.7	20.0 (32.6)	11.6	13.2
TIS3017	11.8	12.3	23.3 (36.9)	13.2	15.2
Local Red	8.4	9.6	18.0 (32.2)	10.9	11.7
Sauti	10.6	9.7	19.5 (36.5)	14.3	13.5
Dugbadza	10.6	9.7	19.8 (33.5)	9.9	12.5
Cape Coast	—	7.1	—	—	—
CV (%)	29.9	26.7	11.0 (6.3)	25.8	—
LSD <sub>0.05</sub>	ns	2.5	3.1 (3.7)	ns	—

<sup>1</sup>Values in parentheses are the per cent dry matter of tubers for the respective clones

C.V. is Coefficient of Variation

LSD<sub>0.05</sub> is least significant difference at the 5% level  
ns is not significant

**Table 3** Fresh tuber yield ( $t\ ha^{-1}$ ) and dry matter content (%) of sweetpotato clones in four agro-ecological zones in Ghana (1996)

Clone	Locations								
	Coastal savannah			Forest	Forest-Savannah transition		Guinea savannah		
	Cape Coast	Ohawu	Pokuase	Fumesua	Akomadan	Wenchi	Nyankpala	Wa	Mean
TIS 8266	14.6	15.2	9.2(29.0) <sup>1</sup>	21.3(32.0)	19.3(33.3)	12.2(30.3)	15.2	18.6	16.0(31.2)
TIS 84/0320	13.8	14.3	8.6(36.0)	20.1(31.0)	14.5(32.8)	9.9(35.3)	12.7	21.3	14.5(33.8)
TIS86/0350	17.0	15.0	11.6(31.8)	16.3(31.0)	13.5(31.8)	9.2(32.0)	15.9	21.1	14.6(31.7)
TIS 3017	17.4	15.8	6.9(37.5)	19.2(32.0)	16.0(36.8)	11.1(36.5)	6.2	24.8	14.0(35.7)
Local Red	8.9	11.1	6.3(37.3)	16.2(35.0)	11.4(38.0)	6.6(35.8)	6.8	23.9	11.9(36.5)
Sauti	10.7	11.8	8.5(35.5)	14.6(35.0)	14.0(37.3)	8.6(38.3)	16.6	17.2	13.3(36.5)
Dugbadza	12.4	12.3	7.3(36.0)	17.9(33.0)	15.9(35.5)	8.8(34.4)	15.8	20.5	14.4(34.7)
Cape Coast	—	—	9.8(35.8)	—	—	—	—	—	9.8(35.8)
CV (%)	26.4	13.3	22.2 (7.8)	18.8 (7.3)	35.5 (8.7)	29.5 (7.2)	23.5	19.6	—
LSD <sub>0.05</sub>	5.3	2.4	1.3 (1.8)	2.4 (2.0)	3.7 (2.1)	2.1 (1.8)	3.96	2.7	—

<sup>1</sup>Values in parentheses are the per cent dry matter of tubers for the respective clones

C.V. is Coefficient of Variation

LSD<sub>0.05</sub> is least significant difference at the 5% level

the locations ranged from  $11.7\ t\ ha^{-1}$  (Local Red) to  $15.4\ t\ ha^{-1}$  (TIS 8266). The differences in the performance of the clones in the various agro-ecologies were probably due to the amount of rainfall and soil fertility levels, being highest in the forest and least in the coastal savannah ecologies. Similar yield patterns have been reported by Otoo *et al.* (1995). Per cent DM recorded at Fumesua was significantly highest in TIS 3017 (36.9%) and least in TIS 84/0320 (29.6%).

In 1996, the yield trend was similar to that of 1995. Forest ecology had the highest yields and coastal savannah the lowest yields (Table 3). At Pokuase (coastal savannah), TIS 86/0350 produced the highest fresh yield of  $11.6\ t\ ha^{-1}$  while Local Red had the lowest yield of  $6.3\ t\ ha^{-1}$ . Clone TIS 3017, which had a low fresh yield of  $6.9\ t\ ha^{-1}$ , produced the highest per cent DM. At Fumesua (Forest), TIS 8266 produced the highest fresh tuber yields of  $21.3\ t\ ha^{-1}$  with Sauti yielding the lowest ( $14.6\ t\ ha^{-1}$ ). Sauti appeared to be a late-bulking variety. However, with respect to DM, Sauti and Local Red had significantly highest (35%) value while TIS 84/0320 and TIS 86/0350 had the lowest (31%).

At the two sites in the Forest-Savannah transition zone, TIS 8266 produced the highest yield. The lowest yielding was Local Red, although it was one of the clones with significantly high DM content. Fresh tuber yields of the clones at Wa were consistently higher than at Nyankpala, although both locations occur in the Guinea savannah agro-ecological zone in Ghana. This observation may be the consequence of better rainfall distribution at Wa. Even clones TIS 3017 and Local Red which produced yields less than  $7.0\ t\ ha^{-1}$  at Nyankpala recorded yields of more than  $23.0$

$t\ ha^{-1}$  at Wa.

Means of fresh tuber yield across the locations ranged from  $9.8\ t\ ha^{-1}$  (Cape Coast) to  $16.0\ t\ ha^{-1}$  (TIS 8266). Clone TIS 8266, however, recorded relatively lower DM per cent values, while Local Red had the highest overall per cent DM (Table 3).

Two-year means of tuber yields of the clones across locations and years and their ranking in the various agro-ecological zones are given in Table 4. In the coastal savannah, the four best-yielding clones were TIS 86/0350, TIS 3017, TIS 8266, and TIS 84/0320. In the forest zone, TIS 8266, TIS 84/0320, TIS 3017, and Dugbadza were the highest-yielding clones. In the Forest-Savannah transition, TIS 8266, TIS 3017, Sauti, and Local Red were the best four in that order, and in the Guinea savannah, TIS 86/0350, Dugbadza, TIS 84/0320, and TIS 8266 were the best in that order. While TIS 8266 performed among the best in all four

**Table 4** Two-year means of tuber yields over locations from the various agro-ecological zones

Clone	Coastal savannah	Forest	Forest savannah	Guinea <sup>2</sup> savannah	Mean	Rank sum
TIS 8266	12.0(3) <sup>1</sup>	24.4(1)	14.9(1)	16.9(4)	17.1(1)	9
TIS 84/0320	10.6(4)	23.0(2)	11.9(5)	17.0(3)	15.6(3)	14
TIS 86/0350	12.9(1)	18.2(5)	11.4(7)	18.5(1)	15.3(4)	14
TIS3017	12.8(2)	21.3(3)	13.4(2)	15.5(5)	15.8(2)	12
Local Red	8.9(7)	17.1(6)	12.0(4)	15.4(6)	13.4(7)	23
Sauti	10.3(6)	17.1(6)	12.3(3)	16.9(4)	14.2(6)	19
Dugbadza	10.5(5)	18.9(4)	11.5(6)	18.2(2)	14.8(5)	17
Cape Coast	8.5(8)	—	—	—	—	—

<sup>1</sup>Values in parentheses are the ranking of the means in the column

<sup>2</sup>Two-location means for one year

**Table 5** Per cent *Cylas* infestation of sweetpotato clones in three agro-ecological zones in Ghana (1996)

Clone	Location				Mean
	Coastal savannah	Forest	Forest-Savannah Transition		
	Pokuase	Fumesua	Akomadan	Wenchi	
TIS 8266	1.5	1.4	1.3	1.1	1.3
TIS 84/0320	1.4	2.3	0.8	0.9	1.4
TIS 86/0350	2.6	1.4	1.6	0.8	1.6
TIS3017	1.1	1.6	1.0	0.7	1.1
Local Red	2.2	1.5	0.8	1.1	1.4
Sauli	1.6	1.6	1.2	1.3	1.4
Dugbadza	1.9	2.0	0.7	1.4	1.5
Cape Coast	2.8	—	—	—	2.8
CV (%)	32.5	29.5	42.2	46.0	—
LSD <sub>0.05</sub>	0.7	ns	0.6	ns	—

C.V. is Coefficient of Variation

LSD<sub>0.05</sub> is least significant difference at the 5% level

ns is not significant

agro-ecologies, TIS 84/0320 and TIS 3017 were among the best four in three agro-ecologies. The mean tuber yields for the four clones over all agro-ecological zones showed the following yield trend in decreasing order: TIS 8266, TIS 3017, TIS 84/0320, and TIS 86/0350. This order was also similar to the rank sums.

Tuber infestation by *Cylas* in 1996 was generally low (Table 5). At two of the four sites where data on infestation levels were taken, there was no significant difference among the clones in their reaction to attack by the sweetpotato weevil. At Pokuase (Coastal savannah) and Akomadan (Forest-Savannah transition) where there were some significant differences, the range of infestation was 1.1 to 2.8% and 0.7 to 1.3%, respectively. The low levels of *Cylas* infestation were the result of cultural practices adopted, use of vine cuttings not in contact with soil in the multiplication fields, re-shaping of ridges during weeding to bury developing tubers deep in the soil, and timely harvesting. All these practices have been reported to assist in the control of *Cylas* infestation of tubers in the field (IITA, 1982; Missah and Kissiedu, 1994).

## Conclusion

Although the majority of the clones performed well within the limits of each agro-ecological zone, TIS 8266 out-yielded all others at two out of four and five out of six locations in

1995 and 1996, respectively. In relative terms, the forest ecology seemed to favour sweetpotato cultivation best, although its utilization is not as important compared to other root and tuber crops, such as cassava, yam, and cocoyam. *Cylas* infestation was found to be low, but this pest remains a threat to sweetpotato cultivation in Ghana. It is known that delayed harvesting beyond four months after planting attracts *Cylas* damage. All the agro-ecologies where evaluations were carried out are suitable for sweetpotato production in Ghana.

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# The yield response of two sweetpotato cultivars grown in bags using different soil amendments

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This study investigated the effect of animal manures from cattle, chicken, and horse; plant manures of sawdust, bagasse, grass, coconut coir, and coffee and rice hulls; and inorganic fertilizer, on soil physical and chemical properties and sweetpotato [*Ipomoea batatas* (L.)] yields. Results showed that cv. A28/7 produced a significantly higher tuber yield (254 g plant<sup>-1</sup>) than cv. 049 (211 g plant<sup>-1</sup>) but there were no significant differences between cultivars in response to soil amendments. Animal manures, coffee and rice hulls, and inorganic fertilizer significantly increased tuber dry matter yields (241–442 g plant<sup>-1</sup>) compared to plant manures (35–240 g plant<sup>-1</sup>). Bulk density was improved with the addition of both animal and plant manures and coffee hull but not with rice hull or inorganic fertilizer. Incorporation of residues with high C:N ratios resulted in reduced yields. Bagasse showed markedly reduced plant growth with chlorotic leaves. These results suggest that animal manures and coffee hulls can improve soil physical and chemical properties and can have beneficial effects on sweetpotato tuber yields.

Keywords: Animal and plant manures; Inorganic fertilizer; Tuber yield; Bulk density; C:N ratio

The maintenance and improvement of the properties of tropical soils are important for increasing food production and domestic food security. Root staples like sweetpotato [*Ipomoea batatas* (L.)] are important sources of food and income on the domestic and export market (Ferguson, 1985). However, many fields are poorly fertilized with inorganic fertilizers resulting in low yields and poor tuber quality.

The use of soil amendments has proven to be valuable in improving soil physical properties (Taja and Vander Zaag, 1991), which are important considerations in obtaining desirable tuber shape, size, and weights of sweetpotato. Soil amendments also serve as sources of plant nutrients (Mc Calla, 1975).

Researchers in the Caribbean have found that yam responds positively to farmyard manures (Campbell and Gooding, 1962; Ferguson and Haynes, 1970). Yamada *et al.* (1986) demonstrated high yields of sweetpotato (57 t ha<sup>-1</sup>) when farmyard manures were incorporated before planting. Ferguson and Haynes (1970) suggested that this benefit is made possible because nutrients are released over a longer period of time.

Bagasse is used as a soil amendment on the farms in some Caribbean countries. However, bagasse and other similar type material such as sawdust and coconut coir have high C:N ratios,

over 20–30:1 (Taja and Vander Zaag, 1991) which restrict nutrient availability to crop plants.

Wilson (1974) demonstrated that the sweetpotato cultivars 049 and A28/7 had the genetic potential to produce 30 and 46 t ha<sup>-1</sup>, respectively, in coconut coir. Average yields of these varieties in Trinidad are 8.4 t ha<sup>-1</sup> (Seesahai, 1985).

This study was undertaken to determine the effects of soil incorporated organic residues on the yield of sweetpotato cultivars 049 and A28/7.

## Materials and Methods

The experiment was conducted in polyethylene bags 45 cm long and 40 cm wide at the Crop Research Station, Centeno, between October 1991 and February 1992.

Two sweetpotato cultivars, 049 and A28/7, in 10 soil amendments and an untreated control were investigated in a randomized complete block design replicated three times. The soil amendments were cow manure, horse manure, chicken manure, bagasse, coconut coir, coffee hull, rice hull, sawdust, grass, inorganic fertilizer, and a control. Each treatment consisted of a 1:1 (v/v) mixture of soil and the organic amendment.

The cattle, horse, and chicken manures were

obtained from material stored in the field for approximately five months. The bagasse, coffee hull, rice hull, coconut coir, and sawdust used were all one month old and the grass was cut one week before it was used. Ten grams of 13:13:20 N:P:K compound fertilizer equivalent to 715 kg ha<sup>-1</sup> were applied to the soil at planting supplying 1.3 g N, 1.3 g P, and 5 g K.

Terminal cuttings 24 cm long of the respective cultivars were planted in each unit with approximately 12 cm buried in the soil mixture. Prior to planting, the cuttings were dipped in a mixture of Lannate (2 g L<sup>-1</sup>) and Benlate (1 g L<sup>-1</sup>) for 30 min. A 0.1% Bidrin 240 WSC (dimethyl-2-dimethyl carbonyl-1-methyl-vinyl phosphate) solution was sprayed fortnightly to control the *Megasthes grandalis* stem borer.

The soil type was classified as a clayey, kaolinitic, isohyperthermic, Plinthic Tropaquult. Brown and Bally (1970) described the nutrient status of the 0–8.8 cm layer as containing 1.30 g kg<sup>-1</sup> N, 6 mg kg<sup>-1</sup> P, and 0.00 cmol kg<sup>-1</sup> K, Ca, Na, and Mg. The cation exchange capacity (CEC) of this layer is 3.0 cmol kg<sup>-1</sup> and pH is 4.1.

Two soil samples from the soil mixture and unamended soil were taken on 17 October 1991 at the beginning of the experiment and two samples were taken on 17 February 1992 at the end of the experiment. One set of the soil samples was used to determine the bulk density of the soil (Ahmad and Gumbs, 1973). The other set was used to determine the total N and organic C, available P, and exchangeable K. Nitrogen was determined by the semi-micro-Kjeldahl method described by Allison (1965) and K determined by flame photometry. Organic C was determined by the method described by Mitchell and Rhue (1979).

At the end of the experiment, the plants were harvested, tubers and vines separated, washed, chipped, and dried for 24 h at 80°C, and the dry weights recorded. The numbers of tubers produced by each plant were also recorded.

Statistical analysis of the data was performed using the GLIM and MINITAB 5.1 statistical packages. Residual analyses showed heterogeneity of the data on the original scale and therefore, log-transformed values were used.

## Results

### Total dry matter (TDM) production

Cultivar A28/7 had a significantly higher ( $P < 0.05$ ) TDM than cv. 049 for the different soil amendment mixtures (Table 1). Coffee hull, inorganic fertilizer, and chicken and cattle manure significantly ( $P < 0.05$ ) increased TDM when compared with the unamended soil and the other soil amendment mixtures (Table 1). Chicken manure produced the highest TDM. The grass,

**Table 1** The effect of various soil amendment mixtures and inorganic fertilizer on total dry weight (g plant<sup>-1</sup>) of two sweetpotato cultivars grown in polyethylene bags

Soil amendment mixture	Total dry weight				Soil amendment mean	
	Cv. 049		Cv. A28/7		g plant <sup>-1</sup>	
	g plant <sup>-1</sup>	Log	g plant <sup>-1</sup>	Log	g plant <sup>-1</sup>	Log
Cattle manure	653	6.46	781	6.65	717	6.56
Coffee hull	510	6.24	870	6.74	690	6.49
Inorganic fertilizer	579	6.32	616	6.40	596	6.36
Chicken manure	653	6.47	830	6.71	740	6.59
Horse manure	411	5.83	660	6.47	536	6.15
Unamended soil	273	5.60	527	6.25	400	5.94
Rice hull	280	5.62	515	6.24	397	5.93
Coconut coir	231	5.44	280	5.63	256	5.53
Grass	167	5.13	205	5.19	186	5.15
Bagasse	77	4.32	274	5.55	175	4.94
Sawdust	95	4.35	140	4.84	117	4.59
Cultivar means	357	5.61	518	6.06		
C.V. (%)			4.1			

LSD ( $P < 0.05$ ) (Cultivar) 0.18 (Transformed value)

LSD ( $P < 0.05$ ) (Treatment) 0.42 (Transformed value)

LSD ( $P < 0.05$ ) (Cv. × trt) 0.59 (Transformed value)

C.V. is Coefficient of Variation

bagasse, and sawdust-soil mixtures gave a significantly lower ( $P < 0.05$ ) TDM than soil or soil-rice hull mixture. Coconut coir produced a significantly higher ( $P < 0.05$ ) TDM than bagasse or sawdust. The soil-rice hull mixture produced results similar to the unamended soil.

### Tuber dry weight

Cultivar A28/7 produced a significantly higher ( $P < 0.05$ ) tuber dry weight than cv. 049 (Table 2). The cultivar × soil amendment mixture interaction means were not significant for tuber dry weight. Some soil amendment means for tuber dry weight showed significant differences. The incorporation of coffee hull, inorganic fertilizer, and cattle and chicken manure gave significantly higher ( $P < 0.05$ ) tuber dry weight yields than the unamended soil and the other soil amendment mixtures. The unamended soil produced a significantly higher ( $P < 0.05$ ) yield when compared with grass-, bagasse-, or sawdust-soil mixtures.

### Number of tubers

Cultivar 049 produced fewer tubers than A28/7 (Table 3) and it produced the highest number of tubers when coffee hull was incorporated in the soil. Chicken manure and sawdust gave the lowest numbers of tubers which were significantly lower ( $P < 0.05$ ) than the unamended soil. The cultivar × soil amendment interaction means were not significantly different from each other.

**Table 2** The effect of various soil amendment mixtures and inorganic fertilizer on tuber dry matter ( $\text{g plant}^{-1}$ ) production of two sweetpotato cultivars grown in polyethylene bags

Soil amendment mixture	Total dry matter				Soil amendment mean	
	Cv. 049		Cv. A28/7			
	$\text{g plant}^{-1}$	Log	$\text{g plant}^{-1}$	Log	$\text{g plant}^{-1}$	Log
Cattle manure	476	6.1	408	5.90	442	6.01
Coffee hull	333	5.81	404	5.98	369	5.90
Inorganic fertilizer	337	5.72	383	5.91	360	5.81
Chicken manure	356	5.83	361	5.88	359	5.85
Horse manure	192	5.14	391	5.93	291	5.54
Unamended soil	180	5.19	265	5.48	223	5.34
Rice hull	170	5.13	230	5.38	200	5.26
Coconut coir	151	4.99	116	4.09	134	4.84
Grass	69	4.05	76	4.12	72	4.09
Bagasse	34	3.53	110	3.86	72	4.06
Sawdust	22	3.06	48	3.86	35	3.46
Cultivar means	211	4.96	254	5.24		
C.V. (%)			8.5			

LSD ( $P < 0.05$ ) (Cultivar) 0.22 (Transformed value)LSD ( $P < 0.05$ ) (Treatment) 0.51 (Transformed value)LSD ( $P < 0.05$ ) (Cv.  $\times$  trt) 0.72 (Transformed value)

C.V. is Coefficient of Variation

**Table 3** The effect of various soil amendment mixtures and inorganic fertilizer on tuber number of two sweetpotato cultivars grown in polyethylene bags

Soil amendment mixture	Tuber number				Soil amendment mean	
	Cv. 049		Cv. A28/7			
	No. $\text{plant}^{-1}$	Log	No. $\text{plant}^{-1}$	Log	No. $\text{plant}^{-1}$	Log
Cattle manure	4.7	1.42	2.7	0.92	3.7	1.17
Coffee hull	5.7	1.37	4.7	1.42	5.2	1.49
Inorganic fertilizer	3.7	1.29	3.0	0.99	3.3	1.14
Chicken manure	2.0	0.59	1.7	0.46	1.8	0.52
Horse manure	2.7	1.92	4.3	1.46	3.5	1.19
Unamended soil	4.3	1.44	5.0	1.60	4.7	1.52
Rice hull	2.7	0.96	2.3	0.83	2.5	0.90
Coconut coir	3.3	1.19	4.3	1.42	3.8	1.33
Grass	3.3	1.19	2.0	0.69	2.7	0.94
Bagasse	3.3	1.16	3.3	1.16	3.3	1.16
Sawdust	2.0	0.69	2.7	0.97	2.3	0.83
Cultivar means	3.4	1.13	3.3	1.09		
C.V. (%)			35.2			

LSD ( $P < 0.05$ ) (Cultivar) 0.19 (Transformed value)LSD ( $P < 0.05$ ) (Treatment) 0.46 (Transformed value)LSD ( $P < 0.05$ ) (Cv.  $\times$  trt) 0.65 (Transformed value)

C.V. is Coefficient of Variation

### Carbon:nitrogen (C:N) ratio

At the start of the study, soil-sawdust treatment had the highest C:N ratio which was significantly higher than all other treatments except bagasse. The unamended soil, soil treated with

inorganic fertilizer, cattle manure, and horse manure had low C:N ratios. At the end of the study the C:N ratio of all treatments except cattle manure had declined. Sawdust still had the highest C:N ratio which was significantly ( $P < 0.05$ ) higher than bagasse and all other treatments except coconut coir.

### Bulk density ( $\text{g cm}^{-2}$ )

No statistical analysis was performed on the bulk density data (Table 4). Samples were taken from one block only because of the difficulty of extracting the cores and the risk of damaging the tubers. It would appear that all the organic amendments reduced bulk density as expected. The initial high bulk density value for the rice hull amendment cannot readily be explained

### Soil N, P, and K

The initial and final soil total N (%), available P (ppm), and K (meq.  $100 \text{ g}^{-1}$ ) values for the soil amendment means for both cultivars are shown in Table 5. Cattle, chicken, and horse manure treatment mixtures had significantly higher ( $P < 0.05$ ) N than the other treatments, and there were no significant differences between cattle, chicken, or horse manure soil amendment mixtures.

Chicken manure gave the highest P values which were significantly higher ( $P < 0.05$ ) than all the other treatments initially. Coffee hull, inorganic fertilizer, cattle, chicken, and horse manure, coconut coir, and bagasse had significantly higher values ( $P < 0.05$ ) than the unamended soil.

Coffee hull, rice hull, and sawdust had significantly higher ( $P < 0.05$ ) P values than the unamended soil at the end of the experiment.

Cattle manure, grass, coconut coir, and sawdust had significantly higher ( $P < 0.05$ ) K than the unamended soil. However, the unamended soil had significantly higher ( $P < 0.05$ ) K than

**Table 4** The initial and final treatment mean values for the soil pH status and bulk density ( $\text{g cm}^{-3}$ ) of the various soil amendment mixtures for the two sweetpotato cultivars grown in polyethylene bags

Soil amendment mixture	Bulk density ( $\text{g cm}^{-3}$ )	
	Initial	Final
Cattle manure	1.06	1.08
Coffee hull	0.85	0.71
Inorganic fertilizer	1.38	1.59
Chicken manure	1.14	1.16
Horse manure	0.73	1.05
Unamended soil	1.37	1.56
Rice hull	1.37	0.92
Coconut coir	0.80	0.90
Grass	1.25	1.20
Bagasse	0.80	1.01
Sawdust	0.85	0.91

**Table 5** The initial and final values for the nutrient status of various soil amendment mixture treatment means for two sweetpotato cultivars grown in polyethylene bags

Soil amendment mixture	Nutrient status					
	N g kg <sup>-1</sup>		P mg kg <sup>-1</sup>		K cmol kg <sup>-1</sup>	
	Init.	Final	Init.	Final	Init.	Final
Cattle manure	0.20	0.22	26.0	23.8	0.57	0.47
Coffee hull	0.09	0.20	25.0	36.6	0.29	0.82
Inorganic fertilizer	0.10	0.12	24.2	29.1	0.53	0.33
Chicken manure	0.19	0.28	29.6	16.8	0.30	0.53
Horse manure	0.20	0.22	25.6	20.8	0.35	0.59
Unamended soil	0.11	0.12	21.9	27.2	0.55	0.33
Rice hull	0.10	0.15	20.0	34.4	0.24	0.65
Coconut coir	0.12	0.12	23.6	25.3	0.36	0.62
Grass	0.01	0.13	18.8	24.7	0.41	0.57
Bagasse	0.09	0.13	24.3	20.1	0.19	0.43
Sawdust	0.09	0.20	23.0	37.8	0.37	0.37
LSD <sub>0.05</sub>	0.014	0.034	1.517	4.025	0.035	0.093
C.V. (%)	20.5	33.8	11.08	25.7	16.1	29.0

C.V. is Coefficient of Variation

coffee hull, rice hull, and bagasse mixtures. At the end of the experiment, coffee hull had significantly higher ( $P < 0.05$ ) K than all the other treatments. Chicken manure, horse manure, rice hull, coconut coir, and grass gave significantly higher ( $P < 0.05$ ) values than the unamended soil.

## Discussion

The results showed that A28/7 produced significantly higher total and tuber dry matter than 049 (Tables 1 and 2). However, the response was higher for A28/7 than 049 but the difference was not significant (Table 3). A28/7 has been shown to produce higher yields than 049 on the River Estate Loam (Lowe and Wilson, 1974; Roberts, 1984). In this experiment, the higher yield of A28/7 was due to high total biomass production and large tuber sizes and not to differences in tuber numbers.

The results indicated that the total plant and tuber yield response for both cultivars were similar for most of the soil amendments. No significant differences were found for the cultivar  $\times$  soil amendment interaction means, but there were significant differences for the soil amendment means. Several workers have reported high variability in sweetpotato from plant to plant and season to season (Haynes, 1970; Lowe, 1971; Wilson, 1973). In this trial, residual analysis of the data was found to be

heterogeneous and the soil amendment mixtures to be variable in composition and nutrient status, which probably accounted for the lack of significance among the interaction means. However, some general differences among the cultivars' responses to the soil amendment interaction means can be observed. The tuber yield response showed that 049 responded better to cow manure and A28/7 to horse manure. For both 049 and A28/7, chicken manure and coffee hull produced the highest plant biomass. The response was higher for A28/7 than 049 but the difference was not significant. Grass, bagasse, and sawdust consistently gave higher plant biomass with A28/7 but the dry weights for both cultivars decreased relative to the unamended soil. Chicken manure, cow manure, coffee hull, and inorganic fertilizer produced higher total plant and tuber dry weights ( $P < 0.05$ ) compared to the unamended soil.

In this experiment, the following limitations should be recognized in explaining the differences found among the soil amendment means. The total amount of nutrients in the unmixed soil amendments were not measured, the nutrient levels in the plant tissues were also not determined, and the decomposition rates of different soil amendments can also vary. These limitations should be recognized whenever comparisons between the different soil amendment mixtures are attempted in this discussion.

Coffee hull produced high N levels and high K levels at the final harvest. This treatment produced high total plant biomass and tuber initiation was encouraged as seen in the high tuber number production. Because of the good response of the sweetpotato plant to this soil amendment mixture, it is possible that had the experiment been extended for a longer period of time, this mixture may have produced the highest tuber yield.

In this study, the higher yield of cattle manure was due primarily to a high tuber yield, in chicken manure to high total plant biomass production, and in coffee hull to high total plant yield when compared with the unamended soil. Inorganic fertilizer also gave high yields due to good canopy and tuber development. The horse manure and unamended soil treatments had lower total plant biomass and yields were comparatively lower for these treatments.

Yamada *et al.* (1986) found no significant increases in tuber weights when farmyard manure was applied to soil even though farmyard manure gave high sweetpotato yields. However, the beneficial effects of farmyard manure application increasing tuber yields have been observed by several other workers (Thiagalingam and Bourke, 1981).

Many workers have suggested that the beneficial effects of manurial applications are possible because nutrients are released over a longer period of time (Edwards, 1967; Ferguson and



Haynes, 1970; Ofori, 1980). Organic matter was maintained at final harvest and the reduction of the C:N ratio indicated that N release, due to the net mineralization of organic N, became available for plant growth. The unamended soil had an initial low N and K status which declined at the final harvest. Cow and chicken manures and coffee hulls had initially higher N levels than soil and available K levels were either maintained or increased at final harvest. These data suggested that nutrients were released and were utilized by the plants, as was indicated by the increases in total plant yield for these soil amendment mixtures when compared with that of the unamended soil.

Coconut coir, grass, bagasse, and sawdust had a pronounced effect on tuber development and number when compared with that of the unamended soil. Significant reductions in total plant yield were noted and soil analyses showed a low available soil N. Taja and Vander Zaag (1991), Avinmelech and Cohen (1989), and Allison (1973) have all suggested that the C:N ratio of manures determines the efficiency of the manure as a soil conditioner. The first two studies suggested the incorporation of nitrogenous fertilizer when incorporating residues into the soil to balance the soil C:N ratio. It is often documented that sub-optimal levels of available soil N reduced total plant growth and tuber development, restricted vine growth, and produced small leaves (Cibes and Samuels, 1957; Spence and Ahmad, 1967). All these symptoms were observed with grass, bagasse, and sawdust amendments, the use of which resulted in reduced plant growth with poor tuber development.

There are suggestions of either toxic substances (Taja and Vander Zaag, 1991) or growth regulatory substances (Famoso and Bautista, 1982) from some plant residues which can negatively affect the growth of crop plants. Taja and Vander Zaag (1991) found that when bagasse was used without the addition of any inorganic fertilizer the yield of potato (*Solanum* sp.) was suppressed. The bagasse treatment produced chlorotic plants that looked unhealthy and tuber yields were reduced relative to the unamended soil.

In summary, therefore, amendments like grass, coconut coir, bagasse, and sawdust with high C:N ratios reduced total plant growth, restricted assimilate production, and the allocation of assimilates into the terminal components of yield possibly by a reduction of N supply to the plant.

The results showed a reduction in bulk density by all the soil amendments, especially sawdust, grass, coconut coir, and bagasse.

On the basis of the results obtained in this experiment, it is recommended that coffee hull, chicken manure, inorganic fertilizer, and cow manure can be used to improve the yield of sweetpotato on the Long Stretch Soil Series.

These amendments were used in a decomposed state before they were mixed in the soil, and the yields contrasted sharply with amendments that were relatively undecomposed.

Further experimentation, therefore, is necessary using these recommended amendments possibly at higher levels or in combination with inorganic fertilizers under field conditions.

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# Comparison of the elemental composition of sweetpotato grown in a bioreactor effluent and modified half Hoagland solution

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Controlled environment chamber experiments were conducted with 'TU-82-155' and 'Georgia Jet' sweetpotato [*Ipomoea batatas* (L.)], grown in either modified half Hoagland (MHH) nutrient solution (control treatment) or in a nutrient medium composed of 20% effluent from an aerobic bioreactor (Filtrate Treatment). Plants were grown for 120 days with a day/night cycle of 14 h at 28°C and 10 h at 22°C. The filtrate used in this study was filtered (0.2 µm) effluent from the bacterial degradation of residual biomass from hydroponic culture of sweetpotato. Plant tissue analysis showed differences in elemental composition between plants grown in control and filtrate-amended crop nutrient media. Control plants for both 'Georgia Jet' and 'TU-82-155' were high in K ( $4736 \pm 167$  and  $3693 \pm 202$  mg 100 g<sup>-1</sup> plant dry weight, respectively). The filtrate-treated plants were higher in Fe ( $3.9 \pm 0.8$  mg 100 g<sup>-1</sup> dry weight), and lower in most macronutrients, for 'TU-82-155'. Generally, filtrate incorporation in the crop nutrient medium resulted in reduced levels of the selected elements (Ca, Mg, K, Zn, Fe, and total Kjeldahl N) in 'TU-82-155'. For 'Georgia Jet', although nutrients levels were also reduced, when filtrate was added to the nutrient solution, the difference was, generally, not significant.

Keywords: *Ipomoea batatas* (L.); Hydroponic; Aerobic bioreactor effluent; Mineral composition

Sweetpotato [*Ipomoea batatas* (L.)] is an important human food source for the proposed long-duration space missions planned by the United States National Aeronautics and Space Administration (NASA). The importance of the sweetpotato is mainly due to its high edible biomass index (EBI) as both foliage and storage roots are available for consumption (Hill *et al.*, 1992). The sweetpotato additionally provides versatility as a menu item and the leaves are a source of vitamins, iron, and protein (Pace *et al.*, 1985). In the scenarios being developed, human presence in space is constrained by the need for continuous supply of food, oxygen, and water. The NASA Advanced Life Support Programme Division has initiated and funds research programmes that investigate regenerative life support systems as a way to reduce requirements of launch mass, energy, and the need for resupply. Inclusion of a higher plant subsystem, in long-duration missions, in addition to reducing launch mass and energy, will ensure a steady and reliable source of food, oxygen, and water for the crew.

The food, air, and water requirements for human exploration of space can be satisfied through biological regeneration systems (Drysdale and Finger, 1997). The regeneration and recov-

ery of materials from waste, though primarily dependent on the cost-effectiveness and available technologies, are also influenced by elemental composition and by human acceptability of the foods produced by a regeneration system.

The inedible plant biomass which may range from 20 to 70% (Wheeler *et al.*, 1996), depending on the crop type, contains nutrients that have the potential to support growth of new plants. Investigations at Kennedy Space Center (FL, U.S.A.) and at Tuskegee University have been examining the use of potential space mission-generated waste for re-use and recycling. One aspect of plant waste recycling has been to degrade inedible plant biomass in aerobic bioreactors using mixed (Mackowiak *et al.*, 1996) or single-culture microbial populations (Trotman *et al.*, 1996).

The low input requirement of sweetpotato makes it a reliable food source. The sweetpotato can, therefore, be considered a food crop for sustainable food security in the next millennium, for land-based, as well as, space-based agriculture. The present study was initiated to measure the concentration of selected minerals and total N in the tissue of sweetpotato grown in effluent-amended or control nutrient solutions and to determine the effect of effluent

incorporation on the mineral composition of two sweetpotato cultivars.

## Materials and Methods

The experimental plant materials consisted of a sweetpotato line 'TU-82-155' and the cultivar 'Georgia Jet'. The plants had been grown hydroponically in two replicated experiments. The experiments were conducted under controlled environment conditions, in Conviron Growth Chambers, during the fall of 1995 through January 1997, at the G.W. Carver Agricultural Experiment Station in Tuskegee, Alabama. A detailed description of the plant growth conditions and experimental set-up have been described elsewhere (Trotman *et al.*, 1997). Briefly, the plant growth experimental design was a randomized complete block with two replications. The materials consisted of the Tuskegee University nutrient film technique growth channels (Morris *et al.*, 1989), each planted with four 15-cm sweetpotato cuttings of the sweetpotato cultivar being studied. There were three channels in each of two chambers during each run of the experiment. Each study used a single sweetpotato cultivar and was replicated in time (two cycles). The plants were grown for 120 days in two crop nutrient solution treatments. The chemical properties of the solutions in which the plants were grown are presented in Table 1. The hydroponic solutions were treatment 1, modified half Hoagland solution, designated 'MHH'; and treatment 2, 20%

filtered effluent + 80% modified half Hoagland, designated 'Filtrate'.

## Chemical analyses

The elemental composition of selected plant parts was determined from harvest biomass. The experimental design was a randomized block design with two replications for each of the two cultivars. The plant tissue samples from 'Georgia Jet' and 'TU-82-155' studies were kept separate for analyses. All four plants in each treatment channel were pooled to remove effects due to location within the chamber. Four replicate 1.0-g samples of each composite plant part for each treatment, from each study (includes two runs), were analysed for Ca, Mg, K, Zn, Fe, and per cent total Kjeldahl N. For mineral analysis, dried harvest biomass was ashed in a muffle furnace (550°C). After weighing, ash was diluted, filtered, and analysed using standard methods for atomic absorption spectrophotometry. The total N was determined by Kjeldahl technique (Nielsen and Sommers, 1973). The data were recorded and statistical analysis performed. Data were subjected to analysis of variance using the Super ANOVA general linear modeling software package. Analysis was conducted for each plant part separately. Treatment means for each element were separated using Fisher's LSD test (Steel and Torrie, 1980).

**Table 1** Chemical properties of effluent and modified half Hoagland (MHH) solution

Chemical property	Filtrate		MHH	
	Mean <sup>1</sup> (mg L <sup>-1</sup> )	SE	Mean (mg L <sup>-1</sup> )	SE
pH	7.80	0.30	6.01	0.10
Salinity	4.60	0.11	0.70	0.02
Electrical conductivity ( $\mu$ S cm <sup>-1</sup> )	4438	188.05	1010	91.00
NO <sub>3</sub> -N	†	—	41.80	2.90
K	128	11.20	118.16	16.80
Ca	25.60	0.74	83.53	1.05
Mg	4.53	0.05	1.23	0.30
Na	2.69	0.10	†	—
Zn	0.29	0.03	0.03	0.01
Fe	0.08	0.01	0.01	0.00

<sup>1</sup>Mean of 7 samples

†, below detection level of assay

## Results and Discussion

The results of selected mineral and total Kjeldahl N analyses are presented in Table 2. The analysis of variance indicated a significant ( $P < 0.05$ ) treatment effect for some of the elements measured in selected plant parts. There was no significant cultivar  $\times$  treatment interaction.

Analysis of the elemental composition of whole plants indicated that there was a significant ( $P < 0.05$ ) treatment effect for all measured elements, except Zn and Fe. The effluent supplied the required amount of the latter elements. This result is in agreement with the findings of Mackowiak *et al.* (1996), who found that the addition of supplemental minerals in a stock to the crop nutrient solution was needed to ensure an adequate supply of the required crop nutrients.

The total N measured in whole plant was significantly ( $P < 0.05$ ) lower for plants from the filtrate-amended solution. This finding is not surprising given the low N content of the effluent used in the study. It may be desirable, in a bioregenerative system, to use filtrate-amended crop nutrient coupled with the N-fixing ability of microorganisms, in the rooting zone, to alleviate the inherent N insufficiency from effluent, in this system.

The incorporation of bioreactor effluent in the crop nutrient medium, had a significant in-

**Table 2** The elemental composition (mg 100 g<sup>-1</sup> dry weight) of selected plant parts from hydroponically grown sweetpotato. The values represent mean  $\pm$  standard error (n = 8)

Plant part	Cultivar	Treatment	Calcium	Magnesium	Potassium	Zinc	Iron	Total Kjeldahl nitrogen
Whole plant	Georgia Jet TU-82-155	MHH	634 $\pm$ 27.9	575 $\pm$ 27.2	4736 $\pm$ 167	3.73 $\pm$ 0.4	2.66 $\pm$ 0.1	8.71 $\pm$ 0.2
			317 $\pm$ 12.8	324 $\pm$ 36.0	3693.6 $\pm$ 202	2.92 $\pm$ 0.6	2.19 $\pm$ 0.6	7.36 $\pm$ 0.2
	Georgia Jet TU-82-155	Filtrate	412 $\pm$ 17.0	282 $\pm$ 23.0	3210 $\pm$ 217	1.51 $\pm$ 0.01	3.90 $\pm$ 0.8	4.71 $\pm$ 0.2
			252 $\pm$ 18.7	197 $\pm$ 11.2	1313.6 $\pm$ 120	1.22 $\pm$ 0.01	3.20 $\pm$ 0.2	3.36 $\pm$ 0.3
Stem	Georgia Jet TU-82-155	MHH	365 $\pm$ 10.4	272 $\pm$ 19.5	2155 $\pm$ 113	0.87 $\pm$ 0.01	1.53 $\pm$ 0.1	2.83 $\pm$ 0.2
			354 $\pm$ 10.3	149 $\pm$ 10.7	2010 $\pm$ 123	0.38 $\pm$ 0.02	1.38 $\pm$ 0.1	2.70 $\pm$ 0.1
	Georgia Jet TU-82-155	Filtrate	254 $\pm$ 15	134 $\pm$ 21.0	1125 $\pm$ 103	0.57 $\pm$ 0.03	1.90 $\pm$ 0.2	2.20 $\pm$ 0.1
			323 $\pm$ 13.8	82 $\pm$ 10.8	1105 $\pm$ 101	0.92 $\pm$ 0.02	0.80 $\pm$ 0.1	1.98 $\pm$ 0.1
Senesced leaves	Georgia Jet TU-82-155	MHH	537 $\pm$ 20.8	303 $\pm$ 16.0	2454 $\pm$ 105	0.34 $\pm$ 0.01	0.17 $\pm$ 0.02	0.69 $\pm$ 0.1
			468 $\pm$ 10.1	279 $\pm$ 20.0	2372 $\pm$ 121	0.29 $\pm$ 0.01	0.20 $\pm$ 0.01	0.52 $\pm$ 0.1
	Georgia Jet TU-82-155	Filtrate	357 $\pm$ 11.5	144 $\pm$ 10.4	2399 $\pm$ 182	0.25 $\pm$ 0.03	0.42 $\pm$ 0.01	0.48 $\pm$ 0.1
			166 $\pm$ 18.6	121 $\pm$ 19.0	2334 $\pm$ 100	0.10 $\pm$ 0.01	0.11 $\pm$ 0.01	0.34 $\pm$ 0.1
Fibrous mat	Georgia Jet TU-82-155	MHH	395 $\pm$ 11.8	213 $\pm$ 16.9	1406 $\pm$ 125	1.38 $\pm$ 0.11	1.40 $\pm$ 0.01	0.33 $\pm$ 0.0
			173 $\pm$ 7.7	109 $\pm$ 27.0	1184 $\pm$ 113	1.31 $\pm$ 0.12	1.30 $\pm$ 0.02	0.31 $\pm$ 0.0
	Georgia Jet TU-82-155	Filtrate	212 $\pm$ 10.2	107 $\pm$ 23.0	1134 $\pm$ 102	0.80 $\pm$ 0.06	3.40 $\pm$ 0.2	0.24 $\pm$ 0.1
			91.3 $\pm$ 9.6	46 $\pm$ 5.2	1080 $\pm$ 110	0.58 $\pm$ 0.02	2.10 $\pm$ 0.1	0.18 $\pm$ 0.0

fluence ( $P < 0.001$ ), regardless of cultivar, on the measured mineral and total N content of senesced leaves, except for K. There was a non-significant response to treatment for both 'TU-82-155' and 'Georgia Jet' for K levels measured in senesced leaves. This is in agreement with the work by Mackowiak *et al.* (1996) with potato cultured in bioreactor effluent.

The data showed that the elemental composition of the fibrous mat, although numerically lower for filtrate-treated plants, was not significantly different. Also, the composition of the selected elements from 'TU-82-155' generally was lower than measured in 'Georgia Jet' sweetpotato. The difference in means was not significant. The result suggests that the fibrous mat (composed of feeder roots) was supplied with adequate amounts of nutrients, in the filtrate-amended nutrient solution for both sweetpotato genotypes.

In the present study, elemental composition in the sweetpotato plant or plant part was reduced by filtrate incorporation, but this reduction was not significantly different for all elements measured. It can therefore be concluded that the incorporation of aerobic bioreactor effluent in the crop nutrient solution may be used for sweetpotato production with the addition of supplemental Ca, Mg, and K. The source of these minerals may be from harvest biomass and waste from food preparation, of other crops in the proposed bioregenerative system.

This research in sweetpotato as a crop for bioregenerative life support systems can be applicable to land-based agriculture. In areas where mineral fertilization is cost-prohibitive, recycling

crop residue in simple bioreactors can be explored as a means of improving yields.

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# Mechanisms of taro resistance to leaf blight

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Five different cultivars of taro and two other related aroids were screened for the induction of pathogenesis-related (PR) proteins in response to infection by *Phytophthora colocasiae*, the fungal pathogen responsible for taro leaf blight. Extra-cellular fluid from infected leaves was tested for PR protein expression by SDS-PAGE analysis and activity gels were used to measure the activity of the known PR proteins, ( $\beta$ -1,3-glucanase, proteinase inhibitors, and peroxidase). Infected plants showed increased levels of PR proteins but this did not correlate with resistance in the most susceptible cultivars. Despite high levels of some PR protein, these cultivars were unable to prevent infection. Successful resistance in other plants was more closely linked to the pattern of expression of proteinase inhibitors which appear to be an important defence strategy in taro and related aroids.

Keywords: Taro; Leaf blight; SDS-PAGE analysis; Activity gels; Proteinase inhibitors

Taro [*Colocasia esculenta* (L.) Schott], a perennial monocot of the Aroid family, is susceptible to a number of diseases among which is taro leaf blight caused by the fungal pathogen *Phytophthora colocasiae*. This foliar disease was first reported from the Philippines in 1916 (Gomez, 1925) and had spread from Java to Hawaii via Taiwan and Japan within the next decade. Australasia and South Pacific regions were also affected in the 1940s (Ooka, 1983). *Phytophthora colocasiae* reproduces asexually and is spread by air-borne zoospores, but free water is required for successful propagation and infection. It attacks leaves causing lesions, reduces photosynthetic tissues, and eventually completely destroys the plant. It is also responsible for some storage rots. Infected fields may suffer up to 30% yield loss (Trujillo and Aragaki, 1964). There has, however, been little research on the mechanism of the taro leaf blight infection by *P. colocasiae* or on the defence responses of the host, *C. esculenta*.

Defence responses in plants typically include constitutive and inductive hydrolytic enzymes, enzyme inhibitors, and phytoalexins as well as systemic events through signalling. The induction of a specific set of proteins, preferentially accumulated in the extracellular spaces, called pathogenesis-related (PR) proteins (Van den Bulcke *et al.*, 1989) is supposed to play a significant role in defence reactions. Two well described PR proteins are the enzymes chitinase and  $\beta$ -1,3-glucanase. They can act synergistically against fungal attack by hydrolyzing the fungal cell wall components, chitin and  $\beta$ -1,3-glucan, and inhibiting fungal growth (Boller, 1993). Peroxidase is another enzyme involved in defence responses. It catalyses the oxidation of membrane lipids during cell necrosis and the initia-

tion of cell wall lignification reactions which are part of the hypersensitive response, where cells adjacent to the site of infection undergo rapid death to limit the spread of the infection (Sutherland, 1991). Enzyme inhibitors can also function as PR proteins. Proteinase inhibitors (PI) are induced by a proteinase inhibitor-inducing factor (PIIF) which is released upon perception of the wound signal (Bishop *et al.*, 1981). This brings about the activation of the pin genes and the accumulation of PIs which possess specificity against trypsin and chymotrypsin activities (Sanchez-Serrano *et al.*, 1993). Many plant proteinase inhibitors are known to inhibit extracellular proteinases of microorganisms (Ryan, 1973), while others inhibit the endopeptidases of feeding insects or other invaders (Ouchi, 1991).

This study examines the leaf blight disease of taro and the defence responses exhibited by the host plant to identify the factors involved in host resistance.

## Materials and Methods

### Materials

Cultivars of taro and tannia (*Xanthosoma sagittifolium*) were obtained from the Philippines and cultivated at the Kadoorie Agricultural Research Centre, Hong Kong in 1996. The five varieties of taro examined were Liposnay (LP), Paagaaga (PA), Ngadaw (NG), Doho (DH), and Mindanao (MD). *Alocasia macrorrhiza* (giant taro) was collected in the wild in Hong Kong. All plant materials were free from infections and injury prior to the experiments.

Enzymes, inhibitors, and general reagents

were purchased from Sigma Chemical Co. Silver Stain Kit was obtained from Bio-Rad. Molecular weight marker calibration kits for molecular weight determination in native PAGE molecular weight [(MW) range 14 200–800 000] and SDS-PAGE (MW range 12 300–78 000) were purchased from BDH.

### Fungal culture

A strain of *P. colocasiae* originating from Taiwan was obtained from the American Type Culture Collection. The fungus was cultured on a nutrient medium which contained 20% (v/v) V8 vegetable juice (Campbell Soup Company), 3% (w/v) CaCO<sub>3</sub>, 1.5% (w/v) agar (Difco), and 75.5% tap water, and was adjusted to pH 7.2 with 1M NaOH. The medium was sterilised by autoclaving at 121°C for 20 min. The fungal culture was maintained on agar slants at room temperature in the dark.

### Extraction of extra-cellular fluid

One leaf from each plant was infected with 2 mL fungal suspension by hypodermal injection using a 2.5 mL syringe connected to a fine-pore filter. When approximately 20% leaf area of the inoculated leaf turned yellow, extra-cellular fluid (ECF) was extracted. For *X. sagittifolium* and *A. macrorrhiza*, leaves were harvested seven days after inoculation as none turned yellow. For each variety, three leaves were tested, one from a healthy plant, one from the infected plant, and one remote leaf from the infected plant free from visible symptoms. Leaves were detached and cut into strips 1 cm wide. Leaf strips were rinsed and vacuum-infiltrated at 5 kPa for 2 h in a solution containing 50 mM Tris-HCl (pH 7.4) and 0.6 M NaCl (1:1), then blotted dry and rolled into 10-mL syringes. The syringes were placed in centrifuge tubes and centrifuged at 1000 × g for 10 min. The ECF was collected and centrifuged at 2000 × g for 1 min to pellet remaining debris, and the supernatant was collected and stored at 4°C.

Protein content of the ECF was assayed according to Read and Northcote (1981) with a BSA standard.

### Preparation of fungal extract

A suspension of *P. colocasiae* was prepared by transferring the fungal hyphae and spores into 0.5 mL distilled water. Fungal structures were lysed by repeated temperature shock to release cell contents for protein analysis. The fungal suspension was freeze-thawed by immersion in liquid nitrogen and boiling water. This procedure was repeated twice before the cell lysate was lyophilized for concentration.

### PAGE and staining

Discontinuous SDS gel electrophoresis was performed on 0.75-mm mini slab gels with a 5%

stacking gel and a 20% separating gel (Laemmli, 1970). Samples were lyophilized and mixed at 1:1 dilution with sample buffer containing 1.54% (w/v) dithiothreitol, 20% (w/v) SDS, 8% (v/v) 1M Tris-HCl pH 6.8, 10% (v/v) glycerol, 0.6% (v/v) bromophenol blue in ethanol, and 75% H<sub>2</sub>O. Samples were heated at 100°C for 4 min before 20 µL was loaded per well. Gels were run at 30 mA constant current for 1.5 h.

The same system was used for native gels except that the SDS was omitted and a 1-mm 7 to 20% gradient gel was used as the separating gel. Samples were not lyophilized and dithiothreitol was omitted from the sample buffer. An amount of 50 µL of diluted sample was applied to each well and gels were run at constant current 20 mA for 2 h.

Gels were stained with 0.125% Coomassie brilliant blue G-250 for 1 h, and destained overnight in methanol:acetic acid:water (10:7:83). Other gels were stained with silver according to the method supplied with the Bio-Rad Silver Stain Kit.

### Detection of PR proteins

Native gels were used, except otherwise stated, for the detection of PR proteins which had enzyme activity. Molecular weight markers were revealed by silver staining.

### β-1,3-glucanase

The method by Pan *et al.* (1991) was used with slight modification. The standard used was driselase. The gel was rinsed with water, incubated with 50 mM sodium acetate buffer (pH 5.0) for 5 min, and then incubated in 0.7% laminarin in the same buffer at 37°C for 2 h. The gel was then fixed in methanol:acetic acid:water (5:2:5) for 5 min and rinsed with water before staining with 0.15% 2,3,5-triphenyltetrazolium chloride in 200 mL of 1M NaOH by heating over boiling water until red bands appeared. The gel was fixed again in methanol:acetic acid:water (5:2:5).

### Peroxidase

The method by Abeles *et al.* (1970) was adapted to assay peroxidase activity. The standard used was horseradish peroxidase. The gel was rinsed with water and incubated in 50 mM sodium acetate buffer (pH 5.0) for 5 min. The gel was transferred to the same buffer containing 250 mM guaiacol and 0.1% hydrogen peroxide until bands appeared. The gel was finally fixed in methanol:acetic acid:water (4:1:5).

### Proteinase inhibitors

The method for the detection of proteinase inhibitors was modified from that described by García-Carreño *et al.* (1993). Samples were separated on a 12.5% SDS gel using a sample



buffer containing SDS but no reducing agents. The samples were not boiled before loading onto the gels and were separated at a constant current of 20 mA for 1.5 h.

Soya bean trypsin inhibitor type II-S was used as the standard. After electrophoresis, gels were rinsed briefly in water and incubated in 100 mL of 0.1 mg mL<sup>-1</sup> of porcine trypsin or bovine pancreatic  $\alpha$ -chymotrypsin type II in 50 mM Tris-HCl (pH 8.2) and 0.3% (w/v) CaCl<sub>2</sub> at 4°C for 30 min with occasional shaking to allow diffusion of enzyme into the gel. The gels were then rinsed in water and incubated with 100 mL of 2% casein in 50 mM Tris-HCl (pH 7.8) at 37°C for 2 h. After substrate hydrolysis and incubation, the gels were washed with distilled water and stained with silver as mentioned above.

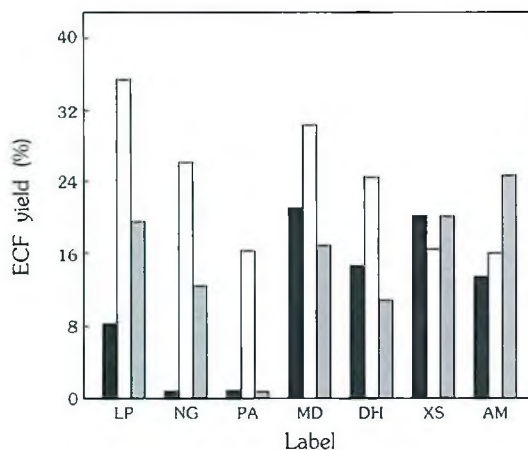
## Results and Discussion

Small, circular yellow spots were observed on the inoculated taro leaves 4–6 days after infection; this was followed by chlorosis and ultimately necrosis. The differing susceptibility of the taro cultivars tested is indicated by differences in the rate of development of the infection. Liposnay deteriorated most rapidly, NG and PA were relatively susceptible, while the development of symptoms in DH and MD was more retarded. Leaves not directly infected remained symptomless throughout the observation period. *Xanthosoma sagittifolium* and *A. macrorrhiza* did not show any signs of disease and are presumed to be resistant to infection by *P. colocasiae*.

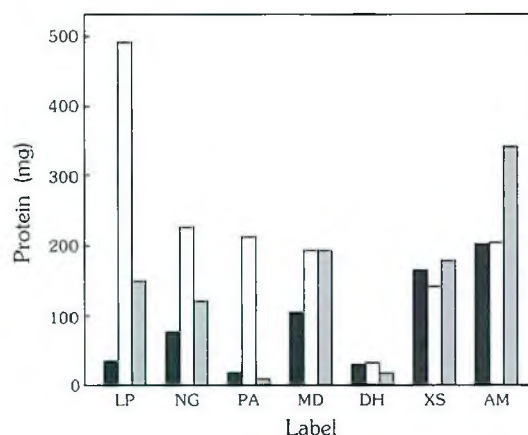
### Yield and protein content of ECF

The yield of ECF collected from leaves varied according to the species and cultivar being used (Figure 1). Yields were consistently higher from infected tissues which is a reflection of the loss of tissue organization during infection. Where there was no apparent disease, as in infected *X. sagittifolium* or remote leaves, there was little difference in the quantity of ECF extracted compared with healthy plants. The protein content of the ECF also showed considerable variation and when the total quantity of protein was considered (Figure 2) a clear distinction was apparent between susceptible and resistant plants which was consistent with the observed loss of leaf area.

The molecular weight distribution of proteins present in the ECF was analysed by SDS-PAGE. Most of the proteins detected were found in all the leaves of each variety regardless of their status. Several low molecular weight proteins of about 24 kD and 47.5 kD were found in healthy and remote leaves but not in the infected leaves. Since these proteins were present in intact leaves but not damaged and yellowed leaves, they may be constitutive



**Figure 1** Weight of extra-cellular fluid (ECF) obtained from different leaves of plants tested expressed as a percentage of initial fresh weight of leaf material used; ■, healthy leaf; □, infected leaf; □, remote leaf



**Figure 2** Quantity of protein present in the extra-cellular fluid of leaves tested expressed as mg protein 100 g<sup>-1</sup> leaf fresh weight; ■, healthy leaf; □, infected leaf; □, remote leaf

proteins which were degraded by fungal action and during tissue damage. This possibility was supported by the two controls *X. sagittifolium* and *A. macrorrhiza*, where no proteins were absent from the infected leaf ECF samples which had not suffered tissue damage.

Proteins present in the infected and remote leaves only may be involved in defence reactions while those found in remote leaves may arise from induced systemic resistance, export of proteins from infected leaves, or through dispersal of the pathogen within the plant. Numerous proteins which were found only in the inoculated leaves of the taro plants were determined to be of fungal origin by comparison with the bands observed on SDS-PAGE of the fungal suspension. In general, inoculated leaves contained more proteins which were absent from the healthy leaves and some proteins were only present in the remote leaves, with the excep-

tion of *X. sagittifolium* and *A. macrorrhiza*. The occurrence of proteins in the three leaves of these two plants was very similar. A possible explanation is that they probably demonstrated non-host resistance without the induction of specialized defence proteins.

Identification of a protein of plant origin which is only present in infected tissues does not, however, necessarily imply a defence function as proteins may be released into the ECF as part of the secondary consequences of invasion and cell death.

### $\beta$ -1,3-glucanase

Two bands were observed from the driselase standard. The sizes of these bands were around 32–44 kD and 49–68 kD, respectively. Since this standard contains a mixture of enzymes including xylanase and cellulase as well as laminarinase, the laminarin substrate might have been hydrolyzed by more than one of these enzymes, giving rise to  $\beta$ -1,3- and  $\beta$ -1,6-oligomers which could be stained. The colour intensity of the bands was used as an index for the  $\beta$ -1,3-glucanase activity. The relative colour intensity of the bands was scored by visual judgement.

Bands were observed in all samples showing that  $\beta$ -1,3-glucanases and (or) similar hydrolytic enzymes were detected. In the five taro cultivars, LP, PA, NG, DH, and MD, the molecular weight of the bands was 30 kD.

In *X. sagittifolium* and *A. macrorrhiza* high molecular weight bands of 380 kD, and 420 kD, respectively, were observed. Glucanases are usually relatively small molecules with low molecular weights, but as these proteins were separated under non-denaturing conditions, this may be a reflection of the low charge of these proteins, or alternatively, that they form part of a larger multitude sub-unit complex.

$\beta$ -1,3-glucanases were found to be constitutively expressed in healthy, non-stressed plants. However, the infected leaves showed higher  $\beta$ -1,3-glucanase levels than the healthy and remote leaves, with the exception of MD, *X. sagittifolium*, and *A. macrorrhiza*. The enzyme activities in different leaves of MD were the same, and in *X. sagittifolium* and *A. macrorrhiza* the highest activity was observed in the healthy leaves. No evidence of systemic induction was obtained as levels of glucanase in remote leaves were the same as in healthy leaves for all plants.

*Phytophthora* species possess complex, branched  $\beta$ -1,3-glucans in their cell walls and contain large amounts of intracellular glucans known as mycolaminarans (Keen and Yoshikawa, 1982) and are susceptible to  $\beta$ -1,3-glucanase hydrolysis. The purified mycolaminarans and the complex wall glucans have been shown to cause wilting symptoms and possibly hypersensitive response in several plant species (Woodward *et al.*, 1980) and these fungus-derived

oligosaccharides can act as exogenous elicitors that trigger the synthesis of  $\beta$ -1,3-glucanase.

### Peroxidase

Peroxidase was found to be present in all the ECF samples. The peroxidase observed in taro varieties DH and MD had a molecular weight of 290 kD. The peroxidases from *X. sagittifolium* and *A. macrorrhiza* were 310 kD and 400 kD, respectively. The difference in the level of enzyme activity in each sample was reflected by the colour intensity of the bands.

Apart from being involved in plant defence responses (Fehrmann and Dimond, 1967), peroxidase also plays an important role in lignification pathways. This enzyme is constitutively expressed at a certain basal level for lignin synthesis during growth and would be expected to be present in all samples. The peroxidase activity under the same treatments varied in different samples. Extra-cellular fluid from inoculated leaves did not always give the highest peroxidase activity, while some samples from healthy leaves showed the highest activity. Only LP, PA, and NG showed the highest peroxidase activity in inoculated leaves, suggesting a possible function in defence reactions. In DH, the highest enzyme activity was found in healthy leaves, but in MD, the same activity was found in remote leaves. It appears that peroxidases are not an important part of defence mechanisms in taro.

### Proteinase inhibitors

Both trypsin inhibitor and chymotrypsin inhibitor activities were detected in the ECF of all the taro varieties. In the trypsin inhibitor assay, three bands were present for MD and DH in all three samples, healthy, infected, and remote. The major band had a molecular weight of 35 kD. Two other bands were also observed and their sizes were 300 kD and 320 kD. In the chymotrypsin inhibitor assay, a 35 kD inhibitor was found in healthy, infected, and remote from DH, but this band was not present in samples from MD where only a 280 kD band was found. In LP, PA, and NG a defence related pattern of induction was found with no inhibitors being present in the healthy samples. A trypsin inhibitor of 35 kD was observed in the remote samples of LP and PA and not in the directly infected samples; however, a chymotrypsin inhibitor of similar size was present only in the infected samples and not in the remote samples. Variety NG presented a different pattern with both trypsin and chymotrypsin inhibitors of approximately 60 kD being observed in both infected and remote samples.

It is possible that the same inhibitor could show both trypsin and chymotrypsin inhibition where the pattern of inhibition overlaps, but in the case of LP and PA the activities were clearly separate. The main inhibitors appear to

have a size about 35 kD and other bands were of high molecular weights (280 kD, 300 kD, and 320 kD) and may be aggregates involving the inhibitor peptide.

For *X. sagittifolium*, only trypsin inhibitor activity was detected in the ECF. One band appeared in healthy leaves and three bands were stained in infected and remote. The major band, present in all ECF samples, had a molecular weight of 35 kD, while the two other bands, present only in infected and remote, were 45 kD and 67 kD in size. No chymotrypsin inhibitor activity was detected showing that the plant is not adapted to the production of this inhibitor.

In *A. macrorrhiza*, none of the two types of proteinase inhibitors (PI) was found in the ECF. No band appeared in any lane that contained the *A. macrorrhiza* samples and this showed that PIs were completely absent from the leaves.

Proteinase inhibitors in leaves are generally induced upon wounding by predators and pathogens. Plant-derived PIs do not act on endogenous proteinases but are effective against insect and microbial enzymes. Proteinase inhibitors are encoded by multi-gene families and are usually small proteins of sizes ranging from 5 kD to 30 kD. In the case of fungal invasion, PIs inhibit enzymes secreted by the fungal hyphae and prevent further destruction of host cells. The majority of PIs found from taro were about 35 kD in size and this is close to the value obtained from other species (Sanchez-Serrano *et al.*, 1993). Although these PIs were present in healthy leaves, relatively higher levels were found in inoculated and remote leaves suggesting that these proteins were produced and accumulated to a higher level after stimulation by pathogenic attack.

## Conclusion

Taro is a compatible host for the leaf blight disease, while *X. sagittifolium* and *A. macrorrhiza* are non-compatible hosts possessing non-host resistance. In taro, although the inoculated leaves died, the rest of the plant survived and continued to grow which demonstrated that the infection was not systemic.

The analysis of proteins present in the ECF did not identify any major induced band that would have provided a clear indication of a possible PR protein. Several minor differences were observed but the low levels of expression will make it difficult to isolate these proteins further. Peroxidase also failed to show any distinct defence related pattern of activity and the conclusion is that these enzymes are not an important part of defence mechanisms in these species.

All the plants showed some  $\beta$ -1,3-glucanase activity and in most cases there was higher activity in the infected leaves, except in the case

of MD and *A. macrorrhiza*, and, as these were both more resistant plants, this did not agree well with a role for  $\beta$ -1,3-glucanase in disease resistance. Although  $\beta$ -1,3-glucanase was induced on infection in LP it clearly failed to prevent the spread of the pathogen in the leaf.

The most interesting results were obtained with the assays of proteinase inhibitors. It is suggested that proteinase inhibitors are an important factor in disease resistance in taro and that increased disease resistance is associated with the presence of inhibitors in healthy leaves. This was not the case, however, for *A. macrorrhiza* where no proteinase inhibitors were observed at all.

Further research on the proteinase inhibitors produced by these aroids will provide useful information on the key determinants for controlling leaf blight in taro. In future, this could permit rapid screening of taro varieties for resistance by testing for the presence of PI genes and it could hold the possibility of improving the resistance of susceptible varieties by PI gene transfer.

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# Diagnosis of yam viruses

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Diagnosis of virus diseases of yams at the International Institute of Tropical Agriculture (IITA) is based on the use of indicator plants, insect vectors, serological diagnostics, and electron microscopy (EM). Yam I (YVI), yam mosaic (YMV), and *Dioscorea dumetorum* (DdV) potyviruses can be identified even in mixed virus infection. Yam mosaic virus is mechanically transmissible to *Nicotiana benthamiana* and YVI is vector-transmissible to *Vigna unguiculata*. The three viruses can be visualised by EM and their identities confirmed serologically. *Dioscorea alata* badnavirus (DaV) can be graft-transmitted to *V. unguiculata* and its presence confirmed by EM and serology. Cucumber mosaic cucumovirus (CMV), reported to occur in yams in West Africa, is readily mechanically transmissible to a range of indicator plants and can be detected serologically. Other isometric viruses have recently been found in Nigeria, and these can be isolated and differentiated by mechanical and vector transmission to indicator plants. These diagnostic techniques have enabled initiation of surveys of viruses of *Dioscorea* spp. in West Africa and substantial progress has been made on the production of virus-tested planting material for international distribution.

Keywords: *Dioscorea* spp.; Yam viruses; Diagnosis; ELISA; Electron microscopy

Virus diseases of yam (*Dioscorea* spp.) have been reported from many countries of the Commonwealth Caribbean, Africa, south-east Asia, and the Pacific. The first reports of yam diseases attributed to viruses were in 1936 in Puerto Rico and Sierra Leone (Cook, 1978), with subsequent reports in 1938 from Nigeria, 1956 from Liberia, and 1957 from Côte d'Ivoire (Thouvenel and Fauquet, 1978). Virus diseases have been reported from numerous yam species including *D. cayenensis* (Mohamed and Mantell, 1976), *D. rotundata* (Mohamed and Terry, 1979), *D. esculenta* (Mohamed and Mantell, 1976), *D. alata* (Thouvenel and Fauquet, 1978), *D. floribunda* (Hearon *et al.*, 1978), *D. composita* (Hearon *et al.*, 1978), *D. trifida* (Thouvenel and Fauquet, 1978), and *D. friedricksthali* and *D. spiculiflora* (Ruppel *et al.*, 1966).

The consequences of virus infection include the development of leaf symptoms which will in turn affect photosynthetic ability and may cause stunting and yield losses. Additional consequences are the vegetative transmission of viruses through vegetative propagules and, therefore, restrictions on the international movement of germplasm and the implications for germplasm improvement. This paper describes virus diseases of yams in Nigeria, the diagnostic techniques currently available, and the application of these techniques for virus surveys and in the production of virus-tested plants.

## Virus Diseases of Yams in Nigeria

Several viruses have been identified and characterized in Nigeria (Hughes *et al.*, 1997). These include yam potyvirus I (YVI), yam mosaic

potyvirus (YMV), *D. dumetorum* potyvirus (DdV), *D. alata* badnavirus (DaV), *Dioscorea* mild chlorosis virus (DMCV), *D. alata* mottle virus (DMV), and *D. alata* necrosis virus (DNV). In addition, cucumber mosaic cucumovirus (CMV), previously reported only in *D. alata* in Côte d'Ivoire (Fauquet and Thouvenel, 1987), has also been found in *D. alata* in Nigeria (Dongo, unpubl. data).

Yam potyvirus was detected in germplasm from Nigeria by Hughes (1986) and confirmed by Hughes *et al.* (1997). It occurs in *D. alata* and *D. rotundata* and causes leaf mosaic and mottling in both yam species.

Yam mosaic potyvirus was reported in Nigeria by Terry (1977) and Mohamed and Terry (1979). The presence of YMV has since been confirmed in *D. alata* and *D. rotundata* in Nigeria (Hughes *et al.*, 1997). Typical symptoms are mosaic, chlorotic mosaic, mottling with occasional leaf distortion, and crinkling.

*Dioscorea dumetorum* potyvirus (Brunt, A.A. pers. commun.) was detected in *D. alata* but not *D. rotundata* in the derived savannah of Nigeria (Hughes *et al.*, 1997). The virus occurred in mixed infection with YVI.

*Dioscorea alata* badnavirus was found in *D. alata* in Nigeria (Hughes *et al.*, 1997) with characteristic symptoms of leaf distortion, blistering, and crinkling.

*Dioscorea* mild chlorosis virus DMV, and DNV have recently been isolated in Nigeria from *D. alata* (Hughes *et al.*, 1997; Dongo, unpubl. data). They are isometric viruses which have been isolated in herbaceous indicator plants but are, at present, incompletely characterized.

Several viruses which have been described from elsewhere in the world have not been

found in Nigeria. These include *D. alata* virus (Porth *et al.*, 1987) and *Dioscorea* green vein-banding mosaic potyvirus (Reckhaus, 1980) from Togo, *Dioscorea* latent potexvirus from Puerto Rico (Waterworth *et al.*, 1974) and a 'carla-virus' from Japan (Fukumoto and Tochihara, 1978).

## Virus Detection

Detection of viruses can be by several methods ranging from biological methods (use of herbaceous indicator hosts or vector transmission), serological detection methods [including agar gel double-diffusion, enzyme-linked immunosorbent assay (ELISA), and Western blotting], electron microscopy, and molecular techniques such as cDNA probes and polymerase chain reaction (PCR). Appropriate techniques and screening methods have been developed at the International Institute of Tropical Agriculture (IITA) for field surveys, identification of unknown viruses, and quarantine diagnostics.

Virus detection techniques which have been developed, and are routinely used, for the detection and identification of yam viruses at IITA are described below.

### Biological tests

While symptom expression by yams can often be indicative of virus infection, other factors such as nutrient depletion or other pathogens may also be responsible for causing leaf symptoms or stunting. Vector or mechanical transmission to herbaceous indicator plants can be used to isolate viruses.

Yam mosaic virus DNV and DMV can be transmitted mechanically to *Nicotiana benthamiana*, and *Vigna unguiculata* (IT84S-2114) and *V. unguiculata* (TVu76), respectively. Yam mosaic virus causes systemic mosaic in *N. benthamiana*. *Dioscorea alata* mottle virus causes systemic leaf puckering, chlorosis, and bleaching while DNV causes mild systemic leaf mottling in their respective cowpea hosts.

*Dioscorea* mild chlorosis virus can be transmitted by the aphid *Aphis craccivora* to two lines of *V. unguiculata*, IT84S-2114 and TVu2657. In both of these cowpea lines, mild systemic chlorotic mosaic is observed.

### Serological detection methods

Many of the antibodies have been raised at IITA. The exceptions are the antibodies raised against viruses which are not endemic to, or have only just been identified in, Nigeria. The antibodies against DaV, YMV, YVI, DbV, and DdV were initially provided by Prof. A.A. Brunt (Horticulture Research International, Wellesbourne, U.K.). However, these have since been superseded by antibodies against YMV, YVI, and DaV raised at IITA. While polyclonal antibodies are generally used, monoclonal antibodies

raised at IITA against YMV are also available. Polyclonal antiserum has also been raised against DaV.

The main serological detection method used at IITA is ELISA. For testing all the viruses using polyclonal antiserum, protein A-sandwich (PAS) ELISA is used. When using monoclonal antibodies, a triple antibody-sandwich (TAS) ELISA is generally used.

### Electron microscopy and immunosorbent electron microscopy

Electron microscopy is routinely used for checking leaf samples which appear to test negative for known viruses by ELISA. For electron microscopy, pieces of leaf tissue are macerated in the negative stain potassium phosphotungstate (aqueous 2%, pH 6.8). The expressed sap is dried on to carbon-coated copper grids and visualised by transmission electron microscopy. This technique is generally useful to detect filamentous virus-like particles in sap preparations, although spherical viruses may sometimes be seen.

Immunosorbent electron microscopy (ISEM) is sometimes used to confirm the presence of a virus. The carbon/formvar-coated grids are floated face-down on antiserum diluted 1:1000 with PBS for 2 h at 27°C then excess antibodies rinsed off the grids with PBS and the grids blotted dry. The grids are then floated face-down on sap extracted in PBS for a further 2 h at 27°C. The grids are then rinsed again with PBS, stained with potassium phosphotungstate, and observed under the transmission electron microscope.

The technique of 'decoration' can also be used to identify viruses in mixed infection on the grids. When a mixture of viruses which has been adsorbed on the grid is floated on antibodies which are homologous to only one of the viruses, the homologous virus will be 'decorated' with antibodies. When stained, the antibodies appear as a darker line around the virus than the 'undecorated' virus, thus identifying the virus in question in the mixture.

### cDNA probes and PCR

These techniques are available and can be used for the detection and identification of some yam viruses, in particular, YMV. However, for routine use, the techniques are rarely used due to the costs of the reagents and the stringent conditions needed to ensure reproducibility.

## Survey of Yam Viruses in West Africa

A preliminary survey of yam viruses occurring in Nigeria has been completed (Hughes *et al.*, 1997). Yam viruses were found in both *D. alata* and *D. rotundata* in the humid forest, derived savannah, and southern Guinea savan-

nah yam-growing agro-ecological zones in Nigeria. Yam mosaic virus was the most common virus, occurring in both yam species. Yam potyvirus also occurred in both species although it was less common. *Dioscorea alata* badnavirus and DdV were found only in *D. alata*. Three previously uncharacterized viruses DMCV, DMV, and DNV were found as a result of the preliminary survey. The survey is being expanded to cover more areas within Nigeria and also to cover other areas within West Africa. This will identify the viruses naturally occurring in yams in the area, determine their geographic distribution, and enable an assessment of the potential for germplasm enhancement through the distribution of virus-tested germplasm.

### Production of Virus-tested Plant Material at IITA

Plantlets have been regenerated from meristem culture of yams (Ng, 1984) and virus-tested white yam (*D. rotundata*) plants were obtained through a combination of heat treatment and meristem culture or meristem culture alone followed by rigorous virus indexing (Ng, 1988). Micropropagation using nodal cultures has been reported and used for micropropagation of yam plantlets for distribution (Mantel *et al.*, 1979; Ng, 1992). Three systems for the multiplication and production of virus-tested white yam propagules for international distribution were described (Ng, 1994). These systems include the production of *in vitro* plantlets, micro-tubers *in vitro*, and minitubers in the screenhouse in sterile soil.

The IITA has a yam germplasm collection of more than 2500 accessions maintained in the field and *in vitro* (Ng and Ng, 1994). Through its selection and breeding efforts, improved germplasm of both *D. alata* and *D. rotundata* with high yield and resistance to major diseases were produced. In order to be able to distribute virus-tested material of both species, IITA has a comprehensive indexing and tissue culture programme to produce healthy material from field or screenhouse-grown plants.

Candidate plants selected for the production of virus-tested material through tissue culture are first indexed while growing in the screenhouse. Visual observations on symptom expression are taken, then leaf samples are tested by ELISA for YVI, YMV, DdV, DaV, and DMV. If symptoms are observed but the ELISA tests are negative, the plants may be checked by electron microscopy and mechanical or vector transmission tests done.

After meristem-tip culture, the regenerated plantlets are again indexed. If YVI or YMV are found, it is possible to do some preliminary screening using leaf trimmings from the tissue culture containers when subculturing. Representative plantlets obtained after subculturing are hardened and grown in an isolation room and

are re-tested for the viruses which were found prior to meristem-tip culture. Plants and their clonal lines which test positive for virus are discarded.

Prior to distribution, lines which have tested negative for virus are inspected and retested by Nigerian Plant Quarantine officials. After inspection and certification, certified plants are micropropagated for distribution. Currently, a total of 32 white yam genotypes are virus-tested and available for distribution (Ng and Hughes, 1996). During 1996, a total of 3689 virus-tested white yam plants and 5500 minitubers were distributed to national programmes in nine and seven countries, respectively (Ng and Asiedu, 1996). Efforts are now focussed on *D. alata* and, in 1998, virus-tested *D. alata* plantlets will be available for distribution.

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# Use of household disinfectants to suppress *Pratylenchus coffeae* and dry rot of yellow yam (*Dioscorea cayenensis*)

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*Pratylenchus coffeae* and other nematodes cause a dry rot in the skin of yam (*Dioscorea* spp.) tubers which can compromise their usefulness as planting material. Dettol antiseptic and bleach, which are household disinfectants, were as effective as the nematicide oxamyl in suppressing *P. coffeae* populations and development of the dry rot in yellow yam (*D. cayenensis*) heads. There was somewhat earlier and a higher level of sprouting of heads dipped in these three chemicals, and these heads bore the heaviest sprouts. These heads, and those dipped in Jeyes Fluid, sustained their integrity for longer, compared with heads dipped in water or alcohol. The Jeyes Fluid dip appeared to injure the growing points on the heads, but by 14 weeks, all had recovered and sprouted. In a field trial, the Jeyes Fluid dip caused delayed sprouting of the heads, while the oxamyl dip encouraged early sprouting. However, by 25 weeks, all Dettol-, Jeyes Fluid-, or oxamyl-dipped heads had sprouted, and these plants produced the greatest weights of tubers; plants from Dettol-dipped tubers bore significantly greater tuber yields than all other treatments.

Keywords: Household disinfectants; Nematicides; Yam; Sprouting; Tuber yields

Several destructive plant nematodes affect yam (*Dioscorea* spp.) production worldwide (Acosta and Ayala, 1975; Coates-Beckford and Brathwaite, 1977; Badra and Caveness, 1979; Degras and Mathurin, 1980). *Pratylenchus coffeae* is considered to be the greatest hindrance to yam production in Jamaica, and for the very severe decline in production of the yampie (*D. trifida*) and white yam (*D. alata*) (Hutton *et al.*, 1982, 1985). This nematode is always present in yellow yam (*D. cayenensis*) tubers. Nematode damage to yam tubers appears as cracking and a dry rot in the skin and underlying tissues. As nematode populations increase, the dry rot spreads and penetrates deeper into the tuber. On tubers selected as planting materials, the tissues that will form stem and root primordia, can be damaged or destroyed by dry rot, affecting their potential for sprouting, and for producing thrifty and highly productive plants (Degras and Mathurin, 1980). Since traditional yam planting materials (heads) are usually affected by the dry rot, one way of reducing losses from nematode damage of tubers is to select those with the least evidence of dry rot. Hutton *et al.* (1985) found that there was less sprouting of severely dry-rotted yellow yam heads, compared with lightly dry-rotted heads, and that plants from the severely dry-rotted heads were often unthrifty and under-productive. It

appeared that reduced yields from plots planted with nematode-infested heads were sometimes due to poor stands rather than to poor production by the plants from the infested heads.

Investigations have proven that dipping infested heads in solutions of oxamyl or other chemicals, or in hot water, suppressed populations of nematodes and development of the dry rot, caused increased levels of sprouting and plant thriftiness, and improved tuber production (Acosta and Ayala, 1976; Coates-Beckford and Brathwaite, 1977; Coates-Beckford *et al.*, 1978; Badra and Caveness, 1979; Hutton *et al.*, 1982, 1985; Hutton, 1987, 1995). However, oxamyl is extremely toxic. Furthermore, after use, the dips must be properly disposed of to prevent environmental contamination. Hot water treatment of yams, if not properly done, can cause severe tissue damage on the one hand, or ineffective nematode kill on the other. Therefore, these techniques must be competently handled.

Two trials were carried out, one in a greenhouse to test the effectiveness of three household disinfectants, or alcohol, in reducing *P. coffeae* in, and dry rot of, yellow yam tubers, and on sprouting of the tubers. The other was a field trial to measure the effectiveness of these dips on sprouting of heads and on increasing tuber production.

## Materials and Methods

### Greenhouse trial

The experiment was conducted in a greenhouse at The University of the West Indies, Mona Campus, Jamaica, for 14 weeks. Yellow yam heads (12 heads per treatment, replicated thrice) were dipped for 40 min in either ambient water (control), 10% ethanol, 1250 ppm bleach (NaOCl), 2500 ppm Dettol antiseptic (chloroxylenol), 4.5% Jeyes Fluid (a blend of high boiling tar acids and washed neutral oil, solubilized in vegetable soap), or 1000 ppm oxamyl [Methyl N', N'-dimethyl-N-[(methyl-carbamoyl)oxy]-1-thioxamimidate]. They were then placed in 50 cm x 30 cm plastic cases and covered with shredded newspaper which was kept damp by wetting lightly every day. The yams were kept in a greenhouse at 28–32°C. Before dipping, and at intervals over 14 weeks, a piece of paring about 3.5 cm x 2 cm was taken from each tuber, cut up, and *P. coffeae* in the skin from each replication extracted by just covering the parings with water in a 15-cm dish, leaving for four days, then recovering the nematodes. The greatest depth of the nematode-related dry rot was measured where the paring was taken on each head. Records were made of heads which had sprouted. At 14 weeks, the sprout produced by each head was removed, and the sprout and the remaining head were weighed separately.

### Field trial

The field trials were conducted in Coleyville, Manchester, for one year. Heads dipped as previously described were held for three days, then planted in hills 1 m x 2 m apart. There were 40 yams per treatment in five replications arranged in a randomised complete block design. Eight heads were planted, three, two, and three, in the three hills which constituted a plot. Thirteen weeks after planting, about 200 gm of an 11:22:22 (N:P:K) fertilizer were applied to each hill. Sprouted yams were counted at intervals up to 25 weeks after planting. The plots were harvested one year after planting and every harvested yam was weighed.

## Results

### Greenhouse trial

Water, alcohol, and Jeyes Fluid did not suppress *P. coffeae* populations and development of the dry rot over the 14 weeks of the trial (Table 1). Nematode populations remained low, sometimes undetected, in Dettol- and oxamyl-dipped tubers for the duration of the trial, and for the first 10 weeks in bleach-dipped tubers. Dettol and bleach suppressed

**Table 1** Efficacy of alcohol, three household disinfectants, or oxamyl in suppressing *Pratylenchus coffeae* infestation of yellow yam (*Dioscorea cayenensis*) planting pieces (heads)

Treatment <sup>†</sup>	No. of nematodes g <sup>-1</sup> yam skin			
	Weeks			
	0	6	10	14
Control	52 a	66 a	47 a	1432 a
Alcohol (10%)	23 a	154 a	56 a	315 b
Bleach (1250 ppm)	14 a	0 b	0 b	168 b
Dettol (2500 ppm)	36 a	0 b	0 b	11 c
Jeyes Fluid (4.5%)	48 a	25 a	158 a	424 b
Oxamyl (1000 ppm)	37 a	15 b	0 b	19 c

Treatments with different letters within columns are significantly different ( $P < 0.05$ )

<sup>†</sup>Heads dipped for 40 min

development of the dry rot more effectively than the water, alcohol, and Jeyes treatments. Oxamyl caused significant suppression of the dry rot compared with all other treatments (Tables 1 and 2).

Oxamyl, bleach, and Dettol dips promoted earlier sprouting of tubers, and at 14 weeks, these treatments had induced the heaviest sprouts, and the remaining heads were heavier. Growing points ('eyes') on heads dipped in Jeyes Fluid appeared to have been injured by the treatment, and early sprouting of these heads was lower than the control, bleach, Dettol, and oxamyl treatments. However, by 14 weeks, all Jeyes Fluid-dipped heads had sprouted and the heads remaining from this treatment were heaviest. The remaining heads from the Jeyes Fluid, bleach, oxamyl, and Dettol treatments were significantly heavier than those from the control and alcohol treatments (Table 3).

**Table 2** Efficacy of alcohol, three household disinfectants, or oxamyl in suppressing *Pratylenchus coffeae*-related dry rotting of yellow yam (*Dioscorea cayenensis*) planting pieces (heads)

Treatment <sup>†</sup>	Depth of the dry rot (mm)			
	Weeks			
	0	6	10	14
Control	0.17 a	0.4 a	0.7 a	2.9 b
Alcohol (10%)	0.28 a	0.5 a	0.7 a	4.7 a
Bleach (1250 ppm)	0.11 a	0.2 b	0.6 a	1.4 c
Dettol (2500 ppm)	0.17 a	0.1 b	0.1 b	1.1 c
Jeyes Fluid (4.5%)	0.17 a	0.4 a	1.2 a	3.4 b
Oxamyl (1000 ppm)	0.17 a	0.2 a	0.6 a	0.5 c

Treatments with different letters within columns are significantly different ( $P < 0.05$ )

<sup>†</sup>Heads dipped for 40 min

**Table 3** Efficacy of alcohol, three household disinfectants, or oxamyl in encouraging sprouting of *Pratylenchus coffeae*-infested yellow yam (*Dioscorea cayenensis*) planting pieces (heads), and maintaining head substance

Treatment <sup>†</sup>	Sprouted heads (%)			Avg sprout wt (g) at 14 weeks	Head wt week 14/ week 0 (%)
	Weeks				
	2	6	14		
Control	33 a	67 a	78 a	25.8 a	50 a
Alcohol (10%)	22 a	56 a	67 a	25.6 a	49 a
Bleach (1250 ppm)	44 a	78 a	89 a	46.2 a	69 b
Dettol (2500 ppm)	56 b	78 a	100 b	60.1 b	62 a
Jeyes Fluid (4.5%)	22 a	67 a	100 b	33.1 a	73 b
Oxamyl (1000 ppm)	33 a	89 a	100 b	58.4 b	67 b

Treatments with different letters within columns are significantly different ( $P < 0.05$ )

<sup>†</sup>Heads dipped for 40 min

### Field trial

At seven weeks, only 15% of Jeyes Fluid-dipped heads had sprouted, compared with 28% of water-dipped and 33% of oxamyl-dipped heads. However, by 25 weeks, all Jeyes Fluid- and Dettol-dipped heads had sprouted, compared with 93% of oxamyl-dipped, and 90% of alcohol-dipped heads. Tuber weights of plants from Dettol-dipped heads were more than 25% heavier than tubers of plants from oxamyl-dipped heads, and 45% heavier than those of plants from the control heads. Ethanol-dipped heads produced the lowest weights of yams (Table 4).

### Discussion

It has been shown that as dry rotting of yam planting material becomes more extensive, the potential for planting material to sprout and bear thrifty and productive plants is diminished (Hutton *et al.*, 1982, 1985). It seems that the advancing dry rot damages, and can destroy the cellular blocks that differentiate to form the stem and root primordia (Degras and Mathurin, 1980). Any treatment that suppresses development of populations of destructive nematodes and development of the dry rot will enhance the vitality and viability of the planting material. Hot water and (or) oxamyl dips have proven to be the best treatments for achieving this (Acosta and Ayala, 1976; Coates-Beckford and Brathwaite, 1977; Coates-Beckford *et al.*,

**Table 4** Sprouting of and bearing from yellow yam (*Dioscorea cayenensis*) planting pieces (heads) disinfested by *Pratylenchus coffeae*, using alcohol, three household disinfectants, or oxamyl (field trial)

Treatment <sup>†</sup>	Sprouted heads (%)			Wt of yams borne by 40 planted heads (kg)
	Weeks			
	7	15	25	
Control	28 a	88 a	95 a	70.3 a
Alcohol (10%)	23 a	83 a	90 a	59.5 a
Bleach (1250 ppm)	23 a	95 a	98 a	72.0 a
Dettol (2500 ppm)	23 a	93 a	100 a	102.1 b
Jeyes Fluid (4.5%)	15 a	88 a	100 a	83.2 a
Oxamyl (1000 ppm)	33 a	93 a	93 a	81.0 a

Treatments with different letters within columns are significantly different ( $P < 0.05$ )

<sup>†</sup>Heads dipped for 40 min

1978; Hutton *et al.*, 1982, 1985; Hutton, 1987, 1995). In this trial, Dettol and bleach dips were as effective disinfectants as oxamyl. The Jeyes Fluid dip was not effective in controlling *P. coffeae* and dry rot, but this chemical, oxamyl, bleach, and Dettol dips all enhanced the vitality and viability of the yam planting material. In both the greenhouse and field trials, a very high proportion of all of the yam heads dipped in these chemicals sprouted. Better stands and more bearing plants are important factors related to increased quantitative yields from yam plantings.

Hutton (1993) reported that destructive nematodes can reduce production of yams in Jamaica by over 40%. One way of mitigating these losses is to use planting material with the least evidence of dry rotting. Disinfesting the planting material using hot water or nematicides such as oxamyl is another option. However, using hot water to disinfest yam heads and other plant materials of nematodes and other pests and pathogens requires competence and trained persons. Oxamyl is a very toxic chemical and its use is restricted. When it is used for dipping plant materials, precautions must be taken for user protection, and methods of properly disposing of the used dip must be devised in order to prevent contamination of the environment. If further investigations should confirm the effectiveness of 'safe' chemicals like the household disinfectants Dettol, bleach, and Jeyes Fluid, then these chemicals could more readily be put into the hands of farmers themselves

with pertinent recommendations. Their use would not present the several difficulties related to use of hot water or toxic nematicides.

In current trials, Dettol, bleach, and Jeyes Fluid are proving to have strong activity against *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Helicotylenchus* sp., and other plant-parasitic nematodes in soil. If further investigations confirm the usefulness of these materials in treating infested soil, then they could be recommended for use in household gardens for this purpose. Traditional nematicides are not recommended for use in household gardens in Jamaica. It seems that there is a place for these "non-traditional" nematicides in nematode control in Jamaica and other countries.

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# Yam anthracnose in the English-speaking islands of the Eastern Caribbean: Successes and research advances in disease management

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Anthracnose (*Colletotrichum gloeosporioides*) has been the single most important factor responsible for the decline of 'White Lisbon' a white yam cultivar (*Dioscorea alata*) from the beginning of the 1980s in Barbados and the English-speaking Caribbean. The outstanding success achieved, to date, in the management of the disease is the selection of tolerant cultivars introduced from Guadeloupe, Puerto Rico, and Costa Rica. Five such cultivars have shown superiority to all others including the two cultivars indigenous to Barbados, 'Oriental' and 'Welch'. The five superior clones are 'Plimbite', 'Kinabayo', 'Belep', 'Pacala', and 'Gunung'. The challenge, however, may lie in the application of biotechnology techniques to study the pathogen (*C. gloeosporioides*) as well as the host White Lisbon (the preferred cultivar) so as to better understand the host and (or) pathogen relationships and the genetics of the pathogen and host at the molecular level.

Keywords: Anthracnose; White Lisbon; Tolerant cultivars; Biotechnology

Anthracnose disease (*Colletotrichum gloeosporioides* Penz.) Sacc. & Penz. telemorph *Glomerella cingulata* (Stonem.) Spauld & Schrenk has been the single most important factor responsible for the decline of *Dioscorea alata* (L.) cv. White Lisbon yam production in Barbados and the rest of the Eastern Caribbean States (ECS). At the beginning of the 1980s, anthracnose severely reduced the production of White Lisbon yam in these countries. Over the last five years, the epiphytotic level of the disease caused almost complete eradication of this cultivar.

Since the outbreaks of the 1980s and the near destruction of the yam industry in Barbados, the Caribbean Agricultural Research and Development Institute (CARDI) has been conducting research on the agronomy of yam. However, no studies on yam pathology were undertaken until the 1990s. It is, therefore, regrettable, that no regional approach was adopted until recently, to conduct research on anthracnose of white yam (local yam) in any focussed and sustained way.

This report highlights the economic importance of anthracnose in White Lisbon yam, reviews briefly what has been achieved so far, and outlines a research plan. The two premier agricultural institutions of the region, CARDI

and The University of the West Indies, Cave Hill Campus, have agreed to collaborate in the research effort.

## Economic Importance of Yam Anthracnose

In Barbados and the rest of the Eastern Caribbean, white yam (water yam) (*D. alata*) is one of the important tropical root crops used as a staple food and grown extensively (Ferguson, 1974). The cultivar White Lisbon is the preferred yam and known for its soft texture, flavour, white flesh (cortex), and good storage qualities. Production figures of Barbados were estimated at 15 500–18 000 tonnes during 1970 (Gooding, 1970). White Lisbon comprised most of this production in the country.

Production in St Vincent during the same period was estimated to be less than 500 tonnes. In the other Eastern Caribbean islands of Dominica, St Lucia, St Kitts & Nevis, and Antigua, the cv. White Lisbon was not grown in significant quantities in the early 1990s, but this cultivar has always been identified as a potential major root crop not only as a part of

the staple diet but also as an important foreign exchange earner (Anon., 1988). It might be reasonably argued that anthracnose disease severely hampered the expansion and growth of White Lisbon yam production in the five islands in the late 1980s.

White Lisbon yam production decreased in Barbados in the 1990s to 1800 t yr<sup>-1</sup>. Consequently, exports fell significantly to less than 100 tonnes in 1991 (BMC, 1991). A fair estimate of Bds \$3.0 million (U.S. \$1 million) of foreign exchange was lost in 1991 alone. This loss can be regarded as a good benchmark for estimating foreign exchange losses per year over the period 1984–91 due to anthracnose which caused a significant drop in the acreage of White Lisbon from 283 ha to 25 ha (BMC, 1984, 1991). This decline in yam production and exports can be attributed to the severity of anthracnose disease on White Lisbon. Given the present understanding of the disease, fungicidal application does not guarantee economic control. Hence, White Lisbon is presently a non-factor in yam production of Barbados. The disease has virtually eliminated a cultivar that was once thought to be pivotal in crop diversification in Barbados and the English-speaking Caribbean.

## Review of Research on Yam Anthracnose

By 1984, the severe decline in yam production in Barbados and the rest of the Eastern Caribbean signalled the apparent extinction of the White Lisbon cultivar. A few farmers in Barbados have, however, continued to maintain small acreages.

The CARDI has adopted several research approaches to revive White Lisbon in Barbados and the rest of the Eastern Caribbean. In Barbados the main thrust was the introduction and testing of white yam cultivars reported to have field tolerance to anthracnose. In 1984–87 CARDI identified five introduced and two local cultivars reported to have tolerance to the disease. 'Plimbite', 'Belep', 'Kinabayo', 'Pacala', and 'Grand Cayman' were the introduced selections. Oriental and Welch were the local ones. A scheme was also developed by CARDI in Barbados to have certified growers who received certified planting material (FAO, 1997a) of the introduced cultivars for bulking through the minisett technique. The materials were then multiplied by sale to other farmers. A group of five farmers started the scheme and, to date, this number has grown to eight. A brief description of the agronomic and economic characteristics of two of the four important introduced *D. alata* cultivars grown in Barbados during the 1990s are given in Table 1 together with the two local cultivars and the susceptible White Lisbon type (FAO, 1997b).

Presently, Plimbite and Belep are the two most widely grown cultivars of the introduced yams in Barbados. Of the local cultivars, Welch is the most commonly grown with superior cooking quantities to those of Oriental.

Cultivar Gunung has recently been introduced into the production system on a limited scale. Cultivars Diamond 22 and DOMI are the most recent accessions from Costa Rica and are anthracnose-tolerant. They are being bulked for testing in Barbados. The latter cultivar has been growing successfully in Dominica for the past three years; its cooking qualities, flavour, and shelf life are second only to White Lisbon. The situation is dynamic with respect to the introduction of cultivars tolerant to anthracnose in the production system. The farmers of Barbados and the Organization of Eastern Caribbean States are resilient and eager to try tolerant cultivars, when available. This attitude in Barbados can be considered by some as a departure from that of the late 1970s when the predominant cultivar was White Lisbon which occupied 95% of all yam acreages (Chandler *et al.*, 1983).

Farming systems research was conducted region-wide during the epiphytotic years of anthracnose 1985–89 (Rao *et al.*, 1989) with the purpose of managing the disease. This research included the introduction of tolerant cultivars and the development of intercropping systems. Intercropping did not seem to be compatible with yam production systems in Barbados, although crop rotation was important. However, a three-year rotation system has been in practice by plantation farmers over the past 10 years (Chandler, F.L., pers. commun.)

Rao and George (1987) reported that intercropping yam with cowpea, snapbean, and dasheen demonstrated an increase in yields of yam and a synergistic effect between yam and intercrops. The incidence of anthracnose was also found to be lower because of intercropping.

Green (1994) reported that intercropping White Lisbon with other yam cultivars and (or) species known to be tolerant or immune to *C. gloeosporioides* was ineffective in the reduction of the severity of anthracnose disease when compared with pure stands of White Lisbon. On the other hand, there was a significant difference in disease severity between the two sites where the experiment was conducted. The site with medium rainfall had a higher disease severity than the site with a lower rainfall regime (Green, 1994).

## Recent Advances

All research in anthracnose (*C. gloeosporioides*) in *D. alata* cv. White Lisbon over the past 13 years in the English-speaking Caribbean was conducted using standard procedures in mycology and plant pathology. During recent times molecular biology techniques have been used to

**Table 1** Agronomic and economic characteristics of two introduced *Dioscorea alata* cultivars, two local *D. alata* cultivars, and susceptible White Lisbon yam

Description	Cultivar				
	Susceptible	Local		Introduced	
	White Lisbon	Welch*	Oriental*	Plimbite†	Belep†
<b>Tuber</b>					
Shape	variable	ellipsoidal	globular	branched	branched
Length	6-20 cm	6-20 cm	6-20 cm	6-20 cm	6-20 cm
Skin thickness	thin (<1 mm)	thin (<1 mm)	thin (<1 mm)	thin (<1 mm)	thin (<1 mm)
Texture of tuber skin	rough	rough	rough	rough	smooth
Hairiness of surface roots	profuse	na	absent	absent	profuse
Tuber head cortex colour	yellow	purple	purple	cream	cream
Central transverse section flesh colour	white	cream	cream	cream	cream
Time of maturity	12 months	6-7 months	6-7 months	6-7 months	7-8 months
<b>Leaf</b>					
Phyllotaxy	opposite or alternate	opposite	opposite or alternate	opposite and (or) alternate	opposite and (or) alternate
Type	simple, entire, shallowly lobed	simple, entire, shallowly lobed	simple, entire, shallowly lobed	simple, entire, shallowly lobed	simple, entire, shallowly lobed
Shape	cordate	cordate	cordate	cordate	cordate
Hairiness of upper surface	none	none	none	none	none
Hairiness of lower surface	none	none	none	none	none
Waxiness of upper surface	waxy	non-waxy	waxy	waxy	waxy
Waxiness of lower surface	waxy	non-waxy	waxy	waxy	waxy
<b>Stem</b>					
Twining habit	twining	twining	twining	twining	twining
Direction of twining	clockwise	clockwise	clockwise	clockwise	clockwise
Plant type	climbing	climbing	climbing	climbing	climbing
Hairiness of stem	na	na	na	none	none
Stem pigmentation at emergence	green and purple	green	green and purple	green and purple	green and purple
Susceptible or tolerant to Anthracnose	very susceptible	tolerant	tolerant to moderately susceptible	tolerant	tolerant

na, Not applicable

The two other introduced cultivars Gunung and Kinabayo had leaf, stem, and tolerance to anthracnose characteristics that were identical to the introduced cultivars Plimbite and Belep. The tuber characteristics of Gunung were similar to Plimbite except that the texture of the tuber was smooth. The tubers of Kinabayo had an ellipsoidal shape, thick skin, purple tuber head cortex and white flesh colour, and matured in 7-8 months

study yam anthracnose and the fungal pathogen *C. gloeosporioides*.

The Department of Biological and Chemical Sciences, Faculty of Science and Technology, The University of the West Indies, Cave Hill Campus, Barbados, has formed a Microbiological Pathogenicity Research Group that is studying the yam anthracnose disease on White Lisbon yam at the molecular level.

Alleyne (1996) partially purified and characterized the phytotoxic products exuded by the pathogen *C. gloeosporioides* isolated from

White Lisbon yam grown in Barbados. The water-soluble, host-selective glycoprotein-type toxic compounds are composed of large polysaccharides mainly mannose, galactose, rhamnose, and glucose of which 80-85% were mannose and galactose.

The protein fraction of the complex compound was to demonstrate host selectivity of the toxin by screening a wide host range including lime, mango, anthurium, and three yam cultivars, namely, White Lisbon, Plimbite, and Welch. Leaves of yam cultivars were observed

for symptoms of anthracnose after 2–3 days. These included severe leaf necrosis leading to eventual death of the leaf. Necrotic lesions were always associated with the immediate area of application of the toxin. Aqueous solutions of the toxic compound were also used to screen cell suspensions of yam, pepper, and tomato. Electrolyte leakage and cell death were determined. The latter was assessed by measuring cell viability using fluorescein diacetate (FDA) staining of all tested suspensions. White electrolyte leakage was measured by use of conductivity readings ( $\text{mho cm}^{-1}$ ). The viability of yam cell suspensions containing  $7.5 \times 10^5$  cells  $\text{mL}^{-1}$  treated with aqueous solutions of the crude precipitate from isolates of *C. gloeosporioides* from White Lisbon yam declined by 80–90% after treatment.

The University has also begun to do molecular characteristics of various yam species and cultivars by polymerase chain reaction–random amplification of polymorphic DNAs (PCR–RAPDs) analysis under the supervision of the Department of Biological and Chemical Sciences. This kind of characterization by amplification of DNA markers is useful for the various species and cultivars of the genus *Dioscorea*; they can be placed in groups thus indicating genetic similarities among the species and any cultivars.

Preliminary RAPDs analysis of yam has been conducted by Bateson (1996) using 11 yam accessions from the CARDI yam germplasm collection. This enabled the construction of a dendrogram showing the genetic similarities of the different accessions. The patterns revealed by this molecular technique generally confirmed the traditional classification based on phenotypic characters. Thus, all the *D. alata* samples (with the notable exception of cv. Diamond 22) were seen as a closely related group, distinct from *D. trifida*, *D. esculenta*, *D. rotundata*, and *D. cayenensis* groups.

Close examination of the dendrogram revealed an interesting pattern. A number of *D. alata* cultivars which showed significant tolerance to anthracnose disease viz., 'Oriental', 'Plimbite', 'Belep', and 'Kinabayo' were seen to be genetically closely related. Expansion of the study to compare a larger number of both tolerant and susceptible cultivars is necessary to substantiate this correlation. If it is confirmed, it is suggested that RAPDs analysis may provide an effective method of predicting anthracnose tolerance or susceptibility of a given *D. alata* cultivar. This would obviate the need for large-scale planting of potentially commercial cultivars over several seasons to assess this degree of tolerance to anthracnose.

### Outline of Research Plan

It has been accepted that the application of molecular biology techniques will assist in better understanding of the disease. However, there is

still the need for strategic research to be undertaken in combination with molecular biology, mycology, and plant pathology to enhance the knowledge of anthracnose disease of the preferred cv. White Lisbon in Barbados.

Forecasting systems should be developed based on the spatial and temporal dynamics of the disease in combination of the weather data of a given locality. This would inform an efficient spray programme.

A disease index should be developed based entirely on disease severity which should correlate with the yam (host) yield. This would serve as a good measure to index all new yam accessions exhibiting field tolerance.

A sustainable production system should be developed for producing planting material of all commercial yam cultivars field-tolerant to anthracnose through micropropagation (tissue culture). The aim of the process should be twofold:

- 1) Provision of clean, disease-free planting material including viruses; and
- 2) Renewal of vigour in planting material of selected cultivars on an annual basis.

Finally, there must be economic analyses of the crop management required for the control of anthracnose disease and of the production system to multiply yam planting material by tissue culture.

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# Discovery of new diseases of cassava in West Africa

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Two new diseases of cassava were discovered in Benin, Ghana, and Nigeria. *Curvularia* leaf and stem blight [*Curvularia lunata* (Wakker) Boedijn] was discovered in all three countries. In the field, incidence of *Curvularia*-infected plants ranged between 0 and 80%, and severity between 2 and 25 lesions per plant. The effect of the fungus on growth was investigated using six cultivars, all naturally infected. For all cultivars, when buds were completely colonized, sprouting was completely inhibited. However, partially colonized buds sprouted, but growth was reduced by 20–50% (depending on genotype), compared with healthy stems. On artificially inoculated leaves, *C. lunata* induced lesions of up to 1.5 cm and 40% leaf abscission, compared with healthy leaves. *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko root and stem rot was discovered in Benin and Nigeria, with field incidence ranging between 0 and 54%. For all four cultivars, *N. mangiferae* significantly reduced the number of shoots, shoot growth, and number of roots. Two of the cultivars died three weeks after planting.

Keywords: Cassava; *Curvularia lunata*; *Nattrassia mangiferae*; Growth reduction; West Africa

The importance of cassava (*Manihot esculenta* Crantz) as a major food crop for Africa, Asia, and Latin America has been enumerated many times (Hahn *et al.*, 1989; El-Sharkawy, 1993). Cassava production is affected by biotic and abiotic constraints. Among the biotic constraints, pests and diseases cause significant yield reduction. For example, Lozano and Terry (1976) estimate that a total of 30 different bacterial, fungal, and viral diseases attack cassava, and that diseases alone or in combination, may reduce yield by up to 90% (Lozano and Booth, 1974). In addition, new ones continue to arise. For example, field surveys in Benin, Ghana, and Nigeria found that *Curvularia lunata* (Wakker) Boedijn, hitherto unreported to cause disease on cassava, was in fact causing a stem blight (Msikita *et al.*, 1997a). Similarly surveys conducted in Benin and Nigeria found *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko root and stem rot to be causing germination failure of cassava less than three months old (Msikita *et al.*, 1997b). Following the discovery of the diseases, intensive diagnostic surveys were conducted in all three countries (Benin and Nigeria for *N. mangiferae*) to determine the extent and severity of the disease. This paper provides information on the prevalence and severity of *C. lunata* stem blight and *N. mangiferae* root and stem rot, in the West African sub-region.

## Materials and Methods

*Curvularia lunata* stem blight was discovered on cassava at Bunso, (6°17' N, 0°27' W) in

the southern part of Ghana, in March 1995. *Nattrassia mangiferae* was discovered on cassava in Save (8°01' N, 2°27' E), Benin in January 1996, and in Lafia (8°31' N, 8°30' E), Nigeria in February 1996.

## Field surveys (*C. lunata*)

Following the discovery of *C. lunata* in Bunso, intensive diagnostic surveys were conducted during part of the dry season in 1996 (November to December) in Benin (66 fields, randomly selected between lat. 6°25' N and 11°25' N), Ghana (60 fields between lat. 4°55' N and 8°16' N), and Nigeria (47 fields between lat. 4°50' N and 7°56' N) to determine the prevalence and severity of *C. lunata* on cassava. Only plants  $\geq 9$  months were surveyed because the disease is easily visible at  $\geq 9$  months. In each field, 30 plants were randomly selected in a diagonal transect across the field, and plants in the diagonal were examined thoroughly for symptoms of the disease. Disease incidence (per cent of total number of plants per total sampled) and severity (number of lesions per stem) were determined for each field. Also, cassava germplasm collections in Benin (located 6°40' N, 2°7' E at Niaoli), and in Ghana (located 6°17' N, 0°27' E at Bunso) were assessed for incidence (per cent of total number of plants infected per plot) and severity (number of lesions per stem) of the disease. The Benin collection was assessed in November 1996, and September 1997, while the Ghana collection was assessed once. In both countries, accessions were planted in 2 m  $\times$  6 m long rows spaced 1 m apart, and were 9 and 10 months old,

respectively, for the germplasm collections in Benin and Ghana at the time of disease assessment. Means of disease incidence and severity per field or plot were calculated, and compared using the least significant (LSD) method of SAS (1986).

### Greenhouse studies (*C. lunata*)

#### *Bud germination*

**Naturally infected stems** — Thirty-centimetre long, naturally infected (3–5 lesions) stems of cassava of local cultivars, 'Lombo', 'Axotonon', 'Odungbo', 'Amazon', and 'Okayao' were obtained from nine-month old field-grown plants, and planted in sterilized (autoclaved for 1 h at 1.06 kg cm<sup>-1</sup> pressure) sand, and shoot growth measured (with a ruler) weekly for four weeks. Treatments comprised stem buds completely, partially, or non-colonized (control stems) by *C. lunata*. After planting, the stems were maintained in a greenhouse under natural light at 28 to 30°C, and watered two times per week. The experiment was repeated three times in a completely randomized design with five replications per treatment. Mean shoot growth per treatment per week was calculated, and compared by the LSD method of SAS (1986). Due to the difficulty in obtaining sufficient number of infected stems, field germination of infected stems was studied only for cultivars 'Odungbo' and 'TMS 30572'. The experiment was planted in two localities (Amusho and Kotopo), located 20 km apart in Abeokuta (7°10' N, 3°25' E), in Nigeria. Infected and uninfected stems (30 cm long) were planted (1 m × 1 m) separately in 6 m<sup>2</sup> plots, arranged in a completely randomized design with five replications for each of the control and infected stems. Treatments comprised naturally infected (3–5 lesions, partially or completely colonized), and uninfected stems (control plants). Four weeks after planting, germination between infected and non-infected stems were determined and means of germination between treatments were compared as described above.

#### *Artificially inoculated stems*

Prior to the experiment, *C. lunata* (isolate Cl 1), locally collected from cassava (6°17' N, 0°27' E), was cultured on potato dextrose agar (PDA) acidified to pH 4.5 with 0.4% (v/v) lactic acid, and frozen at -6°C for three months. A week before the experiment started, the fungus was thawed and sub-cultured on PDA, and incubated for 10 days under cool-white fluorescent light at 30 µmol m<sup>-2</sup> s<sup>-1</sup> and 25 ± 2°C, and 12 h photoperiod. These cultures were used to inoculate cassava stems.

Stems (30 cm long) from cultivars 'Agric', 'Tchukunochi', 'TMS 30572', and 'Ben 86052', were disinfested in hot water (52°C, 5 min), transplanted in sterilized sand, and maintained in a greenhouse under natural light at 28–

30°C. Before planting, five stems were wound-inoculated (sliced with an epidermal scalpel) just above nodes, and a 1-mL mycelial suspension (prepared by placing a 5-mm diameter PDA mycelial plug cut out on the edge of the petri plate by cork borer number 2, were added to 2 mL sterilized distilled water and the conidia adjusted to 10 × 10<sup>3</sup> conidia mL<sup>-1</sup>) of *C. lunata* was applied to each wound. Stems were then kept in a plastic bag for 24 h before planting. For each cultivar, five control stem cuttings were similarly wounded but treated with sterilized distilled water. All plants were maintained under ≥90% relative humidity, and were watered two times per week. The experiment was a completely randomized design with five replications for each of control and inoculated stems. Shoot growth per week were calculated, and mean growth separated and compared as described in (a) above.

#### *Leaf infection*

Stems from cultivars, 'Agric', 'Amazon', 'Axotonon', 'Ben 86052', 'Lombo', 'Odungbo', 'Tchukunochi', and 'TMS 30572' were sown, and maintained for four weeks in the greenhouse, as described above. On each plant, the two lowermost leaves on the stem were wounded using four pins affixed to a cork, and 1 mL mycelial suspension of *C. lunata* (adjusted to 10 × 10<sup>3</sup> conidia) were applied to the wounds. Control plants were similarly wounded but treated with sterilized distilled water. All plants were maintained at >90% relative humidity, and watered two times per week. There were five replications for each of control and inoculated plants, and all pots were arranged in a completely randomized design. Disease progress was monitored weekly, and means of growth per treatment were compared and separated as described above.

#### *Field surveys (N. mangiferae )*

During part of the dry season in 1996 (November to December), surveys were made on cassava plants (≤3 months old) for incidence of root and stem rot [typified by wilted and (or) dead plants] in 99 fields of cassava randomly selected between latitudes 6°36' N and 7°49' N in Benin (79 fields) and Nigeria (20 fields). In each field, 60 plants were examined in two diagonals (30 plants per diagonal) cutting across the field. Wilted and (or) dead plants showing termite damage were neither sampled nor included in the incidence of root and stem rot. In the laboratory, infected root and stem portions (0.5 to 1 cm) were cut out, surface-disinfested (10 min) in 10% bleach (0.6% sodium hypochlorite), rinsed in sterilized distilled water, and cultured on PDA acidified to pH 4.5 with 0.4% (v/v) lactic acid. Cultures were incubated at 25°C, under 12-h daylength provided by cool-white fluorescent lamps. After one week, mycelia, conidiophores, and conidia were

observed at  $\times 30$  to  $\times 40$  magnification under a compound microscope.

### Greenhouse studies

Pathogenicity of *N. mangiferae* was tested on cassava cultivars 'Agric', 'Ben 86052', 'Tchukunochi', and 'TMS 30572'. Two weeks prior to the experiment, inocula for pathogenicity tests were prepared by incubating 5-mm diameter mycelial plugs of *N. mangiferae* (isolate Nm 14) locally collected from cassava (8°01' N, 2°27' E), with 500 mL autoclaved rice seed for 10 days at 25°C, followed by air-drying in a laminar flow hood for 2 days. Five 30-cm long stem portions were cut from healthy plants of each cassava cultivar, surface-disinfested in hot water (52°C, 5 min), and transplanted into sterilized (autoclaved, 1 h) sand in 1-L pots to which 10 mL of the *N. mangiferae*-colonized rice inoculum had been added. There were five control stems for each cultivar, similarly treated, but not inoculated. Plants were maintained in a greenhouse under natural light at 28–30°C. Thirty days after planting, plant height, lesion length, and number of shoots and roots were recorded.

## Results

### Field surveys (*C. lunata*)

On infected plants, the disease was expressed as greyish-brown superficial mycelial mats (stroma) predominantly on the lignified stem portions. The mats always originated on the buds located on the lignified portion of the stems, and spread to completely colonize the infected buds and contiguous areas. Under severe infection, the mycelial mats extended to buds located on the green portion of the stems, but not the shoot tip.

In Benin, the disease appeared to be confined to two localities (Savalou and Bantè). Similarly, the disease appeared to be clustered around two localities in Ghana (the area around Bonsu and Kumasi). In south-west Nigeria, however, the disease was more widespread. Of the 66 fields of cassava surveyed in Benin, the disease was found only in 6 fields (9% of fields surveyed). In Ghana, of the 60 fields visited, the disease was found in 8 fields (13% of fields surveyed) while in Nigeria, of the 47 fields visited, the disease was found in 18 fields (38% of fields surveyed). However, in all three countries, incidence and severity varied significantly within and among cassava fields. The highest incidence (93%) was recorded in Abeokuta (Nigeria) while the highest severity (21.7 mean lesions per plant) was recorded in Ghana. The lowest field incidence (3%) was also recorded in Nigeria, while the lowest severity (<1%) was recorded in Benin. Overall, disease severity was lowest in Benin.

The germplasm collection in Benin comprised 340 accessions, whereas the Ghana collection had 118 accessions. No *Curvularia* stem blight was found in the Benin collection. However, in the Ghana germplasm collection, 19 accessions were infected by the disease. For the 19 accessions showing the disease, incidence and severity of disease varied significantly among accessions. Plot incidence of infected plants ranged between 17% in accession 'MC 90/010' and 80% in accession 'JK 006'. Severity ranged from a mean of one lesion per stem (accessions 'JK 001' and 'KAA 90/54'), to 25 in 'MC 90/020' (Table 1).

Field incidence and severity data for leaf infection was difficult to assess because symptoms produced by *Curvularia* blight (blighted patches surrounded by a spreading yellow area) were indistinguishable from those produced by *Cercospora* blight (*Cercospora viscosae* Muller & Chupp).

### Greenhouse studies (*C. lunata*)

#### Bud germination

*Naturally infected stems* — Buds sprouted within one week after planting. In all six cultivars ('Lombo', 'Axotonon', 'TMS 30572', 'Okoyao', 'Odungbo', and 'Amazon'), uninfected buds grew into large shoots within two weeks after planting. When buds were completely colo-

**Table 1** Disease incidence and severity of *Curvularia lunata* (Wakker) Boed. in the cassava germplasm collection in Ghana

Accession	Incidence <sup>a</sup> (%)	Severity <sup>b</sup> (%)
JK 005	40.00	2.25
JK 021	55.56	5.00
JK 006	80.00	8.00
JK 022	20.00	15.50
MC 90/020	20.00	25.00
MC 90/013	30.00	10.00
MC 90/010	16.67	8.00
KAA 90/54	20.00	1.00
MC 90/033	20.00	2.00
KAE 90/046	25.00	7.00
KAA 90/058	33.33	6.00
JK 026	77.78	3.00
JK 009	50.00	18.40
JK 016	27.27	5.00
JK 001	25.00	1.00
KAA 90/065	50.00	7.50
KAA 90/050	57.14	7.50
KAA 90/053	28.57	4.00
KAA 90/056	57.14	3.00
LSD <sub>0.05</sub>	10.07	3.40

<sup>a</sup>Percentage of plants in the plot symptomatic for *C. lunata*

<sup>b</sup>Mean number of lesions per stem

nized by *C. lunata*, very little (e.g., <2 cm. in 'TMS 30572' and 'Amazon') or no growth was observed even four weeks after planting. For all cultivars, when the fungus partially colonized the buds these sprouted, but shoot growth was significantly reduced by 20–50% (depending on genotype) compared with healthy stems.

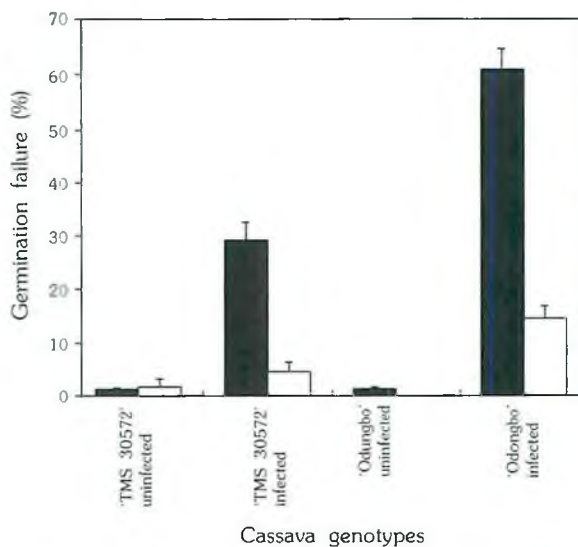
In the field, shoot germination was significantly less for infected stems for both 'Odungbo' and 'TMS 30572', in both locations, compared to their respective control plants. Also, there were significant differences in percentage of shoot germination between the two localities. Overall germination for 'Odungbo' was significantly less than that for 'TMS 30572' (Figure 1).

**Artificially inoculated stems**

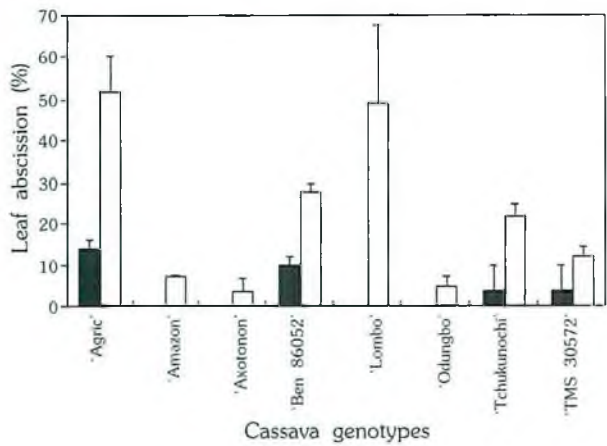
For both control and inoculated stems, buds sprouted one week after planting. In all four cultivars ('Agric', 'Ben 86052', 'TMS 30572', and Tchukunochi), shoots sprouted in both inoculated and non-inoculated stems. However, growth was significantly lower for the inoculated than for the control treatments.

**Leaf infection**

Within two days of inoculation, a blight developed around the inoculated sight. The blight continued to expand to about 1.2 cm in diameter, two weeks after inoculation. There were, however, no significant differences in size of lesions among cultivars. Inoculated leaves turned yellow and abscised. The presence of the fungus on the plant also led to abscission of non-inoculated leaves on the plant. Between 10–50% more leaves were abscised in inoculated plants. Highest leaf abscission (50% of total leaves) was in cultivar 'Agric', and the least (14% of total leaves) was in 'TMS 30572' (Figure 2).



**Figure 1** Effect of *Curvularia lunata* (Wakker) Boed. stem infection on field germination of cassava genotypes in two localities in Nigeria; ■, Anusho, □, Kotopo

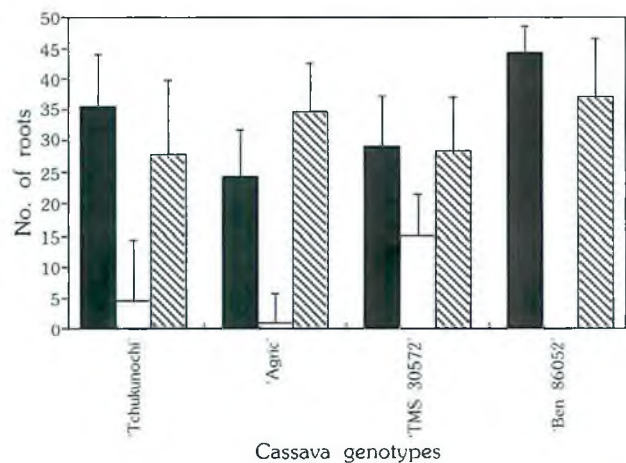


**Figure 2** Leaf abscission of cassava genotypes inoculated with *Curvularia lunata* (Wakker) Boed.; ■, control, □, inoculated

***Nattrassia mangiferae***

A total of 201 samples of wilted and (or) dead plants were collected (from the two countries) for laboratory analysis. Out of 169 symptomatic samples collected from Benin, 9 fungal genera were isolated: *Aspergillus* spp. (1% of fungi observed), *Botryodiplodia theobromae* Pat (7.7%), *Fusarium* spp. (11.8%), *Macrophomina phaseolina* (Tass) Goidanich (14.2%), *N. mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko (56.2%), *Penicillium* spp. (0.6%), *Pythium* spp. (2.9%), *Rhizopus* spp. (1.7%), and *Trichoderma* spp. (2.4%). One per cent of the fungi isolated did not sporulate in culture and was not identified. Out of the 32 samples collected from Nigeria, 4 fungal genera were identified: *N. mangiferae* (40.6%), *B. theobromae* (28.1%), *M. phaseolina* (18.7%), and *Fusarium* spp. (12.5%).

For all four cultivars, *N. mangiferae* significantly reduced plant height and number of shoots and roots compared to control plants (Figure 3). Lesions (3–15 cm long) formed on



**Figure 3** Root formation and disease development of cassava genotypes inoculated with *Nattrassia mangiferae* (Syd. & P. Syd.) B. Sutton & Dyko in a greenhouse; ■, control, □, inoculated, ▨, lesion

the lower stem portions of all inoculated plants, resulting in variable degrees of wilting of the infected plants. Two of the cultivars (Agric and Ben 86052) died three weeks after planting. Control plants remained asymptomatic. *Nattractia mangiferae* was consistently re-isolated from infected plants, and the identification was independently confirmed by the International Mycological Institute, Surrey, U.K.

## Discussions

In all three countries surveyed, *C. lunata* stem infection always originated on buds located on the lignified stem portions suggesting that the fungus may be using the polymer lignin as part of its food base. The significant variability in field incidence of *C. lunata* may suggest variability in cultivation practices, whereby use of infected cuttings, invariably leads to increased field incidence. Such cuttings may be acting as focal points for plant-to-plant spread within the infected fields, and possibly to neighbouring fields. Spread of disease from infected fields to neighbouring fields may also explain the clustering nature of spread in all three countries, and further suggest that farmers use similar cultivation systems, and (or) share planting material with their neighbours. In Benin, the incidence and severity of the disease was lowest compared to Ghana and Nigeria which may suggest that farmers select and plant clean cuttings.

The significant variability in disease severity among fields could be related to the age of the crop, and (or) cultural practice employed. Differences in severity of the disease among fields may also be related to resistance or susceptibility in cassava genotypes. This fact is well illustrated in the germplasm collection at Bunso (Ghana) where 104 accessions were planted in the same field, and only 19 were symptomatic. The significant variability in disease severity may also be partially related to differences in environmental conditions because any observable disease is an interaction of the pathogen, the host, and the environment (Bateman, 1978).

Interestingly, the germplasm collection in Benin was free of the disease although the accessions in the collection were obtained from farmers all around the country. Lack of disease in the collection suggests that clean cuttings were planted, and is a further indication that use of infected cuttings is the quickest way to spread the disease.

Field assessment for *C. lunata* leaf damage was difficult because symptoms produced by *C. lunata* were indistinguishable from those produced by *Cercospora* blight. Thus, leaf infection was only shown under artificial conditions in the greenhouse, and revealed that impact on leaves may be quite significant considering the percentage of leaf abscission. Cultivars varied significantly in the percentage of leaf abscission

suggesting possible cultivar variability in resistance to *C. lunata* leaf infection.

On the stem, complete colonization of buds by the fungus completely inhibited germination of the affected buds in the greenhouse, suggesting that the buds were killed. On the other hand, when buds were partially colonized, growth was significantly lower than that for non-colonized buds, suggesting that *C. lunata* impairs affected shoots. Similarly, artificially inoculated stems consistently grew less than the non-inoculated (control) stems suggesting that infection by *C. lunata* weakens affected buds. In the field, however, where moisture availability may be erratic, and the stems exposed to other stressful factors (such as root-rot pathogens), germination of stems infected by *C. lunata* was significantly less than the non-infected stems, regardless of the locality and (or) degree of fungal colonization of the stem buds. This observation may also suggest that *C. lunata* physically weakens infected stems.

*Nattractia mangiferae*, formally *Hendersonula toruloidea* (Sutton and Dyko, 1989) has a wide host range including fruit trees (Calavan and Wallace, 1954; Reckhaus and Adamou, 1987), banana (Meredith, 1963), eucalyptus (Matheron and Sigler, 1994), and man (Obasi and Clayton, 1989; Summerbell *et al.*, 1989). On cassava, the synamorphic arthric state (*Scytilidium* sp.) has been reported to cause up to 85% root yield loss in north-east Brazil (Anon., 1992). Extensive surveys completed for Benin showed that the fungus is the most commonly isolated root rot pathogen of cassava  $\leq 3$  months of age. Pathogenicity studies revealed that the fungus is very virulent on cassava. These observations necessitate developing effective and sustainable control practices for *N. mangiferae* in West Africa.

This study has shown that *C. lunata* is present in Benin, Ghana, and Nigeria, and that the fungus is a primary pathogen of cassava stem and possibly leaf blight. On stems, the disease reduced the ability of infected stems to germinate, while on the leaf, the fungus may cause significant leaf abscission in susceptible cultivars. *Nattractia mangiferae* was present in all surveyed parts of Benin and Nigeria, and was a primary pathogen responsible for germination failure in cassava  $\leq 3$  months of age.

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# Effect of foliage removal on root yield, dry matter, and proximate composition of five sweetpotato genotypes in Ghana

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Preliminary studies were conducted in Ghana in 1995 to identify high-yielding sweetpotato genotypes suitable for foliage removal without reducing root yield. Five genotypes were selected based on yield performance and nutrient content. These genotypes were ITS2, 91/198, and 83/123 which are improved genotypes from the University of Ghana, and 'Agbeyeye' and 'Damadami' which are two local genotypes currently cultivated by the farmers in the Akatsi District. These genotypes were used to test the effect of foliage removal on root yield, dry matter, and proximate composition of sweetpotato in two villages in the Akatsi District of the Volta region of Ghana. Results from the data indicated that there were significant differences between cutting treatments. However, genotypes tested showed variation in yield response for all root grades. There were significant genotypic differences as well as yield differences in location and in proximate composition. 'Agbeyeye' and 'Damadami' showed a better adaptation (based on visual observations), higher root yields, and proximate compositions and, therefore, would be ideal for consideration for foliage removal.

Keywords: Sweetpotato genotypes; Foliage removal; Root yield; Nutrient content; Ghana

Sweetpotato is an important crop in many countries of the world. The FAO (1990) reported that approximately of 137 M t were produced between 1975 and 1977. China accounts for over 80% of the production and is the world's principal producer. Indonesia, Vietnam, Uganda, India, Brazil, Philippines, and Korea produce most of the remaining 20% and yields recorded from 1986 to 1988 were between 596 000 t to 108 M t (Scott, 1991). Sweetpotato ranks sixth among food crops in Africa and third among root and tuber crops in Ghana (Doku, 1988), but, like many West African countries, the adverse socio-economic situations in Ghana have reduced the food and nutritional status of the rural people. Their women and children, in particular, often suffer from the combined effects of iron and vitamin deficiency which could be linked to the lack of consumption of green leafy and deep orange flesh vegetables. Because sweetpotato is relatively easy to cultivate, is drought-tolerant, nutritious, and available all year round, it can play a major role in helping to minimize the food and nutrient deficiency problems in Ghana.

Although sweetpotato is an important crop in West Africa, it is produced on a relatively small scale (Alvarez, 1992). Cassava (*Manihot esculenta*) is a popular staple while sweetpotato is considered as a good food security or famine prevention crop and an insurance against

drought. Sweetpotato is consumed until the more traditional crops become available (Doku, 1988).

Sweetpotato leaves are a source of vitamin A, iron, and protein and they could serve as a good supplement to the already known and consumed green leafy vegetables. They also enable farmers to have a two-in-one cash crop to provide extra income towards the upkeep of their families. This study was conducted to determine if foliage removal could have an adverse effect on the root yield, dry matter, and proximate composition of five sweetpotato genotypes in Ghana.

## Methodology

Five sweetpotato genotypes including three improved genotypes (ITS2, 91/198, and 82/123) from the University of Ghana collection and two local genotypes ('Agbeyeye' and 'Damadami') currently cultivated by the farmers in the Akatsi district in the Volta region of Ghana were grown at four locations in the Akatsi district between March and October 1996 to coincide with the major farming season in the year for the area.

The experimental design was a  $2 \times 5 \times 4$  factorial arrangement using a randomized complete block design with four replications.



Land preparation involved ploughing and harrowing followed by manual bed preparation. Fifteen-centimetre vine cuttings were planted on the raised beds which were 105 cm wide and 360 cm long. The spacing was 30 cm between and within plants.

Foliage removal consisted of cutting foliage of 15 cm length from all the terminal ends of the plant and a control where no foliage was removed. Cutting treatments were initiated 42 days after planting (DAP) and continued thereafter at 30-days intervals until final harvest which occurred at 120 DAP. At final harvest, roots were graded according to current market grades in Ghana. Five roots were selected at random from each treatment and analysed for nitrogen using the Kjeldahl procedure and for moisture, fat, ash, carbohydrate, and dry matter using AOAC methods.

The data collected were analysed by the statistical package SAS (SAS, 1985) and the Tuskegee University Time Share ACDVAX computer system.

### Results and Discussion

The effect of foliage removal, genotype, and location on sweetpotato root yield is shown in Table 1. The results showed that cutting treatments significantly affected sweetpotato yield. Plants with no foliage removed resulted in higher total yield and canner grades than plants

where foliage was removed throughout the season. This suggests foliage removal may have caused a reduction in the total leaf surface area available for light interception which may have resulted in a reduction in photosynthates produced, thus lowering root yields. Also, foliage removal could have coincided with the active period for storage root formation.

The genotypes tested differed significantly for all root grades. 'Agbeyeye' and 'Damadami' were the highest yielding genotypes. Based on visual observations, both showed vigorous vegetative growth throughout the growing period compared to the improved genotypes from the University of Ghana collection. However, the improved genotypes, although they have shown higher yields in other regions of Ghana, (unpubl. data) they were found to be susceptible to viral infection. The yield performance of the two local genotypes showed they may have been more adapted to the soil environment and more resistant to the mosaic virus.

The soil at location 4 had a high clay content which may have restricted root growth and development and may have resulted in reduction in yield. The pH for all the locations were about the same; however, the nitrogen, phosphorus, potassium, and organic matter content were high in locations 1 and 2 which may have contributed to the high yields compared to the other locations.

The analysis of variance for root yield is presented in Table 2. Significant first order interactions were found in the jumbo and canner grades for genotype and location as well as treatment by location. There was a second order interaction in the genotype by treatment by location for canner grades. Proximate composition of sweetpotato roots were also affected by foliage removal, genotype as well as location (Table 3). Plants where foliage were removed

**Table 1** Effect of foliage removal, genotype, and location on sweetpotato root yield

Treatment	Total yield	Grades		
		Jumbo	US #1	Canners
----- t ha <sup>-1</sup> -----				
<b>Cutting treatment</b>				
Foliage removed	20.09	5.47	10.11	4.52
No foliage removed	24.71	7.39	11.26	6.06
LSD <sub>0.05</sub>	2.72	2.11	1.52	0.87
<b>Genotypes</b>				
Agbeyeye	44.27	16.43	18.83	9.01
Damadami	29.48	6.13	14.37	8.97
91/198	20.56	6.77	9.99	3.80
82/123	10.68	2.37	5.91	2.27
ITS 2	10.68	2.37	5.91	2.40
LSD <sub>0.05</sub>	ns	ns	ns	ns
<b>Location</b>				
1	33.93	16.14	12.80	4.99
2	25.09	9.57	12.47	3.05
3	16.74	0.00	8.94	7.80
4	13.85	0.00	8.52	5.34
LSD <sub>0.05</sub>	ns	ns	ns	ns

ns is Not significant

**Table 2** Analysis of variance for root yield (t ha<sup>-1</sup>)

Source	df	Total yield	Grades		
			Jumbo	US #1	Canners
----- Mean square -----					
Genotype (G)	4	7242.50**	1220.94**	1148.56**	377.13**
Treatment (Trt)	1	852.50	147.90*	53.30	94.66**
G × Trt	4	164.59	37.17	19.55	22.55
Rep (G × Trt)	30	80.30	39.64	32.35	5.91
Location (L)	2	2817.04**	2491.26**	205.72**	151.96**
G × L	12	303.88**	443.29**	35.08	19.04**
T × L	3	51.39	91.66	46.93	39.76**
G × Trt × L	12	51.39	27.99	25.36	6.27*
Error	90	128.48	24.99	17.41	8.04

\*, \*\*, Significant at P < 0.05 and P < 0.01, respectively

**Table 3** Effect of foliage removal, genotype, and location on proximate composition of sweetpotato roots

Treatment	Dry matter	Protein	Fat	Ash	Carbohydrate
	----- % -----				
Cutting treatment					
Foliage removed	30.98	1.03	0.11	0.39	18.84
No foliage removed	30.23	1.08	0.22	0.37	17.59
LSD <sub>0.05</sub>	ns	0.01	0.038	0.01	0.10
Genotypes					
Abgeyeye	30.69	1.26	0.25	0.39	17.90
Damadami	30.73	1.22	0.69	0.39	17.60
91/198	31.35	0.97	0.13	0.39	18.76
82/123	31.13	0.97	0.29	0.42	17.60
ITS 2	28.95	1.26	0.08	0.32	19.23
LSD <sub>0.05</sub>	ns	0.01	0.06	0.002	0.17
Location					
1	31.2	0.97	0.11	0.11	18.66
2	30.06	1.19	0.27	0.27	18.23
3	31.00	1.03	0.12	0.12	17.47
4	30.11	1.03	0.15	0.15	18.51
LSD <sub>0.05</sub>	ns	0.01	0.05	0.05	0.15

Mean separation at 5% level utilizing LSD test

showed increases in moisture, ash, and carbohydrate and decreases in protein and fat contents. All the genotypes differed significantly for the protein, fat, ash, and carbohydrate contents of the storage roots. However, ITS2 recorded the highest carbohydrate content among all the genotypes although it had recorded lowest root yields. Also, the dry matter content in 91/198, and 82/123 were highest among the genotypes and the protein content were relatively high in the local genotypes as well as ITS2.

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# Cassava plant population and leaf harvesting effects on the productivity of cassava-rice intercrop on the upland in Sierra Leone

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An experiment was conducted to determine the appropriate cassava plant population to be intercropped with rice and the time of first harvesting of the cassava leaves for improved productivity of the intercrops. The cassava variety 80/40 was planted at four plant populations (3333, 6666, 10 000, and 13 333 plants ha<sup>-1</sup>) in mid-May of 1996 on the upland in Sierra Leone. The cassava was intercropped with the rice variety Rokl6 at a spacing of 25 cm × 25 cm one month later. For each of the cassava populations, there were four leaf harvesting regimes: no leaf harvesting and first leaf harvesting at two, three, and four months after planting (MAP). Subsequent leaf harvesting was done after every two months. A total of three leaf harvests were carried out for each regime. The results revealed that root and leaf yield of cassava did not significantly increase after 10 000 plants ha<sup>-1</sup>. Grain yield of rice decreased as intercropped cassava plant population was increased from 6666 to 13 333 plants ha<sup>-1</sup>. Harvesting the cassava leaves at 2 and 3 MAP which was at mid-vegetative stage and panicle initiation stage of intercropped rice, respectively, resulted in similar rice grain yield which was significantly higher than when first leaf harvesting was at 4 MAP cassava, which was at late flowering stage of the rice. Tuberos root yield of cassava increased as the time of first leaf harvesting was delayed from 2 to 4 MAP. The highest net return was obtained when rice was intercropped with 10 000 cassava plants and leaf harvesting started at 3 MAP.

Keywords: Cassava; Leaf harvest; Cassava-rice; Productivity; Sierra Leone

In many countries in Africa particularly in West and Central Africa, cassava is grown for both the leaves and the tuberous roots. The leaves are used as vegetables while the tuberous roots can be boiled and eaten directly or processed into food products like *gari* and *fofofo*, and other products like starch and alcohol. In Africa, cassava is commonly grown in association with other crops. According to the 1984-85 census of Agriculture of Sierra Leone, about 70% of the total area under cassava is in mixture with other crops (Government of Sierra Leone, 1986).

Adetiloye (1985) reported that the yield advantage of intercropping has not been so marked in several situations possibly due to the use of either superoptimal or suboptimal plant population for component crops. Jalloh (1995) reported that optimum cassava plant population for intercropping with rice was 6666 plants ha<sup>-1</sup> and that increasing the cassava plant population beyond 6666 cassava plants ha<sup>-1</sup> decreased Land Equivalent Ratio and net returns. This recommendation was, however, based on intercropping rice and cassava without harvesting the leaves of cassava. The progressive decrease in the grain yield of intercropped rice was attributed to the increase in shade as a

result of the increase in cassava plant population in the mixture (Jalloh *et al.*, 1995).

The leaves of cassava are commonly harvested for home consumption and market. Osiname and Landu (1992) reported that farmers started harvesting the leaves when the plants are about three months old and that income from leaf harvests can reach 70-100% of revenue from fresh root sales depending on the cassava clone. The harvesting of cassava leaves which are usually above the rice reduce the shade on the intercropped rice. This may provide the opportunity to further increase the intercropped cassava plant population beyond the 6666 plants ha<sup>-1</sup> identified by Jalloh *et al.* (1995) without any adverse effect on the rice yield.

The time of first harvesting the leaves relative to the developmental stage of the rice is also crucial in determining both the productivity of the cassava and the rice. Yoshida and Paraw (1987) reported that shading during the vegetative stage had little effect on grain yield, whereas, shading during the reproductive stage had even greater effect than during grain ripening. This study was, therefore, conducted to determine the appropriate cassava plant population and time of first harvesting of the cassava

leaves for improved productivity of the rice-cassava intercrop system on the upland of Sierra Leone.

## Materials and Methods

This experiment was conducted during the 1996-97 cropping season at the experimental site of the Institute of Agricultural Research at Rosinth village in the northern Province of Sierra Leone. The village is near the coast and like the rest of the country, experiences a distinct dry and wet season. The wet season starts in May and extends to October while the dry season starts in November and lasts until April. Mean annual rainfall is between 3500 mm and 4500 mm while the average daily temperature ranges between 20 and 30°C. The soil in the experimental area was sandy loam while the pH (water) and organic matter content were 5.3 and 5.39%, respectively.

The experiment consisted of two factors: (i) cassava plant population which had four levels (3333, 6666, 10 000, and 13 333 plants ha<sup>-1</sup>) and (ii) time of first harvesting the cassava leaves which consisted of no leaf harvesting, and first leaf harvesting at two, three, and four months after planting (MAP). Harvesting the cassava leaves at 2, 3, and 4 MAP coincided with the vegetative period, panicle initiation, and flowering stages of rice, respectively. The treatments comprised each of the cassava plant populations combined with each of the leaf harvesting regimes.

The experimental area which had not been cropped for at least five years was first brushed and the dried vegetation burnt. Most of the stumps were then uprooted. Individual plot size measured 16 m × 4 m (64 m<sup>2</sup>). The treatments were laid out in a randomised complete block design and replicated three times.

The cassava was planted in mid-May on small mounds. Planting was in rows with a distance of 1 m between rows while the distance between hills within a row was determined by the respective plant population with a spacing of 3 m, 1.5 m, 1 m, and 0.75 m to obtain 3333, 6666, 10 000, and 13 333 plants ha<sup>-1</sup>, respectively. Rice was dibbled at a spacing of 25 cm × 25 cm one month after planting the cassava. Three rice seeds were planted in each hole. As the cassava plant population was increased from 3333 to 13 333 plants ha<sup>-1</sup>, the intercropped rice population was correspondingly reduced.

At each cassava leaf harvesting, the entire plants of each plot were detopped at about 25 cm to 30 cm below the apex of the branch of each plant. The fresh weight of the entire harvest per plot was then recorded. Also, at each harvest, the price of the fresh leaves was monitored in the local market to determine the value of the leaves. After the first harvest, the interval between subsequent harvests was two

months. A total of three harvests were carried out for each harvesting regime.

The rice was harvested when about 90% of the grains per panicle were yellow. In each plot, an area of 20 m<sup>2</sup> demarcated by a string was used as the harvesting area to determine grain yield. It was originally intended to harvest the cassava roots at 12 MAP, but harvesting was done at 8 MAP due to fear of plundering. A total of 16 guarded plants were harvested in each plot to determine cassava root yield. The prices of both rice and the cassava were also monitored at the market at the time of harvesting.

## Results

### Cassava leaf yield

The first and second leaf harvests on the plants which were first harvested at 2 and 3 MAP were carried out in the rainy season (July to October). It was only the first harvest of the plants that had its leaves first harvested at 4 MAP that occurred in the rainy season (September). The second harvest was carried out in the dry season (November) (Table 1). For all regimes, the third leaf harvest was done in the dry season, (November, December, and January, respectively, for the plants with first leaf harvest at 2, 3, and 4 MAP).

At the first leaf harvest, cassava that had its leaves first harvested at 4 MAP produced the highest leaf yield, and the leaf yield significantly increased as the cassava plant population increased from 3333 to 13 333 plants ha<sup>-1</sup>. Leaf yield of the plants first harvested at 2 and 3 MAP produced similar yields which did not significantly vary with the cassava plant population (Table 1).

At the second harvest, which took place in September and October after first harvesting in July and August, respectively, the two lower plant populations (3333 and 6666 plants ha<sup>-1</sup>) produced similar leaf yield while the two higher plant populations (10 000 and 13 333 plants ha<sup>-1</sup>) also produced similar leaf yield. At the second harvest (November) of the plants that were first harvested at 4 MAP (September), leaf yield did not significantly vary with plant population.

At the third leaf harvest which took place in the dry season (November, December, and January for the plants first harvested at 2, 3, and 4 MAP, respectively) leaf yield did not interact with cassava plant population and time of first leaf harvest (Table 1). However, leaf yield significantly varied with both plant population and time of first harvesting the leaves. Leaf yield significantly increased from 3333 to 10 000 plants ha<sup>-1</sup>. First leaf harvesting at 2 and 3 MAP produced similar leaf yield which was significantly higher than leaf yield of plants first harvested at 4 MAP.

**Table 1** Leaf yield of cassava intercropped with rice as affected by cassava plant population and time of first harvesting the leaves

Time of first leaf harvest (MAP)	Month of harvest	Cassava plant population (plants ha <sup>-1</sup> )				Mean
		3333	6666	10 000	13 333	
<b>First harvest</b>						
2	July	16.7 d	43.3 d	123.3 cd	73.3 cd	64.2 b
3	August	76.7 cd	150.0 cd	260.0 cd	203.3 cd	172.5 b
4	September	340.0 c	680.0 b	953.0 b	1466.7 a	860.0 a
Mean		144.4 c	291.1 bc	445.6 ab	581.1 a	
<b>Second harvest</b>						
2	July	223.3 d	426.7 cd	993.3 ab	1080.0 a	705.8 a
3	August	293.3 de	436.7 cd	883.3 ab	683.3 bc	574.2 a
4	September	0.5 e	0.7 e	1.5 e	1.5 e	1.1 b
Mean		205.7 b	288.0 b	626.1 a	588.3 a	
<b>Third harvest</b>						
2	July	0.4	0.7	1.1	1.6	0.9 a
3	August	0.5	0.9	1.6	1.2	1.1 a
4	September	0.3	0.4	0.9	0.8	0.6 b
Mean		0.4 c	0.7 b	1.2 a	1.2 a	

For each set of means, those with the same letter(s) are not significantly different from each other at LSD<sub>0.05</sub>.

### Cassava tuberous root yield

Tuberous root yield of cassava was significantly affected by both cassava plant population and the time of first harvesting the cassava leaves (Table 2). Tuberous root yield of cassava significantly increased as cassava plant population increased from 3333 to 10 000 plants ha<sup>-1</sup>. The 10 000 and 13 333 plants ha<sup>-1</sup> produced similar root yield. Cassava without leaf harvest pro-

duced significantly higher root yield than those with leaf harvest. Starting leaf harvest at 3 and 4 MAP resulted in the production of similar root yield, but these yields were significantly higher than root yield produced by cassava that had its leaves first harvested at 2 MAP (Table 2).

### Rice grain yield

Intercropped cassava plant population and leaf harvesting regime significantly affected the grain yield of rice (Table 2). Rice intercropped with 3333 and 6666 plants ha<sup>-1</sup> produced similar grain yield. Grain yield of rice significantly decreased as intercropped cassava plant population was increased from 6666 to 13 333 plants ha<sup>-1</sup>. Rice that was intercropped with cassava that had its leaves first harvested at 2 and 3 MAP produced similar grain yield. Rice intercropped with cassava without leaf harvesting and that intercropped with cassava that had its leaves harvested at 4 MAP produced similar grain yield (Table 2).

### Revenue from intercropped rice and cassava

At the lower cassava plant populations (3333 and 6666 plants ha<sup>-1</sup>), the revenue from intercropped rice was higher than the revenue obtained from the intercropped cassava (both leaves and roots) while at the higher cassava plant populations (10 000 and 13 333 plants ha<sup>-1</sup>) the reverse was true. The revenue from rice decreased as cassava plant population increased, while at each cassava plant population, the highest revenue was obtained from rice

**Table 2** Intercrop yields (t ha<sup>-1</sup>) of cassava roots and rice grain as affected by cassava plant population and time of first harvesting the cassava leaves

Cassava plant population (plants ha <sup>-1</sup> )	Time of first leaf harvesting (months after planting cassava)				Mean <sup>††</sup>
	0 <sup>†</sup>	2	3	4	
<b>Tuberous root yield (t ha<sup>-1</sup>)</b>					
3 333	3.3	1.4	1.5	2.2	2.1 c
6 666	6.2	2.5	3.0	3.6	3.8 b
10 000	7.9	4.8	6.8	7.6	6.8 a
13 333	8.6	5.4	5.8	6.5	6.6 a
Mean <sup>††</sup>	6.5 a	3.5 c	4.3 b	5.0 b	
<b>Grain yield (t ha<sup>-1</sup>)</b>					
3 333	1.5	1.7	1.8	1.6	1.7 a
6 666	1.3	1.5	1.6	1.4	1.5 a
10 000	1.0	1.3	1.5	1.1	1.2 b
13 333	0.7	1.0	1.1	0.8	0.9 c
Mean <sup>††</sup>	1.1 b	1.4 a	1.5 a	1.2 b	

<sup>†</sup>No leaf harvest

<sup>††</sup>For each set of means, all those carrying the same letter(s) are not significantly different from each other at LSD<sub>0.05</sub>.

intercropped with cassava that had its leaves harvested at 3 MAP.

The revenue from cassava leaves generally increased as the intercropped cassava plant population increased. Gross and net revenue increased from 3333 to 10 000 cassava plants ha<sup>-1</sup> and the highest revenue was obtained when rice was intercropped with 10 000 cassava plants ha<sup>-1</sup> and leaf harvesting started at 3 MAP.

## Discussion

In many countries of Africa, cassava is increasingly being grown for both the tuberous roots and the leaves. Osiname and Landu (1992) reported that farmers start harvesting the leaves when the plants are about three months old and that there are usually three leaf harvests on each crop before root harvest. In this study, the third leaf harvest produced very low yields as did the second harvest after the first harvest at 4 MAP. These very low yields were all obtained from dry season harvests. The lack of rain during this season could account for such low yields.

The root yield of cassava increased from 3333 to 10 000 plants ha<sup>-1</sup>. Increasing the cassava plant population to 13 333 plants ha<sup>-1</sup> did not increase root yield further. Dahniya *et al.* (1994) reported similar results when the intercropped cassava leaves were not harvested. Leaf yield also did not significantly increase beyond 10 000 plants ha<sup>-1</sup>. It may, therefore, not be economical to increase the intercropped cassava plant population beyond 10 000 plants ha<sup>-1</sup>.

Harvesting the cassava leaves significantly reduced the root yield of the cassava. Osiname and Landu (1992) reported that leaf harvesting significantly reduced fresh root yield, wherein root yield fell by 35% without fertilizer and only 10% with it. In this study, the most severe reduction (46%) was when first leaf harvesting was done at 2 MAP. The effect of leaf harvesting declined from 34 to 23% as first leaf harvesting was delayed from 3 to 4 MAP, respectively. These results are therefore suggesting that for improved root yield, cassava leaf harvesting should not be done before 3 MAP.

Even though leaf harvesting reduced the yield of cassava, the practice significantly increased the yield of intercropped rice. Jalloh (1995) observed that shading of the rice by cassava was probably the major factor in reducing grain yield of intercropped rice since the cassava was taller than the rice throughout their period of association. In this study, the grain yield of rice decreased as the intercropped cassava plant population increased from 6666 to 13 333 plants ha<sup>-1</sup>. Since the experiment involved replacing equal hills of rice by cassava, the increase in the cassava population in the first instance led to a direct reduction in the population of rice and consequently reducing the final

yield of the rice. Increasing the number of cassava plants also may have caused an increase in both intra- and inter-specific competition for the growth factors.

Doku (1985) reported that under mixed or multiple cropping situations, several competitive forces of the atmosphere and rhizosphere are brought into play. The most important is that of shade from the neighbouring plants growing in association. Dahniya (1985) observed that the shading effect of the new cassava clones had adverse effects on the productivity of the crops in the mixture. The improved cassava variety 80/40 used in this experiment branches profusely. Moreover, based on the recommendation of Jalloh *et al.* (1995), the cassava was planted one month before the rice. This resulted in the cassava canopy being taller than that of the rice. Trebanth (1974) had earlier concluded that from all experiments involving competition for light, the component with its leaf area higher in the canopy is at an advantage. In this experiment, therefore, increasing the cassava population not only reduced the rice population, but the amount of shade above the rice also increased, and this could have affected the grain yield of the intercropped rice.

There was an increasing reduction in the grain yield of rice as cassava plant population was increased. Increasing cassava plant population from 3333 to 6666 plants ha<sup>-1</sup> caused a reduction of 12% in the yield of rice while increasing the cassava plant population from 6666 to 10 000 plants ha<sup>-1</sup> and then to 13 333 plants ha<sup>-1</sup>, caused a reduction of 20 and 25%, respectively, in rice grain yield.

Harvesting of the cassava leaves significantly affected the grain yield of the intercropped rice. Harvesting the leaves at 2 MAP cassava removed the shade over the intercropped rice at the tillering stage while harvesting the leaves at 3 MAP meant that the rice was shaded throughout the vegetative stage and the shade removed at the panicle initiation stage. However, the rice yields obtained were similar for the two harvesting regimes. These results suggest that leaving the cassava leaves during the vegetative state of the rice may not be detrimental to the crop.

When cassava leaf harvesting was delayed until at 4 MAP, the rice was shaded up to the late flowering stage of the rice. The yield obtained from the rice was similar to that obtained from rice intercropped with cassava without leaf harvest. The grain yield was significantly lower than the yield obtained from rice, where the shade of the cassava leaves was removed at both tillering and panicle initiation stages. These results, therefore, suggest that leaving the cassava leaves without harvest up to the reproductive stage of rice is detrimental to the productivity of the crop.

These results are similar to those of Yoshida and Paraw (1987) when the rice variety

TR747-B2-6 was shaded to various degrees for periods of 25 days during the vegetative, reproductive, and ripening stages. Shading during the vegetative stage had little effect on grain yield, whereas, shading during the reproductive stage had even greater effect than during grain ripening. The results of this study, therefore, seem to suggest that in order to minimise the effect of the shade of cassava on intercropped rice, the cassava leaves should be harvested between one and two months after planting the rice.

Jalloh (1995) reported that without leaf harvest the highest net return was obtained when rice was intercropped with 6666 plants ha<sup>-1</sup> and that shading by cassava was probably the major factor in reducing grain yield of intercropped rice. The attainment of optimum net return at 10 000 in this present study could therefore be attributed to the improvement in rice yield due to removal of shade and the additional income from the leaves. The results of this study, therefore, suggest that cassava leaf harvesting is beneficial to the intercropped rice by way of reducing the shade above the rice and also by increasing the income through the sale of the leaves. Leaf harvesting also allows a higher cassava plant population in the intercrop. Thus, higher net returns could be obtained from the rice-cassava intercropping system by intercropping rice with 10 000 plants ha<sup>-1</sup> and first harvesting the leaves at 3 MAP.

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# Variations in root and foliage yields and quality among green mite-resistant cassava clones

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The objective of this study was to determine variations in root and foliage yields, crude protein (CP), and dry matter (DM) degradation characteristics of foliage among 25 cassava clones selected for green mite resistance at Ibadan, south-western Nigeria. Clones were sampled 12 months after planting during the 1994-95 and 1995-96 growing seasons to determine root and foliage yields. Foliage samples were analysed for CP and degradation characteristics of DM in rumen-fistulated N'dama (*Bos indicus*) steers using the nylon bag method. Root yield ranged from 1.26 t DM ha<sup>-1</sup> in clone 92/0397 to 7.67 t DM ha<sup>-1</sup> in clone 91/02322, and foliage yield from 0.18 t DM ha<sup>-1</sup> in clone 91/02316 to 2.21 t DM ha<sup>-1</sup> in clone 92/0429. Crude protein varied from 248 g kg<sup>-1</sup> DM in clone 92/0397 to 303 g kg<sup>-1</sup> DM in clone 92/0067. Variations in the soluble (142-325 g kg<sup>-1</sup>) and degradable (519-740 g kg<sup>-1</sup>), fractions, potential degradability (783-933 g kg<sup>-1</sup>), effective degradability (474-575 g kg<sup>-1</sup>), and rate of degradation (0.0173-0.0565% h<sup>-1</sup>) among clones were significant. Root yield was poorly correlated with foliage yield, CP, and effective degradability of foliage quality. Using root and foliage yields and effective degradability of DM as indices for root and foliage production, some clones, (91/00438, 91/002322, 92/0057, 92/0398, and 92/0427) appeared to have higher potential than others, (89/00250, 91/02312, 91/02319, and 92/0397). The results indicate that both agronomic and nutritive value indices of the root and foliage should be considered as selection criteria in future cassava improvement programmes in developing countries.

Keywords: Cassava; Root and foliage yields; Quality; Green mite; Degradation characteristics

Cassava (*Manihot esculenta* Crantz) is a staple food for millions of people in the tropics. The tuberous root is a good source of carbohydrates and the foliage provides an inexpensive and rich source of protein in human and livestock diets (Devendra, 1977; Smith, 1992). Therefore, selection of dual-purpose genotypes with potential for root and foliage production could increase the utilization of the crop, especially in smallholder crop-livestock systems in developing countries where protein is a major limiting nutrient in both human and livestock diets.

Despite the potential of cassava as a dual-purpose crop for root and foliage production, very little research has been done on the foliage component in cassava improvement programmes in the tropics. As a result, data on variation in root and foliage yields and foliage quality among cassava clones that are essential for selection or breeding of dual-purpose geno-

types are scanty (Lutaladia, 1994). In an attempt to increase the utilization of cassava in smallholder crop-livestock systems in sub-Saharan Africa, a collaborative research project between the International Livestock Research Institute (ILRI) and the International Institute for Tropical Agriculture (IITA) was initiated in 1994 to select dual-purpose cassava clones, and to develop indices of foliage quality that could be used in cassava improvement programmes.

This study was undertaken to assess variations in root and foliage yields, crude protein (CP) content, and rumen degradation characteristics of dry matter (DM) in foliage among 25 diploid cassava clones. These clones were selected for resistance against the cassava green mite (*Mononychellus tanajoa*), an important pest in sub-Saharan Africa, causing up to 40% reductions in yield of fresh roots in susceptible varieties (Nyira, 1976).



## Materials and Methods

### Location

The experiment was conducted at IITA, Ibadan (7°30' N, 3°54' E), in the derived savannah zone of south-western Nigeria. The site has a subhumid climate with a bimodal rainfall pattern. The main wet season extends from May to mid-August and the minor wet season from September to November. Total annual rainfall averages 1250 mm. Mean monthly maximum and minimum temperatures are 32 and 24°C, respectively.

### Soil

The soil is an Alfisol. The experimental soil in 1994-95 contained 91, 4, and 5% sand, silt, and clay, respectively, with a pH (H<sub>2</sub>O) of 6.5, 6.3 g kg<sup>-1</sup> organic C, 0.6 g kg<sup>-1</sup> total N, and 11.0 mg kg<sup>-1</sup> available P (Bray 1). In 1995-96, the experimental soil contained 83, 9, and 8% sand, silt, and clay, respectively, with a pH (H<sub>2</sub>O) of 6.2, 16.3 g kg<sup>-1</sup> organic C, 1.52 g kg<sup>-1</sup> total N, and 32.7 mg kg<sup>-1</sup> available P (Bray 1).

### Root and foliage yields

Treatments were 25 cassava clones planted on 25 May in 1994-95 and 24 May in 1995-96. A compound fertilizer (N:P:K; 15:15:15) was applied at 400 kg ha<sup>-1</sup>, eight weeks after planting in each year. The experiment was laid out as a randomized block design with four replications. Each plot was 10 m long and 4 m wide, with 4 rows of plants spaced at 1 m × 1 m.

Plants were harvested 12 months after planting by uprooting manually the two middle rows in each plot, and weights of the fresh roots were recorded. Sub-samples of the roots were oven-dried at 80°C for 72 h to determine DM content. Foliage yield was estimated from four stands randomly selected from each of the two outer rows in each plot. The number of stems per stand was counted. The stems were harvested at 0.10 m above the ground, weighed, and separated into foliage (leaves + stems <10 mm in diameter) and stem. The stem and foliage components were weighed separately. Sub-samples of the foliage were weighed and oven-dried at 60°C for 48 h for DM determination.

### Crude protein

Oven-dried foliage sub-samples from the 1995-96 harvest were divided into two portions. One portion was ground to pass through a 1-mm screen to determine total N using the Kjeldahl method (AOAC, 1990). Crude protein was calculated as 6.25 × N. The remaining portion was ground to pass through a 2.5-mm screen to determine DM degradation characteristics.

### In sacco degradation

Dry matter degradation characteristics were determined using the nylon bag technique (Orskov *et al.*, 1980) in three rumen-fistulated N'Dama (*Bos indicus*) steers, aged three years with an average live weight of about 250 kg. The steers grazed *Panicum maximum* and were supplemented with wheat bran at a daily rate of 2 kg animal<sup>-1</sup>. They were housed in individual pens with free access to water and mineral salts. About 5 g of the ground sample were weighed into nylon bags measuring 0.09 m × 0.18 m with a pore size of 41 µm (Polymon, Switzerland). The bags were incubated in duplicates in the rumen for 6, 12, 24, 48, 72, and 96 h. After incubation, the bags were washed under running tap water until the rinse water was clear, and oven-dried at 60°C for 48 h to estimate DM loss.

Dry matter degradation constants were estimated by fitting the data to the non-linear model suggested by Orskov and McDonald (1979):  $y = a + b(1 - e^{-ct})$

where:  $y$  = degradation after time  $t$ ;  
 $a$  = zero time intercept;  
 $b$  = insoluble but potentially degradable fraction; and  
 $c$  = rate constant at which  $b$  is degraded.

The potential degradability (PD) was estimated as  $a + b$ . Effective degradability (ED) was calculated assuming particulate passage rate ( $k$ ) of 3.0% h<sup>-1</sup> using the formula:  $ED = a + [b \times c / (c + k)]$ . The degradation constants were estimated by an interactive least square method using the NONLIN procedure (SAS, 1988).

### Statistical analysis

Data on root and foliage yields, foliage:stem ratio, and CP were analysed as a randomized block design with four field replications using the General Linear Models Procedures (SAS, 1988). Dry matter degradation characteristics data were analysed as a randomized block design with steers as replicates. Correlation analysis was used to establish relationships between root and forage yields, CP content, and DM degradation characteristics.

## Results

### Root and foliage yields

The highest root yield was recorded in clone 91/02322 and the lowest in clone 92/0397 in both years (Table 1). In 1994-95, foliage yield was lowest in clone 91/02316 and highest in clone 92/0429. Clone 92/0067 had the lowest foliage yield and clone 91/02322 the highest in 1995-96. Clone 91/02319 had the lowest

**Table 1** Root and foliage dry matter yields ( $t\ ha^{-1}$ ) and foliage:stem ratio (%) of 25 green mite-resistant cassava clones in the derived savannah of southwest-ern Nigeria, 1994-95 and 1995-96

Clone	Root		Foliage		Foliage:stem ratio	
	1994-95	1995-96	1994-95	1995-96	1994-95	1995-96
91/02322	6.90	7.67	0.95	1.50	26	27
92/0326	6.33	7.20	0.52	1.43	17	22
92/0057	5.29	6.60	1.07	1.08	24	25
91/02163	4.67	4.60	0.83	1.15	27	23
91/00417	4.33	5.50	0.80	0.75	20	22
92/0427	4.23	7.17	0.78	1.38	20	24
91/00416	4.21	5.38	1.00	0.99	22	20
92/0398	3.83	5.01	1.60	0.85	21	30
30572	3.77	5.94	0.79	1.00	29	29
91/01363	3.66	4.71	0.58	0.83	27	23
91/00420	3.18	4.42	1.07	1.25	24	18
92/0342	2.96	4.71	0.77	0.93	28	28
91/00438	2.89	6.42	0.57	1.32	26	25
91/00450	2.80	5.61	0.48	1.19	21	20
92/0429	2.73	3.47	2.21	1.21	21	16
91/02312	2.58	3.77	1.19	1.16	18	22
91/02316	2.51	4.16	0.18	0.87	21	15
91/00419	2.32	4.54	0.86	1.03	19	25
91/02319	2.16	3.48	0.69	0.80	16	19
91/0067	1.91	4.13	0.48	0.65	22	23
91/00424	1.83	2.58	0.73	1.18	22	27
91/02317	1.77	3.40	1.02	0.93	21	20
TME 1	1.77	2.75	0.57	0.95	18	28
89/00250	1.71	3.47	0.64	1.26	22	31
92/0397	1.26	1.45	0.89	0.89	26	24
S.E.D.	1.06	0.94	0.26	0.24	4.0	3.0

S.E.D., Standard error of the difference between means

foliage:stem ratio whilst clone 30572 had the highest in 1994-95. In 1995-96, clone 92/0429 had the lowest proportion of foliage:stem ratio and clone 89/00250 had the highest. Root and foliage yields in 1994-95 were generally lower than those in 1995-96.

### Crude protein and *in sacco* DM degradation

Clone 92/0397 had the lowest CP content and clone 92/0067 had the highest (Table 2). The degradation equation proposed by Orskov and McDonald (1979) provided a good fit to the data with coefficients of determination higher than 0.85. The degradation constants varied significantly among clones (Table 2). Clones 91/00420, 91/02312, 92/0326, 92/0427, and 91/00420 recorded the lowest *a*, *b*, *c*, *PD*, and *ED*, respectively. The highest *a*, *b*, *c*, *PD*, and *ED* were recorded in clones 91/00450,

**Table 2** Crude protein (CP,  $g\ kg^{-1}$ ) dry matter degradation characteristics of foliage from 25 cassava clones at 12 months after planting

Clone	CP	<i>a</i> <sup>1</sup>	<i>b</i>	<i>c</i>	<i>PD</i>	<i>ED</i> <sup>2</sup>
91/00450	283	325	541	0.0249	865	570
92/0067	303	307	586	0.0228	893	542
92/02312	259	292	519	0.0280	911	540
91/02319	297	282	537	0.0292	819	547
91/00419	259	282	594	0.0296	876	575
92/00438	272	279	611	0.0185	890	512
92/0326	280	275	596	0.0120	870	510
92/0342	295	273	574	0.0299	847	560
91/02322	279	273	649	0.0205	922	535
91/00416	271	270	663	0.0192	933	525
92/0429	254	260	670	0.0173	929	493
30572	259	259	635	0.0218	895	524
92/0397	248	255	644	0.0238	900	528
92/0057	286	252	572	0.0258	824	512
92/0378	301	251	621	0.0283	872	548
TME 1	258	247	606	0.0214	852	497
91/02163	266	245	630	0.0250	875	525
91/01363	300	244	660	0.0290	904	545
91/02316	276	244	618	0.0302	862	549
91/02317	264	240	574	0.0311	815	533
92/0427	276	221	562	0.0262	783	484
91/00424	273	213	622	0.0277	835	505
91/00417	273	211	597	0.0397	808	551
89/00250	293	177	642	0.0565	819	566
91/00420	265	142	740	0.0244	882	474
S.E.D. ( <i>df</i> = 33)	5.5	50.5	67.1	0.0078	55.8	33.4

<sup>1</sup>Estimates from the non-linear equation,  $y = a + b(1 - e^{-ct})$   
<sup>2</sup>*ED*, Effective degradability =  $a + [b/(c/c + k)]$  where *k* is passage rate at 3%  $h^{-1}$

Note: *a*, zero time intercept; *b*, degradable fraction; *PD*, potential degradability; *a* + *b*; *ED*,  $g\ kg^{-1}\ DM$ ; and *c*, rate of degradation as  $\% h^{-1}$

S.E.D., standard error of the difference between means

91/00420, 91/00417, 91/00416, and 91/00450, respectively.

### Root and foliage correlations

The correlations between root and foliage yields ( $r = 0.24$ ;  $P = 0.230$ ) and root yield and foliage:stem ratio ( $r = 0.31$ ;  $P = 0.134$ ) were not significant. Root yield was also poorly correlated with nutritive value of the foliage (CP:  $r = 0.22$ ;  $P = 0.291$ ; *PD*:  $r = 0.06$ ;  $P = 0.768$ ; *c*:  $r = -0.33$ ;  $P = 0.105$ ; and *ED*:  $r = -0.16$ ;  $P = 0.449$ ).

### Classification of clones

Classification of clones into groups with potential for root and forage production (Table 3) was based on mean root and foliage yields, and *ED*. Average root yield of groups A ( $5.4 \pm 1.09\ t\ DM\ ha^{-1}$ ) and B ( $5.3 \pm 1.01\ t\ DM\ ha^{-1}$ ) was more than 40% higher than that of

**Table 3** Classification of green mite-resistant cassava clones into high (Group A), medium (Group B), and low (Group C) potential groups for root and foliage production based on root and foliage yields and effective degradability

Group A <sup>1,2,3</sup>	Group B	Group C
91/00438	91/00450	TME 1
91/02322	91/02163	89/00250
91/00416	91/00417	91/00420
92/0057	92/0326	91/00424
92/0398	92/0427	91/02317
30572		91/02316
		91/01363
		91/02312
		91/02319
		91/00419
		92/0342
		92/0429
		92/0397
		92/0067

<sup>1</sup>Root yield (t DM ha<sup>-1</sup>): Group A, 5.4 ± 1.09; Group B, 5.3 ± 1.01; and Group C, 2.9 ± 0.75

<sup>2</sup>Foliage yield (t DM ha<sup>-1</sup>): Group A, 1.3 ± 0.38; Group B, 0.92 ± 0.127; and Group C, 0.93 ± 0.295

<sup>3</sup>Effective degradability (g kg<sup>-1</sup>): Group A, 526 ± 13.9; Group B, 528 ± 33.8; and Group C, 534 ± 31.3

group C (2.9 ± 0.75 t DM ha<sup>-1</sup>). Average foliage yield of group A (1.3 ± 0.38 t DM ha<sup>-1</sup>) was more than 25% higher than that of groups B (0.92 ± 0.127 t DM ha<sup>-1</sup>) and C (0.93 ± 0.295 t DM ha<sup>-1</sup>). However, ED varied slightly among the three groups (526 ± 13.9, 528 ± 33.8, and 534 ± 31.3 g kg<sup>-1</sup> for groups A, B, and C, respectively).

## Discussion

Clonal differences in root and foliage yields, and foliage:stem ratio in this study agree with those reported in earlier studies (Dahniya *et al.*, 1981; Ravindran, 1992; Lualadia, 1994). This finding may be attributed to differences in anatomical, morphological, and physiological characteristics associated with acquisition of light, moisture, and nutrients for biomass production. Root yield ranged from 1.26–7.67 t DM ha<sup>-1</sup> which is comparable to that reported elsewhere (Dahniya *et al.*, 1981; Dixon *et al.*, 1994). Foliage yield ranged from 0.18–2.21 t DM ha<sup>-1</sup>. This range is relatively lower than ranges reported by other workers (Ravindran, 1992; Lualadia, 1994) partly because foliage yield was estimated only once at root harvest. The relatively higher quantities of root and foliage produced in 1995–96 than in 1994–95 may be due to differences in climatic and soil conditions between years.

Crude protein content of foliage differed sig-

nificantly among clones due to differences in the ratio of leaf:stem in the analysed samples. Crude protein ranged from 248–303 g kg<sup>-1</sup> DM which is consistent with ranges reported in reviews of the literature (Devendra, 1977; Smith, 1992). The average CP content in the foliage of all clones was above 80 g kg<sup>-1</sup> DM, the level below which voluntary intake of tropical forages is reduced (Minson, 1980). This observation suggests that foliage from all the clones could serve as a cheap source of protein for livestock diets, especially in sub-Saharan Africa and Asia where protein is a major limiting nutrient. However, cassava foliage contains condensed tannins which influence the availability of CP (Reed *et al.*, 1982). In addition, hydrocyanic acid (HCN) toxicity is a deterrent to using cassava foliage as human and livestock feed. Therefore, the potential of cassava foliage as a protein source and associated disorders due to its content of cyanogenic glucosides need more investigation.

In agreement with Adedamola (1995), considerable variations in degradation characteristics among clones were demonstrated in this study. The variations in degradation characteristics could be due to differences in CP as well as configuration of cell-wall polysaccharides and their effect on rumen microbial attachment and colonization of digesta particles (Chen *et al.*, 1984). Degradation characteristics are important determinants of foliage quality (Orskov and McDonald, 1979), therefore, further studies are needed to determine whether the differences in degradation characteristics among clones in this study can be translated into animal output (meat, milk, manure) which is a better determinant of forage quality (Moore *et al.*, 1990).

Root yield was poorly correlated with forage yield, CP, and DM degradation characteristics of foliage, suggesting that cassava breeders could select or breed genotypes with higher biomass (root and foliage) and better foliage quality. Further studies involving several cassava clones in different agro-ecological zones are needed to confirm this observation.

Using the root and foliage yields, and the ED of DM reported in this study as indices for root and forage production potential, clones 91/00438, 91/02322, 92/0057, 92/0398, 30572, 91/00450, 91/02163, 91/00417, 92/0326, and 92/0427 appeared to be superior to clones 89/00250, 91/02312, 91/02319, 91/00420, 91/00424, 92/0342, and 92/0397 (Table 3). Further studies are needed to determine whether the superiority in potential for root and forage production could be translated into net income, which is a better determinant of family welfare.

In conclusion, significant variations in root and foliage yields, CP content, and DM degradation characteristics among the cassava clones indicate that clones could be selected for root and foliage production. Based on root and foli-

age yields and effective degradability, clones with high (91/00438, 91/02322, 92/0057, 92/0398, and 30572) and medium (91/00450, 91/02163, 91/00417, 92/0326, and 92/0427) potential for root and foliage production were identified. These clones could be used for further agronomic and nutritional studies. The study has also given an indication of the variability in foliage yield, CP content, and DM degradation characteristics of green mite-resistant cassava clones being evaluated in sub-Saharan Africa. This information could be used to target clones for smallholder crop-livestock farming systems in the derived savanna zone of West Africa and similar environs in the tropics. Finally, because root and foliage of cassava are an important component of human and livestock diets, both agronomic and nutritive value indices of the root and foliage should be considered as selection criteria in future cassava improvement programmes in developing countries.

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# Alternative manifestations in origin, form, and function of the primary nodal complex of yams (*Dioscorea* spp.): A review

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The involvement of the yam 'head' or primary nodal complex (PNC) in several traditional practices for yam propagation, cultivation, and storage is outlined. Alternative phylogenetic and ontogenetic manifestations of the PNC, including those of different species which produce germinating seedlings and sprouting stem cuttings, bulbils, tuber pieces, and whole tubers (with 'heads'), as well as those in bulbil development are described. Failure to recognize the similarity of this unique organ in its different manifestations may have restricted the scope of research investigations for yam improvement. Previous investigations on micro-tuber production in tissue culture for distribution of disease-free germplasm, minisett techniques for production of planting setts and mini-ware tubers, and GA<sub>3</sub> application for extension of tuber dormancy and storage life are described. It is suggested that development of improved techniques for combination of minisett production and GA<sub>3</sub>-induced enhancement of tuber storage life could lead to recognition of mini-tubers as a new staple tropical product, comparable and competitive with *Solanum tuberosum* in production, quality, and international utilization as a table tuber.

Keywords: Primary nodal complex; Yam head; Manifestations in *Dioscorea* spp.

## Overview of Traditional Applications and Definition of the Primary Nodal Complex (PNC)

Although the 'head' which surmounts the tuber of *Dioscorea* spp. varies greatly in size in different species, e.g., *D. cayenensis* and *D. esculenta*, recent investigations suggest that it is involved in many traditional practices for yam propagation, cultivation, and storage. Thus, the yam head is the source of roots, shoots, and tubers during the sprouting, growth, and development of intact tubers, e.g., *D. alata*, *D. rotundata*, *D. esculenta*, and bulbils, e.g., in *D. bulbifera*, used as propagation setts. It is also generated *de novo*, during germination of true seeds of sapogenin-bearing yams, e.g., *D. composita*, *D. floribunda*, and during sprouting both of stem cuttings, e.g., in *D. floribunda* and headless tuber piece setts, e.g., in *D. alata*. Moreover, the yam head proliferates in the course of early growth and development of unstaked *D. alata* cv. White Lisbon crops in Barbados, to produce multiple erect shoots or 'spires' and it has been shown that staking provides support and exposure to light for these shoots to result in increased tuber yield. Also, the head is left on the plant to generate a new tuber for use as food in West Africa or as planting material, in Jamaica,

when the old tuber is harvested early or 'slitted' in *D. cayenensis*. In addition, during storage of ware tubers, the regenerated head at the base of the sprout is removed with the sprout to extend the storage life of *D. rotundata* and *D. alata* tubers in West Africa.

Despite such widespread manipulation of the yam head in traditional practice, relatively little research work has been carried out towards improving and modernizing the techniques involved. However, it has been suggested that the PNC, might function as the tuber-bearing organ (Wilson, 1990; Wilson and Wickham, 1992, 1994) in yams and might exert some control over the tuber number component (Wilson, 1982) of tuber yield. Accordingly, Ferguson (1973) showed that only a small number of the several PNCs produced during crop development in *D. alata* generated mature tubers. In addition, evidence from the polarity of sprouting in intact tubers of *D. alata* and bulbils of certain *D. bulbifera* varieties (Wickham *et al.*, 1982; Passam *et al.*, 1982) suggested that the PNC might also be involved in the control of tuber dormancy and, hence, storage life of ware tubers of yams.

It is here suggested that a more intensive research agenda has not materialized because similarities in the origin, structure, and function of the different manifestations of the yam head have not always been correctly recognised by

research workers. For example, what will be here interpreted to be the yam head or its initial, has been variously designated the sympodial rootstock, the tubercle, the swollen tuberous hypocotyl, or the corm-like hypocotyl axis in germinating seedlings of *D. glabra*, *D. floribunda*, and *D. trifida* to produce tubers and of *D. deltoidea* to produce a contracted sympodial rhizome by Sharma (1974, 1976a, 1980) and Henry (1967), respectively. The head was also designated the hypopodium secondary body in developing bulbils and tubers of *D. glabra* and *D. melanophyma* by Sharma (1976b, 1978) and as vine cormlets and corm buds in sprouting tubers of *D. rotundata* by Thompson and Bancroft (1996).

Earlier, Burkill (1960) had described the yam head as the contracted vestigial rhizome of an ancestral, rhizomatous, proto-*Dioscorea* species, which gave rise to the yam tuber by plagiotropic lobing. Later, Ferguson (1972, 1973) first suggested the existence of similarities between the yam head and the highly modified basal node of the shoot of sprouting stem cuttings and tuber piece setts of *D. alata*. The modified nodal organ was designated the PNC by Ferguson (1972) because of its origin in the first formed node and its complex vasculature. Thereafter, Onwueme (1973) described the sprouting of buds in yam tuber pieces in *D. alata* and *D. rotundata*, but did not recognize the PNC. After a series of papers over 1974-80, Sharma (1978, 1980) recognized the developmental similarity of tubers and bulbils of *D. glabra*, *D. floribunda*, and *D. sansibarensis* (Rao and Tan, 1976) in what is, here, designated as PNC genesis. Subsequently, Wickham et al. (1981, 1982) defined the PNC as the organ of renewed growth and the only true organ of vegetative propagation in five *Dioscorea* species studied (*D. alata*, *D. bulbifera*, *D. esculenta*, *D. rotundata*, and *D. trifida*). Accordingly, alternative manifestations of the PNC were described in germinating seedlings and in sprouting stem cuttings, bulbils, tuber piece setts, and intact tubers, to produce roots, shoots, daughter PNCs, and tubers, as well as in the course of bulbil genesis in *D. bulbifera* and *D. alata* (Wickham et al., 1982).

Accordingly, the PNC of *Dioscorea* species may be defined as the contracted, vestigial rhizome of the proto-*Dioscorea*, consisting of an apical bud, and one or more axillary buds surmounting a mass of tissue with scattered vascular bundles in a matrix of parenchymatous cells, as well as a meristematic region with the capacity to generate roots, shoots, tubers, bulbils, and daughter PNCs during processes of seedling germination, tuber sett, and stem cutting sprouting, and tuber and bulbil development.

Despite these indications of the critical importance of the PNC in plant propagation crop cultivation and in tuber storage, both from traditional practice and research investigations, little attention has been given to the study of this

unique organ of regrowth and development. Therefore, in order to encourage further research on the PNC, available literature on the organ and related investigations in *Dioscorea* species are now critically reviewed.

## Alternative Manifestations of the PNC

### Phylogenetic manifestations

Burkill (1960) described three ecological constraints in the phylogeny of the Dioscoraceae which exerted selection pressure in the evolution of the rhizome into the yam head (PNC) and tuber as follows:

- (1) the requirement for increasing storage tissue in the proto-Dioscoraceae for seasonal regrowth after perennation led to:
  - (i) thickening and plagiotropic lobing of the rhizome; but
  - (ii) difficulty in rhizome growth through the soil for better protection.
- (2) the necessity for the shoot to climb through a shaded canopy to seek light with limited storage tissue led to:
  - (i) elongated development of one aerial stem from the proximal end of the rhizome;
  - (ii) shortening of the rhizome; and
  - (iii) enlargement of one or a few plagiotropic lobes.
- (3) increasing selection pressure for storage tissue and elongated shoot development led to:
  - (i) transfer of the greater part of the storage space from the shrinking rhizome to the plagiotropically swollen lobe(s) or tuber(s) leading to deeper soil penetration and better tuber protection.

Evidence for occurrence of the above-mentioned or similar phylogenetic transformations exist in the number of rhizomatous and intermediate structures among some 600 species in the genus *Dioscorea* (Coursey, 1967). Four of these are, here, referenced.

- (1) *Dioscorea* species with elongated rhizomes, e.g., *D. villosa*, *D. glauca*, and *D. nipponica* (Burkill, 1960);
- (2) *Dioscorea* species with contracted sympodial rhizomes with digitate branching, e.g., *D. deltoidea* (Sharma, 1976a);
- (3) *Dioscorea* species with a large head (PNC) and normally a single tuber, e.g., *D. cayenensis* (Coursey, 1967; Terauchi et al., 1992);

- (4) *Dioscorea* species with progressively reduced heads and multiple tubers, e.g., *D. rotunda*, *D. alata*, *D. trifida*, and *D. esculenta* (Burkil, 1960; Wickham et al., 1982).

However, the essential transformation from rhizome to PNC or tuber appears to be systemic, since, as will be shown, it is repeated during regrowth in nodal regions of germinating seedlings and sprouting stem cuttings, intact bulbils, tubers, and headless tuber piece setts as well as in the nodal generation of bulbils.

### Ontogenetic manifestations

The origin and development of the PNC is perhaps most completely described in the sprouting of 'headless' tuber piece setts by Onwueme (1973) and Wickham et al. (1981) to include three stages as follows:

1. Resumption of meristematicity in the primary thickening meristem (PTM) to include:
  - (i) localized meristematicity to produce tuber root initials, leading to production of thin ephemeral roots;
  - (ii) general meristematicity to produce the multilayered tuber germination meristem (TGM); single or groups of apical meristems; which develop into apical buds with calyptrae, subtending axillary buds.
2. Formation of the PNC initial, i.e., a mass of meristematic and parenchymatous cells at the base of the differentiating bud, due to meristematic activity in the region of the first node of the bud; [called the hypopodium by Sharma (1976b)].
3. Emergence and differentiation of the PNC initial due to activity of the TGM on either side of the developing bud to generate:
  - (i) thick anchoring PNC roots around the circumference of the PNC;
  - (ii) a complex mass of scattered vascular bundles and parenchymatous tissue in the PNC initial; and
  - (iii) a proliferation of axillary buds.

This organ has the capacity to develop roots, shoots, and tubers in the mature plant.

Although the process of PNC development is modified in alternative manifestations of the organ, the three major stages identified remain essentially unchanged. Thus, in seedlings, the first formed bud is often embryonic, i.e., either the axillary bud subtended by the first plumular leaf in the development of the contracted rhizome of *D. deltoidea* (Sharma, 1976a) or an accessory bud in the axil of the cotyledon, e.g., in the PNC of *D. floribunda* (Sharma, 1980). In both cases, the hypocotyl develops into the PNC initial by the activity of a PTM. The PNC development similar to that in *D. floribunda* is

here interpreted to occur in seedlings of *D. trifida* (Henry, 1967) and in *D. rotundata* (Sadik and Okerere, 1975). It should be noted, however, that in the case of *D. deltoidea*, where a contracted rhizome is produced, the perennial bud is active, producing successive shoots, perennial buds, and sympodial growth of the rhizome.

In sprouting stem cuttings, an accessory bud in the leaf axil initiates PNC development as a small white mass of tissue in *D. spiculiflora* (Preston and Haun, 1962), in *D. alata*, *D. rotundata*, and *D. dumetorum* (Njoku, 1963), and in *D. alata* (Ferguson, 1972). Later, Wickham et al. (1982) recorded accessory bud fecundity in the axils of stressed shoots of sprouting tubers, in which up to 14 buds were produced at one node. It was concluded that each bud initiated the development of a potential PNC.

Interestingly, four manifestations of the PNC occur in bulbil development. Thus, in the most primitive manifestation, the entire PNC develops into the bulbil as in *D. melonophyma* (Sharma, 1978) and in the ridged bulbil of the African *D. bulbifera* var. *anthropropagorum* (Wickham and Wilson, unpubl.). However, in *D. melanophyma*, the PNC develops from a single accessory bud and its subtending hypopodium (i.e., the bud axis below the insertion of the prophyll), whilst in the African *D. bulbifera*, the PNC presents as a longitudinal plane of hypopodial tissue, passing through the point of attachment of the bulbil to the stem and the petiole of the subtending leaf. On this unique PNC, there is a girdle of buds spaced at intervals in its longitudinal plane. This unusual manifestation of the PNC is realized by sequential multiple bud genesis and bud separation by continued growth of the PNC in the area separating them. The body of the bulbil is then formed by diageotropic lobing on either side of the planar PNC, to form three flat bulbil faces demarcated by ridges.

Alternatively, in the Asian *D. bulbifera* var. *sativa*, the PNC presents as a poorly demarcated bulbil head, continuous with the body of the smooth bulbil and on which there are spirally distributed buds (Dale, 1901).

Finally, in *D. glabra* (Sharma, 1976b) and *D. alata* (Wickham et al., 1982), the PNC is manifested as a clearly demarcated proximal bulbil head, which is surmounted by one or more accessory buds.

### Implications and Applications of PNC Function

Because of its location and vascular structure, the PNC must play a critical role in the transport of assimilate and plant growth substances from the shoot system to roots, tubers, and bulbils as well as the reverse transport of inor-

ganic ions from roots to tubers and shoots during crop development. Conversely, the PNC is also involved in the transport of stored assimilate from the planting sett (whether nodal cutting, bulbil, intact tuber, or tuber piece) to roots and shoots during sprouting in plant propagation. Moreover, since the generation of the PNC after tuber dormancy is subject to control by exogenous plant growth substances, e.g., gibberellin and 2-chloro-ethanol, the extension or reduction of tuber dormancy occasioned by application of such substances, could have applications in tuber storage by extension of tuber dormancy and in early cultivation of yams by its premature arrest. Some of the problems involved in bringing the practical applications suggested by the above-mentioned implications of PNC function are now discussed.

### Propagation

Although the sapogenin-bearing yams are propagated by seedlings, this practice has not been developed for edible yams, despite regular recovery of viable seed from both *D. trifida* (Henry, 1967) and *D. rotundata* (Sadik and Okerere, 1975). The problems are variable seed dormancy of three weeks to five months and slow seedling growth. Thus, in *D. trifida*, Henry (1967) reported that the transformation of seedlings from the juvenile to adult foliage conformation took up to three months after seed germination and that the PNC (called a corm-like axis at the proximal end of the hypocotyl) development commenced only when the adult shoot produced abundant, vigorous adult foliage.

Propagation with bulbils has not been widely practised except in *D. bulbifera* and although stem cuttings of *D. alata* and *D. rotundata* have been used experimentally to produce tuber setts by Akoroda and Okonmah (1982), this technique has not been widely adopted in practice, except with sapogenin-bearing species. It should be noted, however, that whereas bulbils of *D. bulbifera* var. *sativa* would be expected to show proximal dominance in germination, those of *D. bulbifera* var. *arthropophagorum* have been shown to germinate from the surface of the bulbil in contact with dry or moist media (Passam et al., 1982; Wickham et al., 1982).

Propagation by yam heads, small tubers, and tuber piece setts is the normally practised method for the edible yams, but here, the propagules vary from 100–150 g tuber piece setts in *D. alata* in Barbados and Trinidad to heads or small tubers weighing 1000–3000 g in *D. rotundata* and *D. cayenensis* in Jamaica (Rankine and Ferguson, 1974). Investigations are needed to determine how sett size and structure affect the function of the PNC to produce roots, shoots, and tubers in the mature plant. For example, studies on the mobilization of assimilate from the sett through the PNC to roots and shoots of different species in relation

to the breaking of tuber and (or) tissue dormancy may either explain or obviate the need for large tuber setts in *D. rotundata* and *D. cayenensis*. Thus, in miniset technology, 25-g *D. rotundata* setts, pre-sprouted under controlled conditions, produced tubers of up to 1000 g in 5–6 months (Otoo et al., 1987). This raises the question as to whether controlled conditions of presprouting (Onwueme, 1977) are required for efficient PNC function in yam propagation.

Micro-tubers up to 100 mg (probably mini-bulbils), have been induced in nodal cuttings of *D. alata* and *D. bulbifera* in controlled tissue culture media by Mantell and Hugo (1989). However, these experiments indicated significant differences between the species in the conditions for micro-tuberization, as would be expected from their different patterns of bulbil genesis (Wickham et al., 1982). Thus, in the low-N tuberization medium used, ammonium ions and long photoperiods (greater than 8) inhibited micro-tuberization in *D. alata*. However, in *D. bulbifera*, micro-tuberization occurred over 8–16 photoperiods, provided that 2.5  $\mu$ M kinetin and 4% sucrose were present in the medium. Alternatively, *D. alata* propagules micro-tuberized under 8 photoperiods, 2.5 g kinetin, and 2% sucrose. Notwithstanding these differences in the requirements for micro-tuberization, such micro-tuber production could facilitate the distribution of elite germplasm as certifiable, disease-free planting material.

The importance of short photoperiods for macro-tuberization was earlier demonstrated in nodal vine cuttings of *D. alata* by Ferguson (1972). Also, Uduebo (1971) had shown that the initiation and axillary proliferation of bulbils in *D. bulbifera* could be controlled by exogenous application of plant growth substances.

### Production

The most important question on production is whether, and how does the PNC, which is the source of roots, shoots, and tubers, control or affect the growth and development of the yam crop. Although little attention has been given to this aspect of PNC function, a few important observations have been made on the subject by Ferguson (1973) These include:

1. The PNC, manifested as the yam head, assumes different sizes in mature tubers of different species, e.g., the *D. cayenensis* head is the largest among edible yams;
2. Head setts of *D. alata* cv. White Lisbon produced higher tuber yield than tail setts and larger setts produced larger PNCs and higher tuber yield;
3. There was a close relationship between PNC size at eight weeks after planting and final tuber yield;



4. In an analysis of the sequencing of PNC and tuber development in *D. alata*, four distinct phases were observed:
- (i) development of the PNC up to 13 weeks after planting;
  - (ii) active tuber development by cell division and mainly by increase in tuber number (weeks 13–19);
  - (iii) rapid accumulation of dry matter in tubers (weeks 19–32), with increase in tuber number up to week 28; and
  - (iv) reduced accumulation of dry matter and apparent resorption of tubers over weeks 32–36.

It would appear that in yams as in sweetpotato (Wilson, 1982) that there is a critical period for the initiation of tuber development on the tuber-bearing organ (Wilson, 1990; Wilson and Wickham, 1992, 1994) i.e., the PNC in yams. In *D. alata* cv. White Lisbon, Ferguson (1973) found that this period was over weeks 13–28 after planting, but that only those tubers which were initiated early in crop development, reached commercial size. Accordingly, tuber yield increased with up to seven tuber initials per plant, but decreased markedly, thereafter, (Ferguson, 1973). Moreover, the apparent resorption of tubers observed over weeks 28–36 (Ferguson, 1973) at a time when *D. alata* is known to show bulbil development indicated that the direction of assimilate transport to tubers might have been reversed at that time either to support bulbil growth or to delay leaf senescence. Such reversal may well be a function of the PNC.

Wide variations in sett multiplication ratios (SMRs) and rates in yams are yet to be satisfactorily explained. Rankine and Ferguson (1974) reported an experimental SMR (tuber yield per plant divided by tuber sett weight) of 46 over a period of 9 months for *D. alata* in Trinidad but only 2–3 for commercial *D. rotundata* in Jamaica and suggested that ratios of 8–15 were achievable in practice. However, the experimental *D. alata* SMR per month (46/9) of 5.1 was less than that for *D. rotundata* (8.0), using the pre-sprouted miniset technique (Otoo et al., 1987), i.e., 1000-g yield from a 25-g sett in five months. Sett multiplication ratios in the range 2.5–134 over a period of seven months, equivalent to a maximum rate of 19.1, were obtained by Akoroda (1984) for seedling tubers of *D. rotundata*.

Some control over PNC development to advance planting date of edible yams has been experimentally achieved by stimulation of sprouting in dormant tubers of *D. alata* by dipping of tuber piece setts in 2 chloro-ethanol and thiourea (Cibes and Adsuar, 1966). The effect of 2 chloro-ethanol and thiourea-stimulated sprouting on SMR in miniset production should be investigated.

## Storage

The involvement of the PNC in yam tuber storage derives from the dramatic extension of dormancy and suppression of sprouting occasioned by the application of gibberellic acid (GA<sub>3</sub>) to bulbils and tubers, first described by Okagami and Nagao (1971) in bulbils of *D. batatas*, *D. japonica*, and *D. bulbifera* and in tubers of *D. alata* by Martin (1977). However, it was Wickham et al. (1984a, b) who first demonstrated sprout suppression and storage life extension of economic proportions of up to 26 weeks by GA<sub>3</sub> in *D. alata* and *D. esculenta* tubers. Moreover, the suppression was linked to the inhibition of the formation of the tuber germination meristem and, hence, PNC and shoot development, both in tubers and in bulbils of *D. alata*; but not in the bulbils of *D. bulbifera*. The action of GA<sub>3</sub> both in temperate and in tropical *Dioscorea* species was linked to its effect in reducing levels of a new class of phenolic inhibitor, termed batatasins; five different forms of which have been isolated from bulbils of *D. oposita* by Hashimoto et al. (1972), Hashimoto and Hasegawa (1974), and Hashimoto and Tajima (1978). Interestingly, Ireland et al. (1981) showed that batatacin II was absent from *D. bulbifera*, which might explain the absence of the GA<sub>3</sub> dormancy response in this species.

The suppression of PNC development by GA<sub>3</sub> was more dramatically demonstrated when it was shown by Wickham et al. (1984b) that dormancy could be re-induced by GA<sub>3</sub> treatment in sprouted tubers, after removal of sprouts. This result was interpreted to mean that the development of bud initials on the tuber germinating meristem could be inhibited by GA<sub>3</sub> treatment, after sprouting had begun. Also, after repeated re-induction of dormancy in de-sprouted tubers, the PNC continued to develop and was capable of bypassing the aerial vegetative phase to produce secondary or daughter tubers, i.e., effectively transferring stored assimilate from the primary to the daughter tuber.

## New products and product quality

It is suggested that improved practices for the production of uniform baby or mini yams of *D. rotundata* of 250–300 g weight, using miniset technology, combined with GA<sub>3</sub> extension of tuber dormancy and storage life could lead to development of a new staple tropical tuber crop product, comparable and competitive with *Solanum* spp. (potato) in production, and international utilization, as a table tuber.

Widespread utilization of improved products will depend on the capacity to produce tubers with different qualities, suitable to different markets, targeted consumers, and methods utilization, e.g., boiled, baked, crushed, pounded, and fried chips. Unfortunately, little work has been done on tuber quality in yams. Thus, although

tuber quality has been linked to large tuber size in *D. alata* and *D. rotundata*, Passam et al. (1982) demonstrated that in *D. alata* plants, prematurely senesced by water stress, small tubers 15–160 g in weight, developed periderm and the scleroid layer characteristic of mature tubers (Wickham et al., 1981). Tuber quality has also been linked with storage. Accordingly, Nindjin (1995) found that whereas *D. rotundata* tubers were of acceptable quality immediately after harvest, those of *D. alata* used for yam paste developed acceptable quality (experimentally attributed to starch hydrolysis) only after a period of storage. Such hydrolysis was also inhibited by GA<sub>3</sub> treatment.

Alternatively, *D. dumetorum* tubers deteriorated rapidly with storage over two weeks and such deterioration was associated with characteristic cell wall lignification, initiated around the intercellular spaces and accompanied by ruptured amyloplasts and apparently starch hydrolysis (Sealy et al., 1985). Such quality deterioration has now assumed greater significance in view of the recent finding by Willmitzer (1997) that increased tuber size up to 2 kg in genetically transformed *Solanum* spp. cv. Desiree was associated with expression of genetically engineered yeast invertase in the intercellular spaces. Alternatively, when invertase was expressed within the cell, tuber number rather than tuber size was increased. The large potatoes were judged by chip experts to be of inferior quality because of their higher water content. These results suggested that tuber quality, as judged by size, water, starch, and lignin contents was associated with relative activities of enzymes involving carbohydrate and lignin metabolism (Wilson, 1970).

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# The effect of salting sweetpotato chips prior to drying on infestation by *Araecerus fasciculatus*

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The efficacy of salting sliced sweetpotato chips prior to drying in reducing and (or) controlling damage levels of dried chips in storage by *Araecerus fasciculatus* Degeer (Coleoptera: Anthribidae) was investigated. Eight salt dosage rates, 0 (controls), 0.25, 0.5, 1, 2, 3, 4, and 5 g 100 g<sup>-1</sup> freshly sliced chips were evaluated. Artificial infestation of dried chips with adult *A. fasciculatus* was conducted. Gravid females were allowed to oviposit for one week and the infested chips incubated until the F<sub>1</sub> generation adults had all emerged under prevailing environmental temperatures of 26.29 ± 1.19°C and relative humidity 68.96 ± 7.39%. The number, weight, and generation time of *A. fasciculatus* that emerged varied significantly depending on the salt dosage rates. Best results were obtained with increasing salt dosage levels. Salt application rate at 2–3% is recommended for on-farm validation.

Keywords: Dried sweetpotato chips; *Araecerus fasciculatus*; Control by salting; Storage; Quasi-staple

Sweetpotato is the third most important source of carbohydrates after banana and cassava in Uganda. With the declining production trend of banana and cassava due to biotic and abiotic factors, sweetpotato is gaining in prominence as a source of food, income generation, and as a component of on-farm food security reserve (Hall, 1995).

Sweetpotato is cultivated in the unimodal and bimodal rainfall areas of Uganda (Hall, 1995). In areas with a bimodal rainfall pattern, sweetpotato is cultivated twice, but in areas of unimodal pattern, cultivation is limited to only one season in a year.

In eastern and northern Uganda where sweetpotato cultivation is limited to one season a year, dried root chips are important components of the diet of the communities, because storage of the fresh roots is not possible over the timescale required.

The most limiting biotic factor of sweetpotato root production in Uganda is the sweetpotato weevil *Cylas* spp. which causes very severe damage at crop maturity especially when harvest period is extended into the dry season. A number of other factors limit the time for

which roots can be left in the ground after they reach maturity, and these include development of root sponginess, rots, sun scorch, theft, and rodent infestation (Agona, 1995). Given the perishability of roots once harvested, farmers in Uganda usually process them into dried chips.

Dried sweetpotato chips constitute very important quasi-staple during the dry months of the year when the other sources of carbohydrates are still out of season (Bashaasha *et al.*, 1993). The dried chips are steeped in water, boiled, and seasoned with salt and eaten, or are mixed with cereals such as millet or sorghum and ground into composite flour for making local bread known as 'atap' (Fowler and Stabrawa, 1993; Agona, 1995). Dried sweetpotato chips, however, succumb to insect pest infestation after about 2–3 months of storage (Agona, 1995).

The insect pests of dried sweetpotato chips include *Araecerus fasciculatus* Degeer, *Rhyzopertha dominica* (Fab.), *Dinoderus minutus* (Fab.), *Sitophilus zeamais* (Motsch.), *S. oryzae* (L.), and *Tribolium castaneum* (Herbst) (Agona, 1995). Of the pest complex, *A.*

*fasciculatus* is the most important (Agona, 1995). Pest infestation is manifested by perforations of dried chips, development of off-flavours, mouldy chips, mite infestation, reduced flour content, frass, and cast skins of dead insects. To reduce damage levels, farmers always conduct routine inspections and re-drying in the sun. The results are often discouraging since damage levels continue to escalate with prolonged storage duration. Farmers respond to the problem by requesting insecticides. The use of insecticides to prevent and (or) control infestation has many problems. Farmers are resource-poor, lack technical expertise in chemical handling and application, very few insecticides are available, and those which are available, have low shelf-life and are prone to user abuse. The need for effective pest management strategies are, therefore, urgently required.

Various pest management methods, especially for dried cassava chips have been demonstrated. The methods include storage of dry chips in sealed containers (Ingram and Humphries, 1972), varietal manipulation (Pillai, 1977), mixing of chips with common salt prior to drying (Kumar and Karnavar, 1986), and parboiling of roots prior to slicing and drying (Nwana and Azodeh, 1984; Pillai and Rajamma, 1987; Nwana, 1993). Other disinfection methods, e.g., use of focussed solar energy (Nakayama et al., 1982; Silim and Agona, 1993) have been successfully demonstrated on other insect pests.

This study was conducted to assess the efficiency of salting of freshly sliced sweetpotato chips prior to drying, for the control of *A. fasciculatus*. No reports have been found on the use of this method for dried sweetpotato chips in storage.

The objectives of the study were to:

- (i) evaluate the efficacy of finely ground common salt in reducing and (or) controlling dried sweetpotato chip damage by *A. fasciculatus*;
- (ii) establish the effect of salt dosage levels on the generation (development) time of *A. fasciculatus*; and
- (iii) establish the effect of different salt dosage levels on the weight of adult *A. fasciculatus* at emergence.

## Methodology

Freshly sliced sweetpotato chips of medium size, approximately 5 mm thick, were thoroughly mixed with finely ground common salt (NaCl) at the rate of 0, 0.25, 0.50, 1, 2, 3, 4, and 5 g 100 g<sup>-1</sup> fresh chips prior to drying.

The chips were spread out to dry in the open sun on a wire mesh tray. Each treatment lot was dried separately. Sun drying was discontinued once an equilibrium moisture content (MC) of approximately 11–12% was achieved. This was attained after three days of drying.

The MC was monitored by oven-drying at 130°C for 24 h.

The chips were bagged in cotton-woven sample bags and fumigated with phosphine at the rate of 3 g m<sup>-3</sup> in a fumigation chamber for four days. This was to rid the chips of any prior infestation which might have occurred during the drying period (Hill, 1984; Nwana, 1993). The chips were removed, aired for 6 h to get rid of any residual undecayed fumigant, and were put in 100-L plastic drums fitted with lids until required in order to avoid any cross infestation.

Artificial infestation was carried out on 100-g samples of dried chips kept in 1-L Kilner jars using three-week old *A. fasciculatus* adults at the rate of eight females and four males for each jar. The gravid females were allowed to lay eggs for seven days after which all insects were discarded. The infested chips were retained in the jars coated at the neck with polytetrafluoroethylene (fluo) to prevent insect escape. Additionally, the jars were fitted with muslin cloth at the neck and a perforated screw-on lid to permit maximum aeration to the developing insects. The cultures were placed on a rack in the laboratory under prevailing environmental temperatures of 26.29 ± 1.19°C and relative humidity 68.96 ± 7.39%, until F<sub>1</sub> adult emergence.

There were eight treatments including the control, each replicated four times. The data were analysed as for a completely randomised design (CRD).

Investigative parameters included: (i) total number of adults in the F<sub>1</sub> generation in each treatment; (ii) weight of emerged adults in each treatment; (iii) generation time; and (iv) final moisture content of the chips.

The weights of the weevils were determined soon after emergence using a Stanton 33 BN analytical balance. The final MC of the chips was determined using the oven-dry method as explained above.

## Results

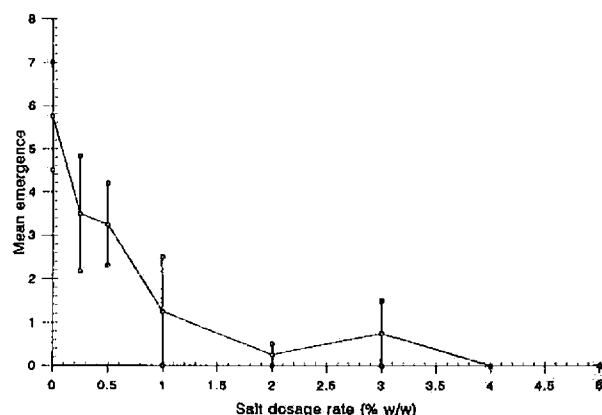
The total adult *A. fasciculatus* emergence and MC mean per cent in the different treatments are summarized in Table 1. The mean number, weight, and generation time of *A. fasciculatus* at different dosage rates of salting sliced sweetpotato chips are presented in Figures 1, 2, and 3. The final MC of the treated chips after about four months of storage is presented in Figure 4.

There were significant differences ( $P < 0.05$ ) in the mean number of adult *A. fasciculatus* that emerged in the chips treated at different salt dosage levels prior to drying. The highest number of emergence was observed in the non-salt treated (controls) and none in those chips treated with salt at dosage levels of 4 and 5% (w/w) (Figure 1).

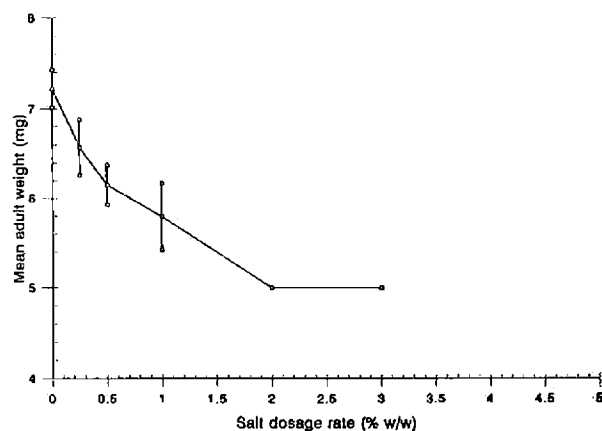
**Table 1** Mean number of *Araecerus fasciculatus* that emerged and final moisture content (MC) of treated chips at different salt dosage levels

Salt dosage level (%)	Transformed means of adults that emerged <sup>f</sup>	Actual means of adult that emerged	Final MC (%)
0	2.462	5.750	13.837
0.25	1.868	3.500	14.112
0.50	1.896	3.250	14.850
1	1.117	1.250	16.225
2	0.837	0.250	16.400
3	0.998	0.750	16.925
4	0.707	0.000	17.200
5	0.707	0.000	17.300
S.E.D. (24 df)	0.372	1.265	0.339
C.V. (%)	39.770	96.990	3.020

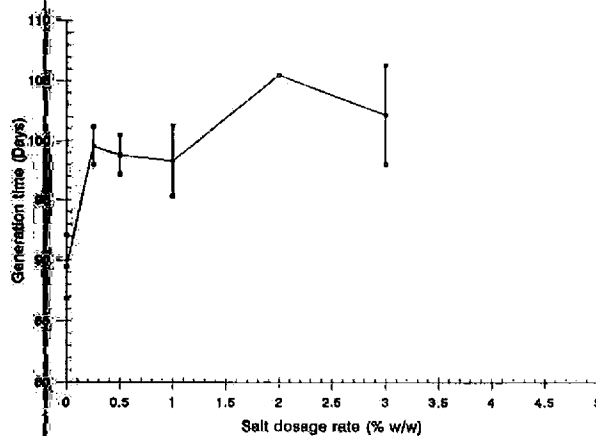
<sup>f</sup>Square root data transformation:  $\sqrt{(x + 0.5)}$ , was adopted  
S.E.D. is standard error of the difference between means  
C.V. is Coefficient of Variation



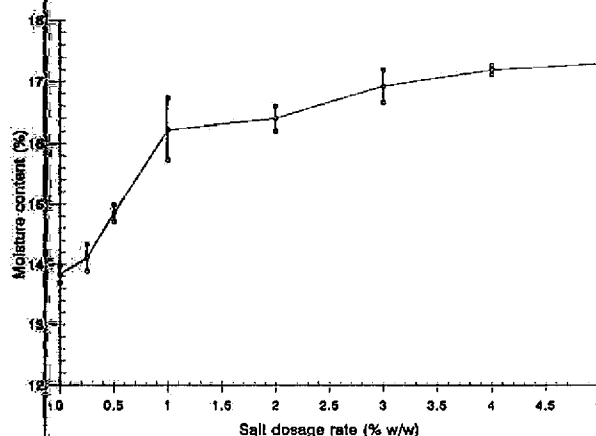
**Figure 1** Mean emergence of *Araecerus fasciculatus* infesting dried sweetpotato chips treated at different salt dosage levels; —■—, Mean emerged



**Figure 2** Effect of salting chips on the weight of *Araecerus fasciculatus* at emergence; —■—, Weight



**Figure 3** Effect of salting chips on the generation time of *Araecerus fasciculatus*; —■—, Time



**Figure 4** Final moisture content (MC) of salted dried sweetpotato chips at different dosage rates; —■—, % MC

There were, however, no significant differences ( $P > 0.05$ ) in the mean number of *A. fasciculatus* which emerged in chips treated at salt dosage levels of 0.25 and 0.50% (w/w). Likewise, the difference in the mean number of adults that emerged in chips treated at salt dosage levels of 1, 2, 3, and also 2, 3, 4, and 5% (w/w) were not significantly different (Table 1).

Salting of the chips had a negative effect on the weight of adult *A. fasciculatus* at emergence. Weevils that were bred on non-salted chips were heavier than those from salted chips. The weights decreased with increasing salt dosage rates (Figure 2).

The generation time, i.e., time from egg laying to adult emergence, was significantly affected by the salt treatment on the chips. The generation time of *A. fasciculatus* increased progressively with increasing salt dosage rates up to 2% and decreased at 3% (Figure 3). The shortest generation time was achieved in the controls ( $89.44 \pm 2.63$  days) and the highest in the chips treated at rate of 2% (w/w)

(105.50 days). There was, however, only a single adult emergence at 2% (w/w) salt level.

The final MC of the chips after about four months of storage increased significantly ( $P < 0.05$ ) with increasing salt dosage level (Figure 4). The highest MC was attained on chips treated at 5% (w/w) ( $17.30 \pm 0.07\%$ ) and the lowest in the controls ( $13.84 \pm 0.29\%$ ).

## Discussion

The results showed that the treatment of freshly sliced sweetpotato chips with common salt prior to drying significantly reduces and (or) controls damage levels by *A. fasciculatus* during storage. The efficacy of salting chips, however, depends on the dosage levels. Best results are obtained at higher dosage levels of 2% (w/w) and above. At these levels insect adult emergence is limited to less than one. The implication of the number of adults that emerged, however, needs to be corroborated with the actual damage as perceived by the farmers. This would require the establishment of a visual scale of damage. The category scale could be based on the number of holes per unit area.

In spite of the favourable results with the prescribed dosage levels, the quality of the final products could be the determinant factor for farmers' acceptability. The organoleptic quality of the product may be affected at high salt dosage levels. In those areas where dried sweetpotato chips are consumed, the chips are first steeped in water, and seasoned with salt during cooking. It is therefore suggested that low salt dosage levels of 2–3% (w/w) could be recommended. Additionally, to reduce salt levels, it is recommended that salted chips are steeped in water for some time, then de-watered and the chips boiled in fresh water. During cooking, seasoning of the chips to taste with salt may be optional depending on salting preference.

The mechanism by which salting reduces the level of damage by *A. fasciculatus* to dried chips may be linked to one or more of (i) choice of oviposition site, (ii) the survival of deposited eggs, or (iii) survival of larvae after hatching. With respect to egg survival, it is envisaged that the content of freshly laid eggs may be destroyed by desiccation before the egg shell hardens as a result of the osmotic potential created by high salt concentrations. At low salt dosage levels of 0.25, 0.5, and 1% (w/w) it is apparent that the process of plasmolysis may not be triggered due to the equilibria in osmotic potential. However, at higher dosage levels of 2% and above, plasmolysis takes place to the detriment of the egg content.

With respect to survival of the larvae after hatching, it is possible that those larvae developing in a salty environment succumb to dehydration since the body's capacity to conserve water becomes weakened due to the hostile environment. Insects are sensitive to the loss

of body water and this is particularly true in environments which increase the rate of water loss (Lessard, 1987). This probably led to the death or reduced rate of growth as reflected by the reduced total number of adults that emerged, reduced adult weight, and prolonged generation time.

## Conclusion

The study has shown that salting sliced sweetpotato chips prior to drying to control damage levels by *A. fasciculatus* is very promising. As a practical method for use by subsistence farmers, an application rate of salt is recommended at 2–3% (w/w) level. It is envisaged that at this level, the culinary qualities of the dried chips is not significantly affected. Additionally, since the treatment has been tested on only one species of the pest complex it is recommended that the method of pest control be tested on-farm on the entire pest complex of dried sweetpotato chips under farmers' storage conditions and management practices.

Additionally, since saltiness tends to increase the moisture content of the dried chips when kept for a long time due to the hygroscopic nature of salt, it is recommended that the chips are re-dried periodically. This may not only help in hardening and drying of the chips, thus avoiding microbial activity, but also getting rid of the adult insects which tend to be photophobic.

## Acknowledgements

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# Cassava in Africa: The root of development in the 21st century

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In the last 20 years, per capita food production in sub-Saharan Africa has been declining, and this trend is likely to continue into the 21st century. However, production of root crops particularly, cassava, has been increasing faster than the population growth rate. In some countries such as Nigeria and Ghana, cassava production has doubled in 10 years. Cassava in Africa is used mainly for direct food consumption by householders. However, there is a new trend to use cassava as a raw material for the formal food industry (fast foods, snacks, and bread) and for other industries (animal feed, starch, chemical, and pharmaceutical). A pilot project to disseminate the production of high quality cassava flour for the baking industry has revealed that farmers participating in such projects experience significant income increase and are motivated to try new technologies. There is need to identify technologies that meet the resource-poor farmer or processor conditions while producing the quality products required by the consumers at a price they are prepared to pay. Such technologies will enable cassava to play a major role in the development of the rural areas where cassava is grown. This study will present scenarios for the use of cassava as a commodity for poverty alleviation and empowerment of the rural areas in cassava-growing countries in Africa.

Keywords: Africa; Food crisis; Food security; Poverty alleviation; Income generation

## Food Crisis in Africa

The international development literature is replete with documents and statistics on the food production crisis in Africa. Declining food per capita availability, rapidly increasing food import bills, and massive food-aid requirements are evidence of the severity and deteriorating situation in food availability. These problems are most acute in sub-Saharan Africa (SSA) where it has been estimated that more than 20% of the population is under-nourished. Sub-Saharan Africa is the only region of the world where per capita food production has decreased during the last two decades.

Food shortage is the result of the combined effect of fast-growing populations and slow growth in the agricultural sector. Africa is the only continent where the annual population growth rate has consistently exceeded food production in the last 30 years. During the period 1970-84, the population grew by 50% whereas agricultural production increased by only 30%. As a result, agricultural production on a per capita basis decreased by 13%. In 1995, Africa's annual population growth rate was projected to be 3.1% compared to less than 2.2% for Asia and 2.3% for Latin America. More importantly, population growth rates have peaked in Asia and Latin America but are still increasing in much of Africa and are not projected to peak until the turn of the century.

Food imports have increased eightfold since 1960. Cereal imports in 1989 totalled 8.2 M t and rose to 11.4 M t in 1991-92. In 1989, the cost of cereal imports reached U.S. \$1 bil-

lion. The inability of most countries to sustain rising import costs has necessitated significant food aid. During the mid-1980s, SSA countries received approximately 50% of all food-aid in the developing countries.

Even under rapid population growth, the population density of Africa is relatively low at less than 20 persons km<sup>-2</sup>. In comparison, the average population density in China is estimated at 108 persons km<sup>-2</sup> and approximately 223 persons km<sup>-2</sup> in India. However, uneven population distribution and migration from rural areas is of major concern. During the 1980s, urban populations increased by 7% a year and when combined with the 3% overall population growth, many cities and rural centres grew at annual rates of more than 10%. It is projected that more than 50% of Africa's population in the year 2010 will reside in urban centres. Rapid urban growth places severe stress on urban services and increases rural labour shortages. It also forces scarce resources to be directed towards upgrading urban infrastructure to accommodate increased population, which in turn accelerates urban growth and restricts development of rural areas. This cycle will continue until quality of life and employment opportunities are improved in rural areas, and this can only happen with agricultural development.

## Food Security and New Trends for Cassava

Since its introduction into Africa in the 16th century, cassava has spread throughout SSA to

**Table 1** Cassava production by region and in selected African countries

Region/ Countries	1988	1989	1990	1991	1992	1993	1994	1995	1996
	----- million tonnes -----								
World	144.9	151.2	152.0	158.7	161.1	163.0	163.1	165.3	163.9
Africa	62.9	64.3	69.7	77.5	80.9	83.1	82.7	84.4	85.0
Asia	52.5	55.0	49.8	49.6	51.4	51.0	49.3	48.5	47.2
Americas	28.4	30.7	31.3	30.3	27.5	27.7	30.2	31.4	31.4
Nigeria	15.4	15.4	19.0	26.0	29.2	29.9	30.9	32.2	31.4
Ghana	3.3	3.3	2.7	3.6	4.0	4.5	6.0	6.9	7.0
Congo D.R.	17.4	18.0	18.7	19.5	20.2	20.8	18.0	18.9	18.8
Tanzania	6.9	6.9	7.8	7.5	7.1	6.8	7.2	6.0	6.0

become one of the dominant starch staples in the diet. Although the crop is grown in every country of the sub-continent, cultivation is concentrated in the humid tropical regions but it is expanding rapidly in the semi-arid zones. In 1994, Africa produced 88 M t of cassava, and since 90% of production is used for human consumption, it is estimated that cassava provides more than 200 calories  $\text{capita}^{-1} \text{day}^{-1}$ . It is the dominant staple particularly in Central Africa where it constitutes over 50% of the dietary caloric intake (FAO, 1996). In the coastal regions of West Africa, from Côte d'Ivoire to Nigeria, cassava is as important as yam, and further along the West African coast cassava is the second most important staple after rice. While rice may be imported to complement local production, cassava is always locally grown. In East Africa, although maize is the dominant staple in most countries, cassava is very important in Mozambique, Tanzania, Uganda, and Burundi.

Cassava is very important in the diet because per capita food production in Africa is declining. After three decades of independence, the continent has become food import-dependent; the unsatisfactory growth in the production of domestic staples has led to the importation of cereals. These imports cause the balance of trade to become increasingly negative which increases the external debt.

Cassava provides a good source of dietary energy and plays a significant role in national food security for several countries in the tropics. Annual per capita consumption averaged 95 kg in 1994. The highest consumption level is found in D.R. Congo with 391 kg  $\text{capita}^{-1}$ , followed by Nigeria with 250 kg  $\text{capita}^{-1}$ . In Nigeria, per capita cassava consumption rose to about 250 kg (which is double the 1980 level) during the ban on the importation of cereals in 1987-90. This was followed by a fall in consumption between 1991 and 1992 when the

cereals import ban was lifted; but since 1993, there has been an upward trend in consumption which is expected to continue.

#### New focus on cassava

In the last 10 years, the world cassava production increased by 22% and Africa's contribution to world production increased from 42.8 to 52.6%. In the same period, Nigeria became the largest producer of cassava in the world, with an estimated 30.9 M t produced in 1994 (Table 1).

Total production of cassava increased from about 35 M t in 1965 to over 84 M t in 1995, an annual growth rate of 2.9%, which matched the population growth rate. However, between 1985 and 1995, cassava production grew faster (3.8%) than population growth (2.5%) and this resulted in an increase in per capita cassava availability.

Contrary to Asia where over 27% of the cassava production is destined for export, and to Latin America where about 33% is used for animal feed, the African cassava production is primarily destined for human consumption. Approximately 89% of the cassava produced in Africa is used as food, particularly in the rural areas where 70% of the African population now live (Table 2).

Food production in Africa is fundamentally based on rain-fed farming systems. This makes

**Table 2** Uses of cassava by continent

Producing region	Food	Feed	Industry	Export	Waste
	----- % -----				
Africa	88.7	1.4	0.1	0.1	9.5
Asia	55.3	2.9	8.6	26.9	6.3
America	42.4	33.4	9.6	0.1	14.0

African farming inherently risky, with large variations in seasonal and annual food supplies. This precarious and highly variable production situation is made even more unstable in areas where land is scarce because of high population growth rate, which either reduces the farm size or induces migration to more marginal agricultural areas.

Cassava's adaptability to relatively marginal soils and erratic rainfall conditions, its high productivity per unit of land and labour, the certainty of obtaining some yield even under the most adverse conditions, and the possibility of maintaining continuity of supply throughout the year, make this root crop a basic component of the farming system in many areas of Africa. Famine rarely occurs in areas where cassava is widely grown, since it provides a stable base to the food production system.

Population growth rate in SSA has exhibited very little variation over three decades but annual output growth rates for both cassava and cereals have fluctuated, ranging from -2.5 to 11% for cassava and from -13 to 32% for cereals. This indicates that cassava is a more dependable food crop than cereals in SSA.

### Wheat in Africa

In the last 30 years, a fundamental transformation has been occurring in the food consumption patterns of many developing countries. Diets have been shifting away from traditional coarse grains and root crops to increasingly include more wheat products. Much of the increased wheat consumption is eaten in the form of commercially supplied bread. Between 1961-65 and 1975-77, developing country consumption of wheat increased at an average annual rate of 2.3% capita<sup>-1</sup> (CIMMYT, 1983). The similar value for rice was 0.4%. The direct consumption of coarse grains other than maize declined by 1.3%. In less than two decades, wheat moved from providing 20% of cereal calories in developing countries to a 27% share. In regions where wheat is not traditionally consumed such as West Africa, consumption of wheat grew at 5% annually. In 1981, total wheat import represented 2.9 M t for West Africa; 50% of that was supplied to Nigeria.

Wheat consumption is closely related to income levels and is significantly higher in urban areas. People prefer wheat products to other staple foods; as their incomes increase, they are likely to substitute wheat products for other staples in their diets, particularly coarse grains. The extent of urbanization is closely associated with national per capita income, and urbanization affects wheat consumption in a number of ways. On the demand side, urban dwellers are often willing to pay a premium for convenience foods (consumed away from home) that require

little or no preparation. This reflects the higher opportunity cost of food preparation time (usually for women) at home due to women working outside their home and high cost of transportation in cities. These factors favour bread consumption even though the price of bread is nearly double than that of wheat flour and it is usually higher than traditional staple foods.

In Africa like elsewhere in the developing world, there has been widespread market interventions by governments seeking to provide low-cost food to urban consumers at subsidized prices. As a result of these policies, wheat products are often cheaper relative to other foods. In the case of Côte d'Ivoire for instance, a subsidy is applied to wheat flour but not to maize grain. As a result, the price of maize is higher than that of wheat flour, whereas in Nigeria, tariffs are higher for maize relative to wheat, presumably to protect domestic maize production.

The high demand for wheat bread can also be explained by cheap import from major world producers (France, Australia, U.S.A., and Canada) using food aid and special institutional arrangements (CIMMYT, 1983, 1992-93). These policies together create a habit for, and dependency on, wheat consumption by West Africans. With a high population growth rate, low or no wheat production capacity, and increasing demand for wheat products, wheat imports have continuously grown in proportion to population growth (CIMMYT, 1983). The low price of wheat products in Africa has discouraged the use of local products such as maize, sorghum, millet, and cassava in bread making.

The trend may be changing. In the 1990s, most SSA countries have been implementing structural adjustment programmes that have been particularly tight on import subsidies. Many of these countries now find themselves unable to keep up with large wheat imports for their growing populations. With continuous balance of trade deficit *vis-à-vis* their major trading partners, it is necessary to find alternative solutions to provide bread to consumers. In the short run, some substitution of wheat flour with local flour (maize, millet, sorghum, and cassava) has been happening and should be encouraged. In the long run, policy makers should encourage research and development of new food products making more use of local raw materials.

### Prospects for Cassava Utilization

While wheat imports decrease in the region, bread is still largely consumed. This shows that people are using local products in bread making. Since 1982, cassava flour has been added to wheat flour directly at the mill in Côte d'Ivoire. In Nigeria, during the ban on cereals import, people were still consuming bread made either from smuggled wheat flour or from local crops such as maize, sorghum, and cassava

**Table 3** Opportunities for cassava in Nigeria in the 21st century (in million U.S. \$)

	Wheat substitution		Alcohol <sup>1</sup>	Starch <sup>2</sup>
	15%	20%		
Foreign exchange savings	14.8	19.5	2.06	—
Net returns to cassava processors	12.7	17.0	1.50	30.12
Gross returns to farmers	4.2	5.7	0.50	12.50

<sup>1</sup> Based on one factory's consumption of 30 t dry cassava chips per day

<sup>2</sup> Based on a production estimate 200 000 t starch yr<sup>-1</sup>

flour. The ban on cereal imports has allowed bakeries to adopt alternative solutions to stay in business. A recent survey in Nigeria and Côte d'Ivoire has shown that the bread consumed in the survey area was from composite flour (wheat mixed with either cassava, sorghum, or maize flour). This explains why per capita consumption of wheat decreases but consumption of bread is still increasing.

In addition to its traditional uses, cassava can be promoted as an industrial food ingredient comparable to wheat and as a modern input in the growing agro-industrial sector. In order to absorb the excess cassava supply and increase farm income, the highly perishable cassava roots can be transformed into high quality chips or flour, with a much longer shelf life than the fresh roots. The opportunities for cassava chips or flour include not only the baking industry but also the alcohol, starch, paper, textile, adhesives, and sweetener industries.

Some simulation results for alternative uses of cassava flour in Nigeria are summarized in Table 3. The analysis of these results show that Nigeria can save foreign exchange which could be then transferred to cassava processors and farmers. For instance, if wheat were to be substituted at a rate of 15% with cassava flour, Nigeria would save \$14.8 million in foreign exchange earnings. This savings would be trans-

ferred to cassava processors (\$12.7 million) and to cassava farmers (\$4.2 million). The main objective of policy makers should be to improve farmers' incomes rather than providing cheap food to urban consumers; such a policy would contribute to improving the quality of life in rural areas, slowing down urban migration, reducing poverty, and promoting national development.

Industrial uses of cassava are still very limited in Africa. Although cassava is potentially one of the most efficient starch-producing crops, cassava starch represents a small proportion of the starch used in African industries. Therefore, the potential for growth of cassava as an industrial raw material is great. Small-scale starch production or provision of raw material to large-scale starch producers could be an avenue for increasing the income of small-scale farmers. Cassava does not offer the same clear-cut possibilities for a major technological breakthrough as occurred through the development of high-yielding varieties of wheat and rice. Rather, improvements in income derived from cassava are likely to occur through complementary and integrated changes in field production methods, processing techniques, and marketing activities. Research will have to pursue these issues simultaneously (Berry, 1993).

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# The causes and consequences of taro leaf blight in Samoa and the implications for trade patterns in taro in the South Pacific region

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Before 1993, the taro export industry in Samoa was a success story. Based on private initiatives, the industry had developed impressively from the late 1960s on the basis of a market focus strategy directed towards an identifiable market segment in specific Pacific rim markets with large expatriate Polynesian populations. However, the taro industry in Samoa was decimated in 1993 with the incidence of taro leaf blight (TLB), caused by the fungus, *Phytophthora colocasiae*. The fungus attacks the leaves and stems of the taro, leading to either stunting or failure to produce a corm. In response, the Ministry of Agriculture, Fisheries, Forestry and Meteorology (MAFFM) instituted a number of initiatives to control TLB, including input subsidies, development of resistant varieties, and food crop diversification. As a short-term measure, it developed a control package combining fungicide applications and sanitation. Given that smallholders are the main producers and exporters of taro, the adoption rate has been minimal and, hence, the level of exports has fallen to almost zero. The medium-term strategy of MAFFM has been to introduce exotic cultivars with some known resistance or tolerance to the disease and reasonable palatability so as to increase future smallholder taro production. The long-term strategy is a breeding programme to build up the resistance in local varieties. The implications of TLB on the economic circumstances in Samoa, and for taro trade and domestic prices in the South Pacific region are also explored.

Keywords: Disease control; Leaf blight; Samoa; Taro; Trade

## Structure of the Taro Industry in Samoa Prior to Taro Leaf Blight (TLB)

### Taro in Samoan agriculture

Samoan agriculture is mainly subsistence in nature with 92% of production used to meet home consumption needs (Anon., 1990). Taro (talo) [*Colocasia esculenta* (L.) Schott] has been by far the most widely planted root crop since the mid-19th century when it replaced yams as the predominant starch staple (Brown, 1970). The agricultural census of 1989 found that 96% of agricultural holdings grew taro, and placed the area under crop at 14 600 ha of which 76% was grown as a monocrop and the rest in mixed cropping mainly with giant taro (ta'amua) [*Alocasia macrorrhiza*] and banana, often under old coconuts (Anon., 1990). Taro is usually the first crop planted on land newly cleared from forest or bush fallow (Wilson *et al.*, 1984).

Reliable estimates of the total production and the domestic consumption of taro are difficult to obtain. Estimates of domestic consumption have usually been derived from assumptions of daily intake per person. They have ranged from 0.5 kg to 0.68 kg person<sup>-1</sup> day<sup>-1</sup> which is between 20 000 t yr<sup>-1</sup> and 37 000 t yr<sup>-1</sup> (Burgess, 1981; Opio, unpubl.). Opio (unpubl.) estimated the total production of taro and giant taro to be about 26 000 t from 1600 ha in 1982. The ADB (1985) also estimated the area to be about 1600 ha in 1982. In 1989, the national agricultural census placed the area under taro at 14 600 ha, a ninefold increase over the 1982 value.

Assuming a yield conservatively at 10 t ha<sup>-1</sup>, the total annual output would be in the vicinity of 16 000 t in 1982 and 146 000 t in 1989. If these estimates were correct, output would have fallen short of consumption and exports in 1982 while far exceeding the quantities consumed locally and exported in 1989, even allowing for a substantial amount of taro that

was exported as cooked corms. In the 1980s, taro was the major agricultural export. Exports peaked at just over 8000 t in 1989 (Central Bank, 1991) and taro was the highest overall export earner between 1991 and 1993. In 1993, 6300 t were exported

### Factors Encouraging Growth of Taro Exports in Samoa

Because taro is the dominant staple crop in Samoa, it has a strong domestic market base. Following the steady emigration of many Samoans to Pacific-rim countries, the domestic market was extended to form a highly efficient commercial marketing system for taro exports. Family ties created strong links at all stages of the marketing process to develop a niche in the export market. The marketing channel was often shortened where family members in Pacific-rim countries were the taro importers. This provided an advantage to the export industry where overseas consumer preferences in colour, taste, and size of taro Niue (*C. esculenta*) were well understood by the producers, exporters, and importers.

Taro has several production advantages which allowed the export sector to expand. It grows all year round and, until 1993, TLB was very resilient to pests and diseases. Ideal for smallholders, the crop required very low levels of purchased inputs and low technology requirements at the post-harvest stage, and achieved relatively high returns to labour (World Bank, 1991). These factors made the domestic and export markets accessible to both smallholders and largeholders.

According to Samoans, taro Niue is superior to other taros grown in the Pacific, with the possible exception of Niue, after which the variety is named. Together with the strong cultural importance Samoan people attach to taro Niue, exporters were able to maintain market share by differentiating their product from other competing taros.

The major commercial disadvantages of taro exports prior to TLB were its low value:weight ratio and short shelf life, plus price volatility in the main export market of New Zealand (Brown, 1995). The potential disadvantages of exporting a crop such as taro are its village-based production and a lack of entrepreneurship among village-based smallholders, yet growth of the taro export industry contradicts these propositions. The fact that taro is a crop of great traditional social significance does not appear to have adversely affected its commercialisation for export. Further, evidence suggests that entrepreneurship can flourish within village-based export production and marketing systems although, in the case of Samoa, the ability to 'quarantine' profits in bank accounts in importing countries (especially New Zealand) may have been crucial (Fleming, 1996).

### Onset of TLB in 1993

Export success brought with it more intensive taro cultivation with fewer rotations and greater resort to monocropping, in contrast to the traditional mixed cropping system with its well-developed crop rotations. This more intensive production system made producers more vulnerable to the damaging effects of pest and disease outbreaks, typified by the onset of TLB in 1993.

### Incidence and causes

Taro leaf blight disease, caused by the fungus *Phytophthora colocasiae* Raciborski, was unknown in Samoa before 1993. It had been present in neighbouring Papua New Guinea and Solomon Islands as well as Hawaii since the Second World War. It is generally believed that TLB entered Samoa with unauthorised imports of infected taro corms or planting material. The disease was first identified in the eastern part of one of the main islands of Samoa, Upolu in July 1993. Aided by unseasonably wet weather and contiguous plantings of highly susceptible local varieties, the disease had spread to all parts of the island and much of Savaii the second main island of Samoa by the end of the year. The disease is now endemic in the country and it survives on the plant during the dry season and becomes active during the wet season. All local taro varieties were highly susceptible and the plant size, number of leaves per plant, and corm yields are significantly reduced if the disease is not controlled during the wet season. In the worst cases, only the youngest leaf remains uninfected.

Infection begins as tiny water-soaked specks on the leaf. These quickly enlarge into roughly circular brown lesions. Typically, a clear brown liquid is exuded at edges of lesions during the night but stops before sunrise. This exudate contains large numbers of sporangia and provides the source of new infection. During the day, the exudate slowly dries to form a reddish-purple crust. The lesion develops rapidly under favourable conditions, causing new infections within two days and a leaf can be completely infected in less than seven days. The disease is spread in rain splash and further afield in wind-driven rain. A few days of continuous rain can very quickly transform a healthy plantation into a severely diseased one.

### Impact on yields

As the name suggests, *Phytophthora* leaf blight is primarily a disease of the leaves and its depressive effect on corm yields is through the reduction of leaf area. The pathogen has also been found to be a cause of corm rot during storage (Butler and Kulkarni, 1913; Jackson and Gollifer, 1975). This is of little concern at present. Because of scarcity, corms are not stored for any length of time and exports are

only to neighbouring American Samoa. Further, the sudden loss of leaf area to the disease causes the corms to become watery and thus unpalatable as stored starch is converted to sugars. Such corms, which Samoans described as being *susu*, are unfit for eating. Losses also extend to the young leaves which are popular as vegetables.

Good control of the disease with fungicides has been reported by Trujillo and Aragaki (1964) and Trujillo (1967) in Hawaii, and Jackson *et al.* (1980) in the Solomon Islands. While these authors reported that fungicides have increased corm weights by 50–60%, they did not indicate how these 'increased' weights compared with taro grown in the absence of the disease. For example, in a trial carried out in Samoa, fungicides increased corm yield by 44% but corms weighed only 339 g (Semisi *et al.*, 1995). Observations in Samoa indicate that corms are now generally smaller due to the loss of leaf area as a direct result of the disease or through the sanitation measures carried out. The reduction depends on the severity of the disease. While a healthy plant would carry up to eight leaves, a plant with four to six leaves is now the norm.

Pre-TLB corm weights in Samoa were between 1.18–1.30 kg (Cable and Asghar, 1981) and 1.5–2.0 kg (Wilson *et al.*, 1984). An average of 1 kg corm<sup>-1</sup> was typically used in yield estimation. With TLB, corm size depends on the severity of the disease. *Ad hoc* checks made on farmers' plots where some effort was being made to control TLB showed that weights of corms varied between 0.4–0.8 kg, which is a substantial loss. Closer spacing to offset the smaller corms is not recommended as it would make the disease more difficult to control.

Levels of inputs necessary to control the disease depend closely on weather conditions. Under high rainfall conditions in Solomon Islands where disease pressure was high, Jackson *et al.* (1980) were unable to control the disease solely by roguing infected leaves. Gomez (1925), on the other hand, was able to control the disease in the Philippines by roguing every two days. The Ministry of Agriculture, Fisheries,

Forestry and Meteorology (MAFFM) recommends a combination of fungicide spray and sanitation at two-day intervals, with spray intervals varying with weather conditions (MAFFM, 1995).

As the introduced varieties are more resistant to the disease, it is expected that levels of inputs would be lower, particularly if grown in the lower rainfall areas of the country. Vasquez (1990) found that the amount of yield reduction depended on the levels of resistance to the disease. With no control, TLB reduced corm weights in resistant varieties by only 2.94–4.67% but reduced those in moderately resistant and susceptible varieties by 31.75–36.51%. Thus, the answer will eventually lie in growing resistant and palatable varieties in the lower rainfall areas of the country, but it is unlikely that taro will return to the dominant position it occupied in Samoan agriculture before TLB.

### Impact on export markets

As a result of TLB, the volume of taro exports fell dramatically in 1993 (Figure 1). For the first half of 1993 the monthly average export earnings were WS \$968 000 (U.S. \$392 000) but, by 1996, averaged only WS \$8000 (U.S. \$3240) per month (Central Bank, 1997). The number of licensed export earners fell from about 160 in the early 1990s to around 25 in 1996 (World Bank, 1991; Central Bank, 1997). In February 1997, taro exports were less than 1% of the total value of exports.

The reduced taro supplies to the New Zealand market caused prices to rise significantly. By 1994 the wholesale price of taro in New Zealand was NZ \$3.15 kg<sup>-1</sup> (U.S. \$2.05 kg<sup>-1</sup>) compared with a retail price in early 1993 of between NZ \$0.90 kg<sup>-1</sup> (U.S. \$0.60 kg<sup>-1</sup>) and NZ \$2.00 kg<sup>-1</sup> (U.S. \$1.30 kg<sup>-1</sup>) (Moody, 1994). Consequently, New Zealand sought alternative Pacific suppliers in Fiji, Niue, and Tonga. By the end of 1994, New Zealand had increased its imports of taro from Fiji 10 times (U.S. \$251 050 in 1993 compared to U.S. \$2.583 million by the end of 1994). The value of taro imported from Fiji continued to rise throughout 1995 and 1996, with nearly

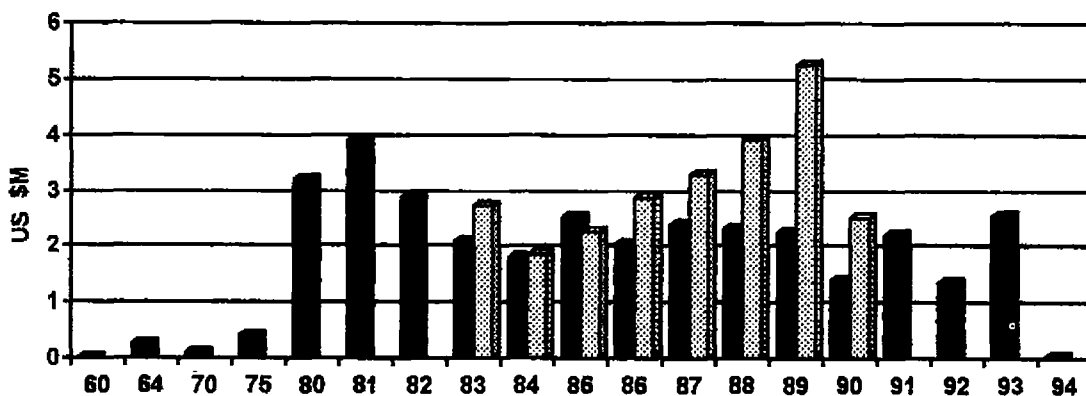


Figure 1 Value of taro exports from Samoa (1960–94); (■), Official estimates, (▨), World Bank Mission estimates Source: Fleming (1996)

NZ \$9 million (U.S. \$5.85 million) imported in 1996, a level similar to volumes imported from Samoa before TLB (SPTO, 1997).

Price data on domestic prices of taro relative to its major competitors, rice and flour, were obtained from the Bureau of Statistics (1997), and the two relative prices were regressed on time for the period during which Fiji has increased its taro exports, from 1993 until August 1997. Results of the two regressions show a negative but insignificant coefficient on the time variable. This suggests that the domestic price of taro relative to its major competitors had not risen during the period of increased taro exports.

Other South Pacific countries have been less successful in exploiting the absence of Samoa from the regional export market for taro. Tonga, which mainly produces a type of taro less preferred by consumers in importing countries and has a less well established marketing network than Samoa (Fleming, 1996), experienced relatively little growth in taro exports. Root crop exports did increase, with a lag, and reached almost T \$1 million (U.S. \$0.80 million) in 1995-96, which was almost double the value of T \$0.54 million (U.S. \$0.41 million) in 1991-92 (National Reserve Bank of Tonga, 1996). Tongan exporters have managed to achieve a greater variety of root crop exports than those from other South Pacific countries and part of the recent upsurge has been due to increases in exports of yam and other root crops. Supplies of root crops to the domestic market in Tonga have been maintained despite this increase in export supply. In fact, the price of domestic supplies of root crops actually fell by 27% between 1993 and 1996 (National Reserve Bank of Tonga, 1996). Because Niue produces the same variety of taro as Samoa, it was able to increase its exports in the wake of TLB in Samoa. However, its small area of agricultural land and poor international transport links mean it has not become a serious competitor in regional trade in taro (Fleming, 1996).

Most of the taro in the post-TLB era has

been exported to American Samoa, with only small irregular shipments to New Zealand. In 1996, Samoa exported only NZ \$65 000 (cif.) (U.S. \$42 250) to New Zealand (SPTO, 1997). Local taro prices in Samoa in April 1997 were still higher than the export prices, indicating a continuing shortage in the domestic market. In 1995, the domestic price for taro was 30 to 35% more than the export price to New Zealand (U.S. \$0.91 kg<sup>-1</sup> to New Zealand compared to U.S. \$1.39-1.53 kg<sup>-1</sup> at the local Apia market). Hence, taro exports are not likely to resume their pre-TLB levels until the domestic demand is satisfied and the production of taro increases, thereby reducing the domestic price and profits to growers.

### Impact on taro output, prices, and income

The impact of TLB is shown quite dramatically in Figure 2. In early 1993, over 20 t of taro were sold every month at the local Apia market. By July 1994, none was reportedly sold commercially. Small amounts were sold in 1995, usually not more than 200 kg week<sup>-1</sup> (Department of Statistics, 1997). As a result of the supply shortage, prices of taro increased sharply from about WS \$1.25 kg<sup>-1</sup> (U.S. \$0.49 kg<sup>-1</sup>) in 1993 to WS \$4.90 kg<sup>-1</sup> (U.S. \$1.98 kg<sup>-1</sup>) at the end of 1996 (Department of Statistics, 1997).

The more commercial farmers have profited from the rising domestic taro prices. With greater access to working capital than smallholders, they have been able to invest in the TLB control package which is a combination of crop sanitation and fungicides, developed by MAFFM. The cost of chemicals has made it difficult for smallholders to replant their traditional staple for cash as well as home consumption.

Since TLB, consumption of bananas and giant taro has increased significantly (Figure 3). However, the willingness of consumers to pay the high price for the limited quantities of taro available suggests that taro is still a strongly preferred staple.

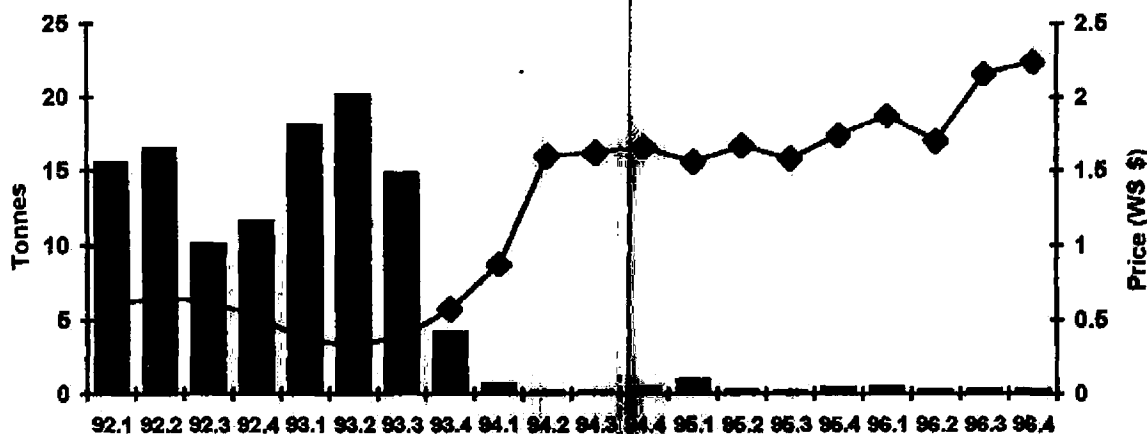


Figure 2 Domestic supply and price of taro (1992-96). (■), Supply, (—◆—), price. Note: Supply figures are in monthly averages for each quarter. Source: Department of Statistics (1997)



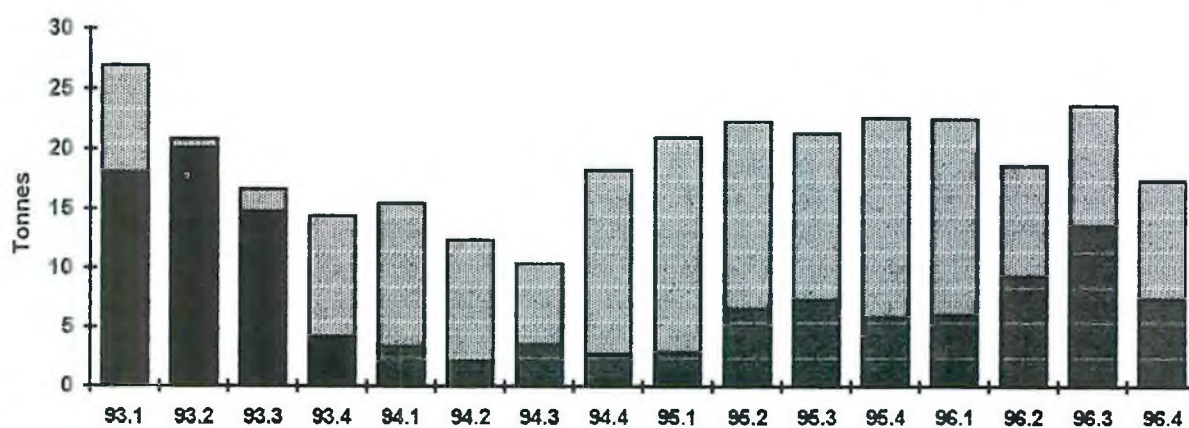


Figure 3 Domestic supply of major staples (1993–96): (▩), Banana, (▨), giant taro, (■), taro  
Source: Department of Statistics (1997)

### Rehabilitation of the Taro Industry

A three-pronged approach has been taken by the Government of Western Samoa and the Western Samoa Farming Systems Project (a USAID funded project) to rehabilitate the taro industry in Samoa. The short-term solution is to control the disease with fungicides, sanitation, and cultural measures. As these recommendations require increased inputs of cash for fungicides and spraying equipment, and labour for spraying and sanitation, they have been incompatible with the traditional Samoan farming methods. Consequently, despite increased returns from the higher taro prices (Wood, unpubl.), it has only been adopted by a small number of commercial growers with the majority of farmers opting to grow bananas and giant taro.

The successful rehabilitation of the taro industry will thus depend on farmers being able to grow the crop with minimal cash and labour inputs. Towards this end, varieties with increased resistance to the disease have been introduced and those with palatable corms have been disseminated to farmers. The aim of such introductions is to provide farmers with a wide range of resistant varieties from which to choose. Farmers have already identified one of the introduced varieties as being better for its leaves than its corms. Lastly, MAFFM is involved in breeding for improved TLB resistance and taste but this is a long-term solution not expected to produce results in less than five years. The options for coping with TLB are discussed more thoroughly in SPC (1997).

### Diversification and future sustainability of the farming system

Since TLB, the clearing of forests for taro cultivation has virtually ceased, with a positive impact on the sustainability of forestry resources. The decline in taro production has also led to a more diversified cropping system. Substitute staple crops have been planted, such as giant taro, bananas, and other root crops. The

MAFFM has also been encouraging the development of vegetable gardens as an alternative cash crop as well as to increase nutritional value in the local diet.

### Recovery of the export market

Taro leaf blight has badly eroded three of the most advantageous attributes of taro as an export crop. The first two events, loss of resilience to pests and diseases and increased requirements of purchased inputs make the crop now relatively less profitable. Thirdly, recent increases in production in competing countries, such as Fiji, of the preferred taro Niue variety have meant that Samoa's traditional advantage as a supplier of taro preferred by consumers in importing countries has been eroded. Loss of resilience to pests and diseases and increased requirements of purchased inputs forced taro producers in Samoa to become heavy users of plant protection inputs. They have forced the taro industry into the high-input category and put it at a disadvantage in other South Pacific countries free of TLB.

Budgets of taro production were compared for Samoa and Fiji, the major competitor in the export market if Samoa were to re-enter in a significant way (ADB, 1996; Milne, unpubl. data). The Samoan budget assumes use of the management regime recommended by MAFFM to combat TLB. Estimates of material and labour inputs and yields are based on information from MAFFM Research and Crop Divisions, the private sector, growers, and buyers. Because of different treatments of labour, it is difficult to compare gross margins per hectare, so it is best to concentrate on returns to household labour. Equivalent farm-gate prices are assumed, based on the value provided in the budget for taro producers in Fiji (ADB, 1996). After adjusting for currency differences, the returns to household labour in Fiji are 1.75 times those in Samoa, even though the suckers for planting were not given a cost in the budget for Western Samoa. Even allowing for the rubbery na-

ture of such values, given variations in yields among farmers, production in Fiji is likely to be more profitable. This should make it difficult for Samoan taro producers to compete with Fijian producers given their need for a high-input regime post-TLB. It could be argued that Samoan taro would likely earn a significant premium over Fijian taro given consumer preferences in import markets. Also, the opportunity cost of labour is higher in Fiji. But the premium would need to be very high and the differential in opportunity cost substantial, to offset the estimated differences in returns.

### Lessons in Niche Exporting from Experiences with TLB

Fleming (1996) gleaned six lessons from the experiences of the taro industry in Samoa over the past two decades. Four of these suggest there is room for optimism in niche exporting in small countries such as Samoa. They are (a) the critical importance of knowledge of production processes, product attributes, and consumer preferences to achieving export success; (b) evidence that the cultural importance of a traditional crop is not necessarily a barrier to its commercialisation for export; (c) evidence that entrepreneurship can flourish within village-based export production and marketing systems; and (d) the impressive performance by strong private marketing networks, reflecting vertical integration or coordination in marketing channels.

On the other hand, the sobering experience of TLB suggests a need for caution in two respects: (a) intensification of production of traditional crops carries with it increased risk of damage to the agroecosystem; and (b) a pest and disease-free status is an advantage possessed by many South Pacific countries that can be critical to export success, and needs to be rigorously protected.

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# Tropical root crops: Sustainable food security for the 21st century

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Tropical root crops are important staples for food security in the 21st century. The major production and consumption of the tropical root crops are concentrated in the regions of Africa, Asia, Latin America, and the Pacific. It is also in these regions where most of the growth in the world population will occur in the 21st century, placing great pressures on food security and environmental management. Tropical root crops have advantages over other staple foods such as grains, for ecologically sustainable production. They are less dependent on agricultural chemicals for production and post-harvest storage, require little irrigation, can be produced with minimum tillage practices, have high energy efficiency ratios, and generate large amounts of food for minimal effort. Tropical root crops provide important policy choices towards food security and poverty reduction through ecologically sustainable development.

Keywords: Food security; 21st century; Root crops; Staples; Population growth

## Tropical Root Crops as Important Staple Foods

Tropical root crops are important staples for food security in the 21st century. Throughout recent human history, tropical root crops have been produced and consumed in the poor regions of the world with heavy concentration in parts of Africa, Asia, Latin America, and the Pacific. This trend is likely to continue in the 21st century. The production of cassava, sweetpotato, yam, taro, and potato by the major world regions in 1995 is shown in Table 1.

Although potato forms the largest share of root crops in the world, only 20% of world production is produced in the tropics. Hence, potato as a staple is not as important as cassava and sweetpotato, but is more important than yam and taro in the tropics. It is an important staple food in poor communities in the tropical highlands of North-Central America and Asia.

Among the other four tropical root crops, cassava (50.5%) and sweetpotato (37.7%) have the highest levels of production, but smaller amounts of yams (10.0%) and taro (1.8%) are produced. Most of the cassava is produced in Africa, Asia, and South America, while sweetpotato is heavily concentrated in Asia. Africa produces most of the yam and taro.

The consumption of root crops (kg capita<sup>-1</sup>) by the major world regions is shown in Table 2. In Africa, the per capita consumption of tropical root crops is 182 kg, with cassava (114 kg) and yam (43 kg) being the most im-

Table 1 Root crop production (1000 t) in 1995

	Cassava	Sweet-potato	Yam	Taro	Potato
Africa	82 776	7 475	31 605	3 564	7 214
North-Central America	1 004	1 069	477	22	25 539
South America	31 450	1 310	315	10	12 484
Asia	48 358	111 556	213	1 759	85 620
Oceania	189	568	288	331	1 441
Europe and USSR	—	56	1	—	148 381
World	163 777	122 034	32 899	5 686	280 679

Source: FAO Production Yearbook (1995)

Table 2 Root crop consumption (kg capita<sup>-1</sup>) in 1995

	Cassava	Sweet-potato	Yam	Taro	Potato
Africa	114	10	43	5	10
North-Central America	2	2	1	—	56
South America	98	4	1	—	39
Asia	14	33	—	1	25
Oceania	7	20	10	12	51
Europe and USSR	—	—	—	—	186
World	29	21	6	1	49

Source: FAO Production Yearbook (1995)

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portant. In North-Central America, 61 kg capita<sup>-1</sup> of root crops are consumed, and potato accounts for the largest share. The per capita consumption of tropical root crops in South America is 142 kg, dominated by cassava at 98 kg. In Asia, sweetpotato and potato are both important in the total per capita consumption of tropical root crops of 73 kg. The per capita consumption of root crops in Oceania is 100 kg with potato and sweetpotato being the most important.

Globally, tropical root crops are important staple foods. If it is assumed that only 10 kg capita<sup>-1</sup> of potato (20% of the world production) is produced in the tropics, the tropical root crops account for 67 kg capita<sup>-1</sup> on the world population basis. Tropical root crops compare very favourably with the per capita consumption of staple cereals such as wheat, rice, maize, barley, and sorghum in the world.

### Staple Food Supply Potential of Tropical Root Crops

Tropical root crops have great potential to alleviate shortages of staple foods in many countries in Africa, Asia, and Latin America. The strategy to increase the supply of tropical root crops as fresh food can only be economically viable if supplies are met almost entirely from local and domestic production. This is because the cost of regularly importing large amounts of basic perishable foods, such as tropical root crops, would be prohibitive for many Less Developed Countries (LDCs). There are also the added problems of bulk, perishability, freight costs, and taxes.

Tropical root crops can produce large amounts of food per unit of labour or per unit of time. This aspect has been well documented by the pioneering works of Coursey and Haynes (1970) and Coursey (1984). The highest recorded edible yields of selected root crops are presented in Table 3. The data indicate the potential of root crops for contributing to the energy requirement of the population. They also show that even after the green revolution in rice and no major improvement in tropical root crops, these crops can still produce greater

**Table 3** Highest recorded edible yield per crop

Crop	t ha <sup>-1</sup>	kJ ha <sup>-1</sup> (million)	Country
Cassava	68	384	Brazil
Sweetpotato	47	212	Taiwan
Yam	36	163	Nigeria
Taro	65	255	Hawaii, U.S.A.
Potato	72	226	Netherlands
Rice	8	118	Japan

Source: Chandra (1994)

yields than rice, both in total edible yield and in food energy. Even when yield per unit time is considered, cassava and sweetpotato can still outyield rice. In terms of total dry matter (DM) production, Doku (1984) estimated that the use of improved varieties under conditions of good husbandry would result in yearly production levels of 140 t ha<sup>-1</sup> for cassava and yam, and 200 t ha<sup>-1</sup> for sweetpotato and taro. Therefore, the highest recorded edible yield per crop shown in Table 3 is only a fraction of the total DM production that can be attained in these crops.

Another advantage of tropical root crops is that some yield components can be harvested and consumed during crop growth, a very desirable characteristic for subsistence and semi-subsistence farmers. On the other hand, cereal grain crops have a specific maturity period which lasts a few days. Therefore, the potential risk of complete crop failure due to natural calamities such as floods, is high.

Most tropical root crops are adapted to a wide range of climatic and edaphic environments, hence, they can be grown in most of the agro-climatic zones present in the tropical and subtropical world. Each crop also has a large number of cultivars, each suitable for a particular locality within an individual country. The plants can also readily adapt to new conditions as has been the historical experience with potato. All tropical root crops are able to produce some yield even under harsh tropical conditions with minimum inputs. They are less dependent on agricultural chemicals for production and post-harvest storage, can be grown without irrigation and with minimum tillage practices, have high energy efficiency ratios, and can produce large amounts of food with minimal effort. No other major staple food can claim these characteristics. The challenge for development policy-makers is to recognise these important attributes of the tropical root crops and to use them to assist poor people to produce more food on their own farms.

Tropical root crops have a strong historic linkage with mankind which dates thousand of years, and indicates their potential for ecologically sustainable development. Farmers would welcome suitable research and development, and extension in tropical root crops is likely to be readily accepted by farmers.

The large investments made in the 1960s and 1970s in rice, wheat, and maize to achieve the objectives of the green revolution and improve the socio-economic conditions of millions of small farmers should be repeated for tropical root crops (Chandra, 1984).

### Food Security and Poverty Alleviation

Tropical root crops have the potential to make major contributions to food security in the LDCs where these crops are important staple

foods. If food production could be increased and sustained at a rate above the rate of population growth, the standard of living of large numbers of rural people will improve. The problem is one of achieving sustainable food production (and agricultural production) at a rate above 3% per annum which is the rate of population growth in many LDCs of Asia, Africa, and Latin America.

Since the 1960s, technological advances in agriculture have substantially contributed to growth in crop production in the LDCs, particularly in Asia. Despite this, food demand has continued to exceed supply and trade has increased as imports rise to meet domestic production shortfalls. Between 1965–95 food consumption in the LDCs grew at a rate of 3.3% yr<sup>-1</sup> while production grew at a rate of 3.1% yr<sup>-1</sup>. Food production growth was slowest in West Africa (<1% yr<sup>-1</sup>) compared to an average yearly increase in the population of 3%. Population growth has been the dominant factor in food consumption growth in the LDCs, but income-induced consumption growth is becoming an increasingly important factor in several countries, especially in East and south-east Asia. The feed and other non-food uses and allowance for waste (estimated to be 15%), and the income elasticity of demand for these commodities are low (Mellor, 1988).

### Ecologically Sustainable Development

Tropical root crops have certain characteristics which promote more environmentally sustainable cultivation practices by avoiding the need for large inputs of chemicals, for fertilization and pest and disease control, irrigation, and mechanization thereby generating a lower fossil fuel usage with a lower impact on global warming.

### Strategies to Achieve Socio-economic Improvements for Tropical Root Crop Farmers

Decreasing poverty and improving the nutritional status of the poor in the LDCs where tropical root crops are important staple foods, must be based on three key strategies.

First, there has to be an efficiently operating research and extension organization. Researchers must produce information that will result in a wide adoption of technologies leading to productivity increases. Such technologies cannot simply be transferred from other countries or international agricultural research centres because they generally require adaptation. For research and extension services to function efficiently, huge investments have to be made into human capital formation and institutional development. Development assistance for tropical root crops can, therefore, be valuable. Second, correct price formation and the development of an effi-

cient market system will enable both the producers and consumers to respond in a manner that will lead to economic growth and equity improvement. Third, investment in rural infrastructure, particularly roads, schools, electricity, telephones, clean potable water, and health facilities will enable improved basic needs for the majority of the population. Improved rural living standards will reduce the tendency for trained personnel to migrate to urban areas in search of better amenities. Such infrastructure development is also essential to ensure better food security in the 21st century.

### Conclusions

One of the greatest challenges of the 21st century will be improving the staple food security of poor and poverty-stricken people in many regions of the world. In those regions where tropical root crops are already important staple foods, there is a great potential to improve the production systems and marketing so that cheap food continues to be readily available to vast numbers of people. Greater research, development, and extension efforts in tropical root crops in countries where these crops are important staple foods, is one way of meeting this challenge. An important characteristic of the tropical root crops is that they have the potential to produce large amounts of cheap food for both rural and urban households. This is recognised by most researchers and extension workers in tropical root crops. What is needed is support from Governments and development policy-makers so that investments into tropical root crops can have high returns which, in the long term, and will make major contributions to the food security and socio-economic improvements for vast numbers of poor and hungry people in the LDCs.

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# Conceptual issues in the use of cassava modelling for improved production in drought environments

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Drought is responsible for large reductions in cassava root yields. On-farm root yields can be improved two- to three-fold and quality of fresh and processed products improved by the adoption of recently improved production technologies. The potential usefulness is high for genetic, morphological, physiological, and agronomic interventions. The use of cassava growth models can be valuable in the decision-making process to improve cassava productivity. The use of a modelling approach to minimise the savannah farmers' risk in cultivating cassava in drought environments is high. However, this approach needs urgent attention to improve on delivery of outputs to cassava growers. Conceptual and logistical issues associated with the use of cassava growth modelling approach to assess the fitness of improved interventions are discussed.

Keywords: Growth models; Simulation; Minimum data sets; Climate; Cassava environments; Abiotic stress

## Status of Cassava in Africa

The African continent is the leader in cassava production, with an annual production of 82 M t of fresh roots. Recent increases in production is associated mainly with an increase in the area of production (Manyong and Oyewole, 1997) and to a lesser extent, an increase in root yield per unit area, and a shift in cassava cultivation from the traditional humid forest to the savannahs. Therefore, it is increasingly grown in suboptimal environments (Dixon *et al.*, 1994; Ekanayake *et al.*, 1996). Recent observations on cassava distribution indicate that cassava is now cultivated more regularly throughout the sub-Saharan region. A severe and prolonged drought in the Sahelian region (Semazzi and Suri, 1997) may have improved the status of cassava as a choice crop by those farmers.

Cassava, in contrast to other staples, grows and produces marketable roots under favourable as well as marginal ecological conditions of rainfall or soil fertility. It is distributed across a wide range of agro-ecozones and across ecological niches within a zone, due to its adaptability to various ecological and sociological conditions. Optimum environmental characteristics for cassava are previously described (Carter *et al.*, 1992; Ekanayake, 1994; Ekanayake and Jagtap, 1997).

## Drought-prone Cassava Environments

Drought-prone agro-ecological zones for cassava in West and Central Africa are forest-savannah transition, southern Guinea savannah,

northern Guinea savannah, mid-altitude savannah, and Sudan savannah zone. Drought is the most limiting factor for cassava cultivation in these areas despite the well known drought tolerance of the crop (Ekanayake *et al.*, 1994, 1996).

Cassava-growing environments are complex (Ekanayake, 1994). The agro-ecological zoning as defined by the International Institute of Tropical Agriculture (IITA) and the Technical Advisory Committee (TAC) uses rainfall distribution, potential evapo-transpiration, and temperature issues for constraint-free environments (IITA, 1992). A consensus has been reached on a global edapho-climatic classification for cassava production by an overlay of IITA approach with the Cassava Atlas classification (Carter *et al.*, 1992) which is now Geographic Information Services (GIS) formatted using evapo-transpiration estimates to determine wet or dry months, and classes are derived from a set of climate conditions for cassava cultivation covering global cassava production. The dominant cassava ecology is the upland in West Africa. A minor cassava ecology is the inland valley swamp ecology.

## Rationale

The use and refinements of crop and system modelling can address the current and anticipated problems associated with cassava production which are common to several agro-ecological zones and ecosystems at different levels of integration. It can predict the efficacy of various mitigation measures to reduce the vulnerability of cassava and improve productivity under different climatic conditions. The objective of this paper

is to examine the conceptual issues associated with the use of cassava growth modelling approach to improve cassava production in drought-prone savannah environment in West Africa.

### Crop Modelling Approach to Improved Cassava Production

Simulation modelling can play an important role in understanding the significance of various environmental factors and specific abiotic and biotic stresses which occur in a given environment on overall cassava plant growth. Complex interactions among various biotic and abiotic stress factors, and their influence on physiological processes can be studied with the use of simulation models. This approach also requires a continual refinement of models as more information and tools are made available.

### Cassava Models — Conceptual Issues

Several comprehensive and dynamic cassava crop simulation models and decision support systems have been assembled in the last three decades by integrating experimental data and

modelling activities (Table 1). These models are used to predict cassava growth, potential production, and its agro-economic viability under various tropical and subtropical conditions and to improve the understanding and decision-making on more efficient use of water, soil, nutrient, and other resources for cassava production. Some of these models are also aimed at predicting production levels under various growing conditions, but not specifically with reference to water and nutrient depletion problems. Accuracy of prediction of cassava growth, pest and diseases dynamics, root yield and quality, and functionality in terms of user friendliness of these models are widely different. Most of these models are used to a very limited extent compared to their potential usefulness and given the number of researchers involved in root crop research, where a modelling approach is useful.

More recently, a few cassava physiologists got together to promote the wider use of cassava growth models in the light of widened availability and use of computer-based tools for the national agricultural research systems. A significant output of this effort is the creation of a global cassava modelling network which will be used to create a pool of trained scientists in cassava crop growth modelling to benefit the end users.

In modelling, the essential processes associated with growth are described by mathematical equations such that a model behaves as a field-grown cassava plant and a plant community. Requirements for developing and using a model are genetic coefficients, climatic data, and systematic observations of various processes (phenology, soil water, and nutrients) under non-stressed (optimum) and stressed conditions or environments. For a given crop model to be generic, a set of coefficients that specify the genotype  $\times$  environment interactions is used. Genetic coefficients make it possible for models to predict the performance of genotypes on a global scale, independent of location, season, and management. These coefficients are usually estimated from field experiments under optimum growing conditions. Therefore, genotype coefficients remain the same for all locations. Software packages are available which are used to determine genotype coefficients to simulate the responses of diverse genotypes to different environmental factors (Hunt *et al.*, 1993).

The fitness of an improved technological intervention to an environment depends on the extent to which it overcomes and provides answers to the limitations of that particular environment. An array of technologies 'on the shelf', that can easily be adopted by small and marginal cassava farmers in savannahs are available. Models can be used to simulate the outcomes at various and increasing levels of complexity. The predictive capacity of a model also depends on the complexity of the environment (genotype, climate, and management factors). Associated with this complexity is the increasing

**Table 1** Summary of modelling activities and growth models available for cassava and *Dioscorea* (yams)

Models and updates	References
Cassava	
DSSAT version 1994	Hunt <i>et al.</i> (1994); GCTE (1997)
DSSAT version 1996	Hunt <i>et al.</i> (1994); GCTE (1997)
CROPSIM-cassava (CSSIM 1995)	Mattews and Hunt (1995) as referred in GCTE (1997)
CSSIM97	Hunt, as referred in GCTE (1997)
HYCAS	Mattews (1997) as referred in GCTE (1997)
Dynamic model for semiarid cassava	Gray (1997)
Tritropic pest interaction model	Gutierrez <i>et al.</i> (1988a, b)
Growth simulation model	Fukai and Hammer (1987)
Modified version SUCROS	Gijzen <i>et al.</i> (1990)
Yam	
Growth model	Spijkerboer (1990)
Growth model approach	Onwueme and Haverkort (1991)

demand for input data for a model to be an efficient predictor. The level of complexity to which a user wishes to simulate a response is also dependent on the availability of input data. Existing data sets can be used for model building, validation, and simulation purposes. Further data need to be collected where potential answers to queries require more information in the modelling process.

### Cassava Models — Logistical Issues

A specific example in which the use of growth-modelling intervention in cassava can be used, is the integration of host plant resistance and biological control of introduced pests and pathogens. Biological control of cassava mealybug through use of a natural enemy, *Epidinocarsis lopezi* parasite, along with improved clones and agronomic practices, provide a cost-effective, sustainable, and environmentally-friendly technology for cassava farmers. Management interventions of complex plant-parasite-pest systems are possible only by understanding the mechanisms involved at the scale of the individuals of the populations and their interactions between various components of the system (Fabres *et al.*, 1994). Systems analysis techniques and simulations, therefore, permit higher-order predictions, i.e., tritropic interactions through quantitative analysis (Savary and Zadoks, 1991).

Cassava green mite (CGM) has had devastating effects in farmers' fields. The impact of improved technologies on sustaining root yields under natural CGM pressures is shown in Table 2. Such tritropic information are

**Table 2** Cassava root yields ( $\text{g m}^{-2}$ ) at various growth stages as influenced by cultivar (host plant resistance) and intervention technology (biocontrol) to control cassava green mite (Ibadan, Nigeria, 1996–97 season)

Intervention technology	Three months of age	Six months of age	Nine months of age
Chemical control ( $\pm$ biocontrol agent)			
Dimethoate	339	1232	1562
Control	364	1320	1393
Permethrin	342	1003	1212
LSD <sub>0.05</sub>	73	138	245
Host plant resistance (cultivar)			
92/0427	538	1582	2044
92/0326	479	1509	1720
91/02327	407	1494	1487
30572	330	1101	1185
TME2	212	935	1150
30001	123	488	745
LSD <sub>0.05</sub>	103	73	346

Source: Hunt *et al.* (1994)

useful in model development in order to control this pest.

### Limitations of Simulation Modelling

Limitation in the use of cassava growth modelling as a tool arises often due to ignorance of the rates of fundamental biochemical and physiological processes of intact plants. For example, the Global Change in Terrestrial Ecosystems (GCTE) cassava network meeting summarized three types of conceptual problems of existing cassava growth models (GCTE, 1997). These are, modelling the plant population effects on growth (both under monocrop and intercrop situations), storage root initiation and dry matter distribution and P and K limitations to growth, and exudation and mycorrhiza (C leakage). Lack of knowledge of the mechanisms of adaptation and acclimatization to various abiotic stresses become a limitation only where these models are used as a framework for predictions and in fine tuning to predict performance in marginal environments. Despite a large body of available information on drought tolerance of cassava, only one attempt to date (Gray, 1997) has been made to predict cassava productivity under drought conditions. Plants grown in the field and under controlled environments respond to similar levels of water stress in a different manner, and field-grown plants experience more than one stress (Amthor and McCree, 1990). Therefore, simulations for growth under water deficit conditions become a difficult task.

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# Assessment of the nutritional value and competitiveness of traditional foods in South Pacific economies: A case study of root crops in Fiji

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The consumption of traditional food crops, dominated by root crops, has been either stagnating or in decline in South Pacific countries. Governments have attempted to reverse this trend through strategies that highlight the importance and health benefits of such crops, but with little success. Inadequate knowledge by policy makers of the major causes of the trend is argued to be an important factor contributing to this lack of success. The most important obstacles to developing traditional food crop industries are identified by examining factors influencing the supply and demand of taro and other root crops in Fiji. On the supply side, attention is given to the constraints on production, storage, processing, transport, and marketing. On the demand side, the focus is on various economic, social, and cultural factors identified as the chief determinants of consumption decisions. Suggestions are made for the development of a strategy to improve the competitiveness of root crops in relation to imported foods (especially rice and flour), that is more effective than current strategies. Policies are suggested to underpin this strategy, with particular emphasis on the encouragement of productivity gains in production and post-harvest activities. Any strategy to make food industries more competitive in domestic food markets will require an improved understanding of the factors changing tastes and preferences of domestic consumers. Marketing research programmes for root crops are needed, but will require government support.

Keywords: Fiji; Nutrition; Research; Root crops

Concerns about declining nutritional status in Fiji have been twofold. First, a growing number of people among both Fijian and Indian populations are not getting sufficient food to meet their nutritional needs (Saito, 1995; Savou *et al.*, 1995). The UNDP (1997) observed that problems of child undernutrition appear to be getting worse, and it is becoming increasingly costly for households to provide a nutritionally adequate diet to children.

The second area of concern is malnutrition in adults. Savou *et al.* (1995) documented changes in the typical diet of the population in Fiji, with obese and overweight adults becoming more common with a corresponding increase in the incidence of so-called 'Western' diseases such as diabetes, hypertension, and heart disease.

The National Food and Nutrition Committee (NFNC) has been charged with the responsibility to coordinate food and nutrition activities to improve dietary balance and nutritional status since its formation in 1982. English (1997)

found that the NFNC has not been successful in these endeavours. In particular, she was critical of the failure to stem the increased consumption of imported foods at the expense of locally produced foods such as root crops.

Five decennial nutrition and health surveys were carried out between 1953 and 1994 in the village of Naduri, in the relatively resource-rich Sigatoka Valley. They show marked trends in food consumption away from traditional foods to imported foods (Seniloli and Tuivaga, 1994).

## Review of Major Factors Influencing Nutritional Status

Four groups of factors influence nutritional status: food supply; choice of foods and dietary balance; poverty and entitlements to food; and health and living style factors. Historically, Fiji has not suffered from shortages or variety of food supplies. Changes in household decisions

about food purchases and planting subsistence crops have been mentioned as a major factor bringing about malnutrition in Fiji (Savou *et al.*, 1995). National household survey results show that the Fijian diet has worsened in both rural and urban areas (Johnson and Lambert, 1982; Saito, 1995).

Entitlement to food has not been a major factor influencing nutritional status in the past, largely because of the strong informal social system in place through family support in both the Fijian and Indian communities. This is no longer the case, and, according to UNDP (1997), around one-quarter of the population in Fiji currently lives in poverty. Food represents a substantial proportion of the budget of poor households (Bureau of Statistics, 1991), and so food costs play an important part in influencing the ability of the poor to improve their situation. Poverty is highly correlated with low education level (UNDP, 1997).

### Historical Importance of Root Crops in Human Nutrition in Fiji

Prior to the arrival of the Europeans and Indians, the staple foods of Fijians were taro and yams which were eaten with the edible hibiscus, hibiscus spinach (*bele*), and other local vegetables like amaranth (*moca*) and fern (*ota dina*). These traditional root staples, considered filling food as they satisfy hunger, were commonly stored as surplus food when preserved in fermentation pits for use in emergency situations such as drought, warfare, or cyclone (Aalbersberg, 1990). Starch flour, made from Polynesian arrowroot (*yabia*), could be stored for months and was often used as an important item of trade.

A departure from the traditional diet occurred with the arrival of European settlers and missionaries, and the establishment of plantation agriculture. Fijians acquired a taste for sugar, flour, and rice, and cassava began replacing taro as the dominant root staple. With the arrival of Indian workers by 1874, more rice, flour, cereals, and dried legumes were imported to satisfy food needs. Increasing affluence reinforced the change in eating habits.

As expected, root vegetables continue to provide the bulk of the staple diet of Fijians. The main staples of the Fijians are cassava, sweetpotato, taro, and yams, which represent 65% of the total amount of food consumed, while Indians mainly consume rice, pulses, Irish potato, eggplants, and green beans, representing 59% of the total amount of food consumed by an adult.

A major problem with a diet based on refined cereals is the decrease in vitamins A, B (thiamine, riboflavin, and niacin), and C, and iron intake which need to be provided from other sources. The nutritive value of the tradi-

tional root staples, such as taro and yams, and leafy green vegetables, like hibiscus, spinach, and taro leaves, is significantly greater than the post-European introduced cereals, rice, and greens like lettuce and English cabbage. Increasing consumption of cassava relative to taro is also of concern as cassava has a much lower protein content (1%), than taro (2%) and yams (1.5%), and also has a lower vitamin B:calorie ratio. Taro is 10 times richer in potassium and 7 times richer in magnesium than cassava, and has higher levels of iron, vitamin A, vitamin B, and vitamin C. Yams are also richer than cassava in potassium, iron, and vitamins A, B, C, and E.

### Root Crop Production Past and Present

Taro is still widely grown on all the islands, and yams are chiefly grown on the drier leeward side of the main islands of Viti Levu and Vanua Levu, the Yasawas, and the Lomaiviti and Lau groups. However, the dominance of the aboriginally-introduced root crops and other staple foods, breadfruit, banana, plantain, and coconut, is under challenge. In addition to cassava replacing taro and yams as the dominant staple in most rural areas, other non-traditional, non-aboriginal root crops (cocoyam, cassava, and sweetpotato) have become important staple food crops.

### The Root Crops Marketing System

The marketing system for root crops in Fiji is unusual for such staple products in that the domestic marketing sector is closely integrated with an export marketing sector. While Fiji has long exported small volumes of root crops, the export industry has developed in recent times because of the incidence of taro leaf blight in Samoa (formerly known as Western Samoa) in 1993. Until that year, the taro industry in Samoa had built up a lucrative export trade to expatriate Pacific islanders living in Pacific rim countries (especially New Zealand) who had largely maintained their traditional eating habits based on root crops (and taro, in particular). The Fijian taro industry has been the most successful among South Pacific countries in filling the breach in this export market left by Samoa.

The value of taro exports in Fiji rose dramatically from F \$0.549 M in 1993 to F \$7.952 M in 1995 in which year it became the second most valuable agricultural export after sugar (Bureau of Statistics, 1996). Development of the export trade was in response to high export prices in New Zealand and the U.S.A., made relatively easy by the pioneering work in establishing an export market by Samoa. Another facilitating factor was that the

attributes of the export market make it essentially an extension of the domestic market in terms of marketing processes and understanding the wants of consumers in importing countries.

Domestic marketing of root crops takes place primarily through fresh produce markets in the major towns. Trade is largely in the hands of Fijian producer-sellers, wholesalers, and retailers. While some specialist wholesalers and retailers exist, the majority of traders in the fresh produce markets tend to be producer-sellers. The marketing processes are rudimentary, with use made of a variety of transport services and packing limited to home-produced baskets made of coconut fronds. Presentation is very simple with the roots, usually in an unwashed state, sold in piles on either the ground or tables that are often lined with taro leaves. The crops are subject to virtually no value-adding processes beyond the space and time utility provided by transport and storage activities.

The taro leaves are also an important item in the fresh produce markets, usually sold separately from the corms. There is some specialist production of leaves which is part of an integrated production, transport, and wholesaling process.

Limited volumes of root crops and taro leaves are sold through supermarkets which rely on wholesale agents. Produce tends to be better presented and cleaner in this channel, but more costly. Members of higher income groups tend to use this marketing channel more than those of lower income groups, although many of the former also buy at fresh produce markets.

### Trends in Outputs and Prices of Root Crops

Estimates of output of the major root crops in Fiji are presented in Figure 1 for the decade, 1986-95. Two features of the estimates are clear. First, output fluctuates substantially, dictated largely by climatic variations which were especially adverse in the early 1990s. Second, there was a general downward trend in output of root crops until 1993, after which there has been a substantial increase. While the increase in taro output would have been expected, given the expansion of the export market, it is quite surprising that cassava output also increased fivefold from 8064 t in 1993 to 40 247 t in 1996. Also, output of sweetpotato almost doubled from 4240 t to 7821 t and output of yams almost trebled from 1588 t to 4401 t over the same three-year period.

Regressions against time were estimated for a longer period, 1976-96, for three of the four root crops in Figure 1 (no data were available on output of sweetpotato prior to 1986). Outputs were expressed per head of population. Results showed that, for all three crops, the coefficient of the trend variable was positive but

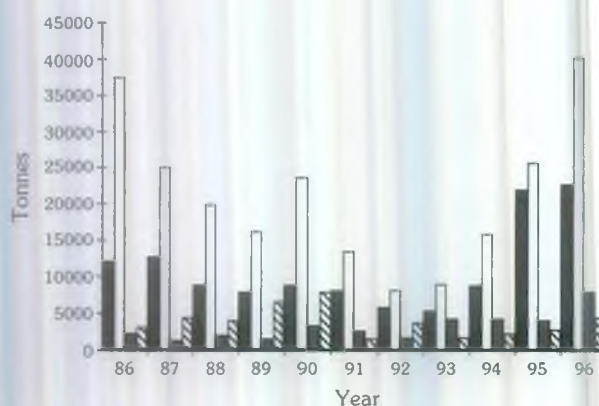


Figure 1 Output of major root crops in Fiji, 1986-96; (■), taro; (□), cassava; (▨), sweetpotato; (▩), yams

not significantly greater than zero at the 5% significance level (although the coefficient in the yams equation only narrowly failed the significance test at this level). Estimated annual rates of output increases are 3.6% for taro (0.0355; S.E. 0.0312), 5.4% for cassava (0.0539; S.E. 0.0412), and 12.4% for yams (0.1245; S.E. 0.0663). This result is surprising given the popularly held belief that output of root crops has been declining along with consumption. It can be largely explained by the boost to output in recent years from higher producer prices, especially as a result of the emergence of a remunerative export market.

Trends in the ratios of retail prices of the two most commonly consumed root crops, taro and cassava, to the two main imported substitutes for root crops, rice and flour are presented in Table 1. These trends are presented as ratios, estimated as regressions with the ratio expressed as a function of time using data in prices per kilogram in monthly averages for the period 1986-96 (Bureau of Statistics, 1997). The price ratio of a kilogram of taro leaves to 500 g of frozen mixed vegetables (a common imported substitute) was also regressed on time using the same approach.

Table 1 Trends in the ratios of root crop prices to flour and rice prices in Fiji, 1986-96

Dependent variable	Intercept	Trend	Adjusted R <sup>2</sup>
Taro-flour price ratio	0.880±0.114	0.0019±0.0014	0.596
Cassava-flour price ratio	0.371±0.096	0.0021±0.0012	0.704
Taro-rice price ratio	0.541±0.094	0.0035±0.0012	0.707
Cassava-rice price ratio	0.226±0.095	0.0026±0.0012	0.790
Taro leaves-frozen mixed vegetables ratio	0.317±0.024	0.0005±0.0003	0.286

Results for the root crops equations show positive trends in relative prices in all cases, indicating that the prices of taro and cassava increased over the study period relative to the prices of flour and rice. However, the relative rates of annual increase in prices of root crops were low: 0.2% and 0.4% for taro and cassava, respectively, relative to flour prices; and 0.5% and 0.6% for taro and cassava, respectively, relative to rice prices. The coefficients of the trend variables were nonsignificant at the 5% level in the taro-flour and cassava-flour and significant at 5% in the taro-rice and cassava-rice equations. The latter is widely attributed to increases in domestic retail prices of taro and cassava with increased taro exports from 1993. However, empirical evidence suggests otherwise. Period dummy variables of the zero-one variety were placed on the trend variables, and found to have negative but insignificant coefficients. This result is at odds with the conventional wisdom that the expansion of the taro export market has caused domestic retail prices of taro to increase relative to the prices of rice and flour.

There is also evidence of a positive trend in the price ratio of taro leaves to frozen mixed vegetables. However, the coefficient on the trend variable was not significant at the 5% level, and the annual rate of increase in the price ratio was very small, at less than 0.1%.

### **Constraints to Development of the Root Crops-based Farming System in Fiji**

Constraints to development of the root crops-based farming system in Fiji can be conveniently categorised at four levels: production, marketing, domestic consumption, and the export market.

#### **Production constraints**

Despite a voluminous amount of research and development activities that have been carried out on many aspects of production, gaps still exist to impede the attainment of optimal farm productivity, efficiency, and sustainability of the root crops-based farming systems. A host of constraints traverse pre-production and post-production systems that need to be surmounted, requiring a holistic approach to improving the farming system.

#### **Marketing constraints**

Marketing constraints for root crops are considerable (Brown, 1995), and are much more binding than marketing constraints facing suppliers of imported competitive foods such as flour-based products and rice. These constraints are manifest at all stages in the marketing chain between the producer and retail. Yet the current system is probably as well adapted to deal-

ing with, and overcoming existing marketing constraints as it could be, given the marketing resources available. Markets are well supplied on a regular basis with a range of produce at reasonable cost and quality, with minimal government support. Solutions to the constraints appear to lie not with significant changes in institutional structure, nor transformation of the fresh produce marketing system, but with research and development work which leads to improved marketing services within the existing system.

#### **Consumption constraints**

Several factors potentially limit domestic consumption of root crops. These constraints are determined by the food purchase decisions based on convenience and perception and dietary considerations of households.

Export market constraints are largely determined by the uncertain competition in future from Samoa if the taro industry in that country overcomes the problems currently faced with taro leaf blight.

### **Suggested Research Agenda**

There is strong circumstantial evidence that continued emphasis on pre-production research will do little to remove the constraints to increased domestic production and consumption of root crops in Fiji. Yet, even if conclusive evidence could be presented to demonstrate an overwhelming need to reorient research resources away from pre-production activities, such a step would be unrealistic in the short term, given negligible capability to undertake research in other areas.

As a consequence, little is to be gained by an immediate reorientation of all research activities to meet what appear to be the higher priorities in research areas other than pre-production. A two-pronged approach would be preferable, with simultaneous research programmes at the consumption and production stages. Market research into consumer behaviour should have the highest priority, because consumer decisions on food purchases have been the greatest constraint on the expansion of root crop sales in the domestic market. There is an urgent need to understand better why consumers make the food purchase decisions they do, the strength of these decisions, and the scope for altering them through actions in the marketing channels. A better understanding of food purchase decisions should identify areas further back in the food marketing chain where research is needed to meet consumers' demands. The thrust in production research should be towards more work in post-production and less emphasis on pre-production activity, except for key areas where continuing research is a priority, such as control of the taro beetle (PRAP, 1996).

For the medium- to long-term objectives, attention needs to be given to developing a suitable cadre of researchers with skills in line with reoriented research requirements. This may mean recruitment of new staff along with some retraining and upgrading of skills of existing staff.

### Policy Implications

The major policy finding of the study concerning the competitiveness of the root crops industry, is the urgent need for the government to establish an appropriate sequence of actions to remedy the problem of a decline in consumption of root crops (and other locally-produced nutritious crops) in Fiji. The government would risk 'firing blanks' if it were to rush into ill-conceived policies before a better diagnosis is made of the current situation and the causes of the problems inherent in this situation.

Two facts seem indisputable from current empirical evidence: there is declining nutritional status and a declining share of root crops in diets. It is also highly likely that these two facts are related. From here onwards, very careful research is needed. First, the extent to which declining nutritional status is caused by reduced quantities of nutritious foods such as root crops in the diet needs to be better established. The current national nutrition plan, termed the Fiji Plan of Action for Nutrition (FPAN) (NFNC, 1996), contains numerous initiatives that are badly in need of prioritisation. Just where counteracting the decline in consumption of root crops lies in this prioritisation, has ramifications for the importance of any research programme to maintain or increase the importance of root crops in diets.

Assuming it is a high priority, the next step in the policy sequence would be to establish the cause of the decline in relative importance of root crops. It appears from the agricultural research strategy currently being pursued, that research planners believe the cause is production-related and specifically related to input-output relationships. As argued in this paper, this supposition is dubious, and needs reconsidering. It is likely that there are a number of factors operating throughout the fresh produce production and marketing systems.

It is therefore concluded that there are numerous gaps in knowledge of the causal factors behind the decline in importance of root crops consumption associated with declining competitiveness of root crops in the domestic retail

markets, and the scope for reversing this decline. It is recommended that a research programme aimed at filling these gaps is the highest policy priority. Research into factors influencing food consumption decisions and dietary balance is probably the most urgent step within such a programme given the current lacuna of knowledge (but plenty of opinions). However, it would be sensible to complement this research with projects aimed at increasing capacity for, and improving efficiency in, production. This would entail a shift in emphasis from current research activities aimed at increasing volume to activities to improve quality and preparation for market, and post-harvest, processing, and marketing activities.

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# Progress towards a global strategy for cassava development

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The development of the Global Cassava Development Strategy was initiated in 1996 at a 'brainstorming' meeting convened by the International Fund for Agricultural Development. Cassava was recognized by the meeting as a food security and commercial crop that lends itself to a commodity-approach to poverty alleviation, given the close connection between the poverty level in many parts of Africa, Asia, and Latin America, and the role of cassava in the cropping and food systems in these areas. However, in order to recognise and meet the full potential of this crop, a Global Strategy was considered necessary. The Global Strategy requires a coalition of stakeholders including cassava producers and their organizations, Governments, the donor community, technical and research agencies and their networks, non-governmental organizations and their networks, and the private sector in order to achieve the objectives of the strategy. The Strategy is being developed from a number of country case studies and regional reviews. A review workshop was held in June 1997. A Forum of representatives of all stakeholders will be held in 1998 to ratify the final strategy and develop a plan for its implementation.

Keywords: Cassava; Global strategy; Rationale; Development workshop

Cassava is one of the most important food security crops in the developing world. It also plays an important role in income generation and stabilisation. However, it has received less attention than the other major food staples. Investments in cassava offer the potential for a commodity-based approach to poverty alleviation, given the close connection between the level of poverty in many parts of Africa, Asia, and Latin America and the role of cassava in the cropping and food systems of households in these areas.

Recognising the potential importance of cassava, it was adopted by the Intergovernmental Group on Grains (IGG) in February 1996. A strategy for its development was required by the Common Fund for Commodities (CFC) to which the IGG submits projects for financing. A strategy which was targeted to the mandate of the CFC was prepared (FAO, 1996) by the 'Cassava Group' [a consortium of the International Institute of Tropical Agriculture (IITA), Centro Internacional de Agricultura Tropical (CIAT), Cassava Biotechnology Network (CBN), Centre de Coopération Internationale en Recherche

Agronomique pour le Développement (CIRAD), and the Natural Resources Institute (NRI)] in consultation with various organizations in Asia, Latin America, and Africa. Recognising the wider potential role of cassava, the International Fund for Agricultural Development (IFAD) took on a facilitation role in the development of a Global Strategy with wider objectives.

This paper reports on the progress made from an initial 'brainstorming meeting' in 1996 to the 10th triennial meeting of the International Society for Tropical Root Crops Meeting in Trinidad in October 1997.

## Strategy Rationale

Globally, over 155 M t of cassava are produced in tropical and sub-tropical areas of the world. The crop is often grown in marginal agricultural areas where it has agronomic advantages over other crops. In these areas it contributes to the food security and income generation of many poor rural households. Investment in cassava development can be an effective means of assisting poor people in fragile environments and is a commodity-based approach to food security and livelihood development.

Research and development investments in the crop over the last three decades have started to make a significant impact in some specific cassava-growing regions. However, physical and (or) economic losses due to biological, edaphic, climatic, social, economic, political, and institutional constraints in many countries persist and many local, domestic, and international market opportunities for the crop still remain undeveloped.

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The investment in cassava research and development has been low in comparison with other important tropical food commodities such as maize and rice. In addition, investments have often been made in isolation, while research and development agendas have been in a research capacity or technology driven. Many of these investments have not, therefore, had the desired impact. Examples of such investments include IFAD's support for cassava multiplication in Nigeria, various investments by a range of donors in overcoming cassava mosaic disease in Uganda (The Gatsby Charitable Foundation, 1977), and the development and dissemination of the low-cost cassava storage technique in Latin America by NRI and CIAT (Wheatley, 1989).

### Strategy Objectives

The initial 'brainstorming' meeting held at IFAD's headquarters in May 1996 agreed that a Global Strategy was required to: (i) identify opportunities for further public and private investments; (ii) set a framework for international technical cooperation for research and technology transfer; (iii) identify more cost-effective institutional mechanisms for rationalising and increasing to the extent possible, the allocation of resources for research and investment; and (iv) set the scene for future debates on global issues relating to the commodity.

### Strategy Development

During the 'brainstorming' meeting in May 1996, an approach for developing the strategy was agreed. It was proposed that IFAD would play a role as a facilitator and catalyse constituency building and the mobilisation of resources. The main needs in terms of facilitation were (i) to call for a coalition of stakeholders from cassava producers and their organizations, national governments, the donor community, technical and research agencies and their networks, and non-governmental organizations (NGOs) and the private sector; (ii) to promote ownership and commitment of partners through sharing of human and financial resources investments; and (iii) to enable the achievement of a consensus on the strategy and an implementation plan developed from it.

### Methods and Organization

Two meetings were held in 1996-97 where key stakeholders in the development of the strategy were brought together: the initial 'brainstorming' meeting of May 1996 and a Workshop to review progress in June 1997.

It was agreed at the May 1996 meeting that the strategy would be developed from three

types of study: country case studies, regional reviews, and thematic reviews. Terms of reference for each of these studies were developed by working groups. It was agreed that country case studies which would be the responsibility of National Programmes would be undertaken in seven countries: Tanzania, Nigeria, Uganda, Vietnam, Colombia, Brazil, and Thailand. Ghana subsequently agreed to also prepare a case study. The principal objective of these studies was to obtain lessons learned from previous investments, research and development programmes, and strategies on cassava that could influence the implementation of the Global Strategy (IFAD, 1996).

Three regional studies were planned and subsequently implemented for Africa, Asia, and South America. The three key elements of these studies were: cassava sector trends; constraints and opportunities analysis; and an inventory of available technologies. These reviews which were intended to provide the major information resource for drafting the strategy had the responsibility for the management of these reviews. The Consultative Group on International Agricultural Research (CGIAR) Centres with mandates for cassava (IITA and CIAT) had the responsibility for the management of these reviews. The CIAT was assisted by CIRAD in undertaking the reviews for Asia and Latin America.

The terms of reference for a drafting team to pull together the various elements of the strategy were developed at the review meeting in June 1997. Although thematic reviews were initially considered as important parts of the strategy, these were included within the terms of reference of the drafting team. The drafting team was charged with (i) reviewing and analysing all the information generated; (ii) developing a draft Strategy; and (iii) developing recommendations for implementation of the Strategy.

After the drafting team has prepared a draft strategy, the document will be circulated to regional bodies for comment. Annexes will be developed to reflect regional priorities. A forum of representatives of all stakeholders will be held in 1998 to discuss the strategy document and reach a consensus on its implementation.

### Financing

The IFAD has mobilised a number of donor agencies for contributions and in addition it has financed several country case studies, meetings, and consultancies. There has been collaboration from the Swiss Development Corporation, IDRC, World Bank, and FAO. Contributions and services have been provided by CIAT, IITA, NRI (supported by the United Kingdom's Department for International Development), CIRAD, and the CBN. Collaboration and services have



also been provided by national governments, research agencies, universities, and regional networks. Several other donor agencies have expressed interest in financing future cassava projects.

## Progress to October 1997

### Studies completed

Six of the eight country case studies have been prepared by national programmes (Table 1), and the remaining two national studies (Nigeria and Vietnam) will be completed in early 1998.

### Regional cassava studies

Three regional studies (Africa, Asia, and Latin America) have been prepared. These are: *Cassava in Africa, Past, Present, and Future* (Spenser and Kainaneh, 1997); *Cassava in Latin America and the Caribbean: Resources for Global Development* (Hershey et al., 1997a); and *Cassava in Asia: Expanding the Competitive Edge in Diversified* (Hershey et al., 1997b).

The FAO has prepared an additional document entitled "*The World Cassava Economy: Recent Trends and Medium Term Outlook*" which analyses the future global trends for the crop.

**Table 1** Country case studies prepared for the Global Cassava Strategy

Country	Title	Authors; Responsible Organization
Ghana	A case of cassava development Ghana	Ofori et al. (1997); Ministry of Food and Agriculture
Tanzania	Status of cassava in Tanzania: Implications for future research and development	Kapinga et al. (1997); Ministry of Agriculture and Cooperatives
Uganda	Cassava development in Uganda	Otim-Nape and Bua (1997); Namulonge Agricultural and Animal Production Research Institute
Brazil	Farmer participatory research: The turning point for cassava in NE Brazil	Pires de Matos et al. (1997); National Centre for Cassava and Tropical Fruit Crops
Colombia	Cassava development in Colombia	Balcazar, V. (1997); Centro de Estudios Ganderos y Agrícolas
Thailand	Status of cassava in Thailand: Implication for future research and development	Ratanawaraha et al. (1997); Ministry of Agriculture and Cooperatives

### Review Workshop (June 1997)

A Review Workshop involving a number of key stakeholders in the process was held in June 1997. The progress on country and regional studies was presented. Working Groups then developed priority issues from the various presentations. It is not the aim of this paper to substitute for the Global Strategy, however, some of the key issues are: importance of a market-led approach to research and development; importance of favourable and integrated government policies; importance of a client orientated (user-orientated) approach to research and development; need for an emphasis on food security in Africa; need for increased attention to technology transfer (and alternative mechanisms of transferring technology); need for increased integration of the private sector in the research and development and technology transfer processes; importance of environmental issues; importance of infrastructure (especially in Africa); and need to explore opportunities for adding value to the commodity and to examine nutrition-related issues.

### Remaining activities

The remaining activities include the review and analysis of all generated information, preparation of a first draft strategy document, and circulation of the draft document to regional bodies and other stakeholders for review and (or) identification of regional priorities. A forum will be held in 1998 for all stakeholders. The objectives will be to present, discuss, and validate the draft strategy document, propose approaches, and discuss and agree with mechanisms for implementation of the strategy and division of responsibilities amongst stakeholders.

## Discussion

The Global Cassava Strategy explicitly involves a commodity-based approach to contribute to food security, poverty alleviation, and livelihood development in developing countries. Many donors, international centres, and some national programmes have recently moved away from commodity-based approaches in favour of production systems or agro-ecological zone-based emphases. However, in the case of cassava, a commodity-based approach should be utilised because of the importance of the crop to poor households in many parts of the world. Returns to investment in cassava offers the opportunity to specifically target poverty alleviation for the most vulnerable. Development of the crop, especially its income-generating capacity for small farmers through the strengthening of their links with growth markets, can be the first and essential step in providing a better livelihood for the inhabitants of cassava-growing regions. The approach needs then to be complemented by the

identification of other and new income-generating opportunities that can build on the experience gained through the investments in cassava.

There are a number of initiatives that are currently aiming to bring together various root and tuber crops research and development activities. These include the CGIAR's Inter-Center Group on Root and Tuber Crops, the European Group on Root and Tuber Crops, and the International Society for Tropical Root and Tuber Crops. Many of the characteristics of cassava in terms of under-funding and their importance to small-scale farmers are common to other root and tuber crops such as yam and sweetpotato. A Global Strategy for cassava provides a starting point for these commodities. Would it be pertinent, in the future, to bring together these various global initiatives on root and tuber crops with the progress made on a common strategy for cassava?

The authority of the Global Strategy is dictated by the extent to which ownership and inputs can be extended to the stakeholders in the commodity and the extent to which these stakeholders use the strategy in making key investment and resource allocation decisions.

The quality of the Global Strategy will serve as incentives for stakeholders, especially donors, to use it as a source of guidance in decision making on future investments.

In time a static Global Strategy will become outdated. Therefore, a dynamic strategy is needed which takes into consideration new information from research findings and the economies of returns on investments. Developing a new strategy is a costly and time-consuming process. Therefore, the implementation plan developed at the forum must ensure that the strategy can be updated periodically.

The Global Cassava Strategy is a starting point towards improving the livelihoods of many people in developing countries. To have impact, a plan which is owned by the stakeholders in the cassava producing systems, has to be put into place. This will be one of the most important products of the forum to be held in 1998.

The minimum expectations of the plan include an increase in the efficiency of the utilisation of the current resources allocated to the commodity, an increased awareness about cassava's potential role in development by key stakeholders including Governments, donor agencies and NGOs, better utilisation of outputs through client/demand-led approach, and the increased empowerment of cassava research by beneficiaries and end-users.

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# The marketing system for fresh yams in Techiman, Ghana and associated post-harvest losses

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In the past, relatively few studies have been directed at the improvement of the post-harvest storage of fresh yams (*Dioscorea* spp.). The problems associated with the trading, transportation, and marketing of yams have been largely overlooked. This paper summarises the initial findings of a market characterization survey conducted at Techiman, one of the largest yam markets in Ghana. The marketing system and the trading practices of the principal agents operating within the system are described. Traders cite transportation costs, seasonality of production, poor market infrastructure, lack of credit, mechanical damage, and rotting of tubers as their main constraints. Observations suggest that, during the early season, the loss of yam quality is associated with certain pre-harvest infestations of nematodes and termites as well as a systemic internal browning; post-harvest tissue damage associated with the stacking of tubers, various rots, and the prolonged exposure of tubers to intense sunlight in the market place are subsequent factors. Such deterioration may lead to price discounting of 25–63% and absolute biological losses of 10%.

Keywords: Yam; *Dioscorea* spp.; Marketing; Traders; Post-harvest; Losses; Discounting; Ghana

Over the past 15 years, world production of yams (*Dioscorea* spp.) has been rising and this is almost entirely due to increased production in Africa (FAO, 1994). Of an estimated global yam production of 30.3 M t yr<sup>-1</sup>, 95–96% is believed to originate from West Africa (FAO, 1994; Onwueme and Charles, 1994) where possibly 60 million people are involved in its production and marketing (Nweke *et al.*, 1992). Much of the research emphasis has been related to pre-harvest concerns (Asiedu, 1996). Periodically, researchers have sought to investigate the factors that influence the post-harvest quality of yams (FAO, 1985; Thompson and Bancroft, unpubl. data). Although various publications have recommended improvements in the grading and packaging of yams for the international export market (Ghartey, unpubl.; Thompson, 1972; Thompson *et al.*, 1977, 1979), there appear to be only very few studies that have tried to either identify the technical and economic problems associated with the trading, transportation, and marketing of fresh yams within West Africa or quantify the biological and economic losses inherent in these activities (Ghartey, unpubl.). The study reported here constitutes one of the first of a series of investigations as part of a project supported by the British Department For International Develop-

ment (DFID) and undertaken in Ghana to characterize the indigenous yam marketing system, develop and test improved handling techniques, and identify and quantify those situations in which both significant physical and economic post-harvest losses occur.

## Methodology

### Market characterization

The location chosen for the study was the main yam market in the town of Techiman in the Brong Ahafo Region of Ghana. Information was gathered over a four-week period in late October and early November 1996 during which time trading practices were described and monitored. To appreciate how the yam market functioned, individual and group interviews were conducted with purposely sampled representatives of all the different categories of market participants. Key informants included producers, producer-traders, various types of market traders, market administrators, and truck operators. Over 30 individuals were interviewed. The topics discussed during these interactions included the production and supply of yams, pricing

strategies, costs, volumes traded, market flows, seasonality, transport, financial arrangements, gross margins, and perceptions of major constraints to the marketing of yams. Detailed analysis of these findings may be found in Bancroft *et al.* (1997). For the purposes of this paper only the salient features of the marketing system are reported.

### Assessment of biological losses

Simultaneous with the market characterization exercise and on seven different trading days, quality assessments of four different species of yams were made of 18 separate wholesale and retail consignments available in the market. Random samples were drawn from all grades of material from those attracting premium prices (Grade 1) to those that had been discarded (Grade 6). The number of roots sampled varied from 4 to 35 depending on the number of tubers per consignment. In each instance, notes were taken of the known history of the different consignments, their retail value, and, for each tuber sampled, both their external and internal physical attributes. Overall, each tuber was assessed for over 40 different indices. The present paper reports preliminary results for only a non-varietal specific subset of the available data with the emphasis being placed on highlighting the incidence of those post-harvest characteristics which would appear to compromise the commercial value of the merchandise. The summary data were generated by the use of the computer program, Statistical Package for Social Sciences (SPSS), Version 6.1.3.

## Results

### Techiman market

Techiman market is both a large municipal and regional market that provides a trading location for a wide range of fresh produce as well as cloth, tools, and cooking utensils. The yam market is considered to be the largest assembly market for yams in Ghana (Kleih *et al.*, 1994), attracting merchants from across the country particularly during the start of the yam season, when Techiman was reputed to be the first market to commence the large-scale trading of tubers. During the season, yam consignments began to arrive in the market on Tuesday with the main wholesale trading days being Wednesday and Thursday. Having secured supplies of tubers earlier in the week, retail sales ensure prolonged market activity until a decline on Friday. From Saturday to Monday, yam trading was virtually suspended.

The infrastructure within the market was rudimentary. The market had no running water or sanitary facilities. Storage structures and shade were limited and the majority of yam traders displayed their produce on the ground in full

sun. At night, each trader arranged for guards to provide security for their remaining yam consignments.

### Yam association

The Techiman 'Yam Association' is run by an executive of seven members led by a 'Yam Queen' and her two deputies. The association has a membership of approximately 600 which represents 90-95% of all those trading in yams at the market. Of the total membership, only 20 are male all of whom are itinerant wholesalers.

The association ensures that only *bone fide* yam traders are allowed to operate within the market. To such traders, the association is able to offer short-term loans for the purchase of yams and also oversees the rental of shelters on a daily basis for those traders who wish to store their merchandise in the shade.

Revenue collected by the association includes a single registration fee of 50 000 Cedis (U.S. \$28) from each trader who wishes to operate in the market on a regular basis. In addition, the association levies a daily tariff of one tuber for every 110 tubers brought into the market. Each itinerant wholesaler or trader entering the market is also charged a fee of 2000 Cedis (U.S. \$1.10) per trip irrespective of the quantity of tubers being traded.

### Yam traders and the marketing channels

Following harvest, yams are graded according to variety and size, and, depending on market demands and the individual needs of the producer, the tubers are either stored or sold directly. Farmers sell their merchandise to itinerant retailers during the harvesting period, to itinerant wholesalers during the yam season, or deliver tubers themselves to sedentary wholesalers or commission agents in the market. When itinerant wholesalers or retailers are not available, this is the only way farmers are able to ensure sales. In a few instances, farmers or their wives may also sell directly to consumers as retail agents. When operating in the market, farmers are free to sell their yams to any buyer except when they have received credit from a particular trader, in which case, they are obliged to continue to supply yams to that trader until the debt is cleared. It is believed that 70% of all yams entering the market do so via the itinerant wholesalers who transfer their consignments to sedentary wholesalers and commission agents for onward sale. Depending on the availability of yams, itinerant wholesalers often spend between two and five days in the production areas collecting yams before returning to Techiman with their consignments. When yams are scarce or roads to the production areas are impassable on account of the rains, itinerant wholesalers may prefer to make a living as sedentary wholesalers in the main market. Within the market, itinerant and sedentary

wholesalers as well as commission agents convey the tubers on to retailers who may operate in the market itself or disperse the yams to satellite trading centres. As the market conditions allow, any trader may operate at a number of levels with sedentary wholesalers retailing part of their consignments when the returns are favourable. Likewise, sedentary wholesalers with established contacts and capital backing may act as commission agents, the role of which is to assist wholesalers and retailers to make contact with itinerant traders. In some cases, commission agents may act as guarantors for retailers in the acquisition of loans in the form of credit purchases. These services are rendered for rewards or 'commissions'. Linkage of the Techiman market to other large regional markets is, again, by means of itinerant traders who probably purchase more than 90% of their supplies from the local sedentary wholesalers and commission agents.

Given the nature of the constraints within which the Techiman market is forced to operate, the yam trading system itself appears to function remarkably well. The various agents suggest that the yam trade is hampered by seasonal fluctuations in the availability of tubers, and the difficulties and expense of organizing appropriate transport from the production areas to the main trading centres. On an individual basis, many traders indicate that they are unable to increase the scope of their business due to lack of credit or appropriate capital. Regarding the market sites, many presently lack the appropriate infrastructure to handle large volumes of yams, and this tends to mitigate against maintaining the quality of the tubers which are prone to mechanical damage and rotting especially later in the season.

### Yam consignments

Discussions with the four main categories of traders suggested that the itinerant and wholesale traders were often more knowledgeable about the provenance of their consignments than the retailers. At the time of the survey, the majority of tubers being imported from known sources was derived from production areas 34 and 54 miles from Techiman. Although the traders did not often know how long the yams had been in the fields prior to harvesting, they had some idea of the length of time between the harvest and transportation, which was variable. Most consignments were held for four weeks with one being dispatched immediately after harvest while others were retained for one to three weeks. At least one was delayed by possibly 7 to 14 weeks. During the interim, the majority of yams was reported to have been stored in pits although a percentage was maintained on traditional platform structures. Only one respondent suggested yams were simply held in the shade. By far, the most popular form of transport from the production areas to

Techiman was by truck with one consignment being conveyed by minibus and another by tractor. Transit times differed considerably from 7 h to 6 days. Once present in the market, approximately 50% of the various consignments were sold within the first two days of trading. If competition was intense, then it might take three days to clear a consignment. Three consignments had been held in the market for eight days. This implied that the quality of the yams was substandard and, therefore, difficult to sell.

### Post-harvest losses

At the point of sale, a range of physical characteristics were observed to be associated with the potential loss in quality of fresh yams, e.g., harvest cuts, non-harvest cuts, grazes, rodents, insects, rots, nematode, heat damage, termite holes, 'spear grass' holes, and internal browning.

Pre-harvest growing conditions were implicated in the frequency of growing cracks and also the incidence of certain benign indentations observed along the length of the tubers. About 32% of tubers had sustained pre-harvest splits and 11.8% exhibited holes caused by stones in the soil. Other superficial punctures associated with the root activity of a local weed called 'Spear Grass' were found on 43.8% of the sample. At this relatively early stage in the yam harvesting season, there was no evidence that these particular features predisposed the tubers to forms of internal deterioration. Predation by potential pests such as various beetles and millipedes was not observed, but damage by vermin (rodents) was 1.5%. In contrast, 21.6% of the tubers exhibited holes left by feeding termites and, although the internal tissue blackening symptomatic of termite activity could be slight, in 2.6% of tubers, up to 10% by volume of the tissues was made inedible as a result. Potentially more detrimental to the saleability of fresh yam tubers, was the incidence of dehydrated and cracked skin caused by nematode infestations. Approximately 30% of all the yams showed nematode damage with 10.8% of tubers exhibiting symptoms on 25% or less of their surface area, and 19.1% being colonised to an even greater extent. Another symptom observed within the tissues of the yams and assumed to be of pre-harvest origin was that of hard, discrete, and generally brown segments of cortical tissue. This condition was found in 12.9% of tubers. In most instances, dissected yams only showed a discrete trace of discoloration. However, in 3.6% of tubers, the volume affected was as much as 10% of the internal tissues. The cause of the latter was not immediately apparent and may have been due to nutritional imbalances, viruses, or systemic infections caused by fungi or bacteria.

Following harvest and temporary storage, although ware yams were offered for sale in the

market with their apical tissues intact, 91% of all yams sampled had had their vines and apical tissues cut away prior to display. In addition to this wound, 50% of all the tubers had sustained other cuts which may also have been inflicted at the time of harvest. It is often assumed that rough handling and compression damage during transportation precipitates breakages and bruising and subsequent decline in yam quality in the market place. The present observations suggest that the repeated stacking of the yams may be more detrimental than their transportation *per se*. The compression bruising of yams (i.e., the crushing of internal tissues without rupturing the surface skin) was almost non-existent while the percentage breakages of tubers generally caused by the dropping of the tubers was limited to approximately 5%. In comparison, when yams were recovered from market stacks, 90% of tubers exhibited superficial grazes and 71% had sustained gashes of some description whereby the surface tissues of the yams had been punctured. These latter symptoms appeared to be derived from the abrasion of yams piled against each other, and the tradition of stacking tubers on their distal ends which often resulted in gashes at this point.

The observations recorded here provide some information on the general condition of yam tubers at a relatively early stage during the season and, hence, the incidence of damage and rots may be assumed to be relatively light in comparison to that which might prevail three or four months later when the yams would be less robust and have been stored for longer. Nevertheless, approximately 18% of the tubers examined exhibited external symptoms of rots. Extensive surface decay (greater than 50% of the surface area) was found on 2% of tubers, whereas, just over 10% of all tubers revealed rots affecting less than 10% of their total surface area. As is often the case, however, the expression of rots on the surface underestimated the true extent of internal damage. On dissecting yam tubers, the percentage found to be infected by rots rose to almost 38%, with 7.7% suffering from severe internal decay which affected >50% by volume of the tuber, and 22% of tubers with symptoms <10% by volume of their tissues.

Yet another cause of loss in the market place was that engendered by heat, for during trading hours, tubers were invariably left exposed to direct sunlight and were occasionally observed to suffer sun scorch. Temperature measurements indicated that, on market days, the internal cortical tissues of the yams could vary from a minimum of 26°C to a maximum of 45°C with a mean of 33 ± 3.9°C. Overall, 2.6% of the tubers exhibited tissue breakdown associated with heat stress with 1% sustaining significant internal destruction which affected 10–75% of their total volume.

The daily grading of yams determined market

prices. Of the 156 graded yams sampled, 46 (approximately 29%) and 41 (26%) were classified as Grade 1 and 2, respectively, with 16 and 13% of tubers being classified as Grades 3 and 4, and 4.5% as Grade 5. Those yams of no residual commercial value were designated as Grade 6 and represented a total biological loss of 9.6% of the total.

When available, on account of its culinary qualities, the *D. rotundata* variety 'Puna' is traditionally the most sought after yam on the market with Grade 1 material attracting a mean retail price of 4000 Cedis (U.S. \$2.21) per heap of three tubers (mean weight of one tuber 2.7 ± 1.1 kg). Relative to Grade 1, the price discounting for Grades 2, 3, 4, and 5 were approximately 25, 37, 50, and 63%, respectively. Yams were graded subjectively according to their general appearance and texture and often with reference to new consignments arriving at the market. In establishing grades the traders ignored the incidence of superficial grazes on the tubers and also the number of wounds caused by cuts. When considering Grades 1 to 3 neither the frequency of gashes nor the occurrence of nematode infestation appeared to influence the level of price discounting, whereas in the Grades 4 to 6, an increase in both these defects appeared to correlate more positively with a reduction in the value of the tubers. Similarly, a marked reduction in the percentage of tubers considered to be free from rots either visible on their surface or internal was particularly associated with the retail Grades 5 and 6.

## Discussion

The market characterization component of the study was successful in defining the main elements of the marketing system operating in Techiman, however, the business relationships between the various agents and the economics operating within the system were found to be extremely complex. Careful analyses of the data collected in Techiman and at other case-study locations will be necessary before researchers will more fully appreciate the intricacies of the various informal credit systems operating in the market place, the gross margins required for successful trading, and the risk factors and commercial constraints inherent in existing marketing practices. The latter two, in particular, will influence the viability or otherwise of any technical or procedural interventions that may ultimately be tested in the marketing system.

The loss assessment data recovered from Techiman provided a brief glimpse of the realities of post-harvest losses in consignments of yams in a regional market prior to their transportation to more distant trading centres. Although other workers have sought to quantify on-farm losses of yams (Anon., 1994; Mück, 1994; Henckes *et al.*, 1995) at various times

during storage, the authors are not aware of published values that specifically refer to losses or the cause of losses within the market system. The present observations suggest considerable variation in the inherent quality of yams even amongst those classified as Grade 1. The range of conditions causing loss in value in the market place is also somewhat different from those often cited with reference to 'on-farm' assessments. The results from Techiman reinforce the view that nematode damage is important and suggest that nematode infestations may be even more prevalent than previously reported. Termite damage too was higher than anticipated. The discovery of internal discoloration within the tubers was unexpected and poses the question whether, given time, such a condition eventually expresses itself as a large-scale internal rot. The more obviously exposed rots have always been cited as important causes of loss in tubers and the present work suggests that, even at an early stage in the yam season, significant amounts of rots are present. Further studies are necessary to determine to what extent the rots observed in the market chain result from chronic infections initiated on the farm, or arise as a consequence of mechanical injury sustained by tubers being conveyed through the system. Although the data suggest that heat damage is relatively minor relative to other conditions, it is one of the causes of post-harvest loss that could be remedied relatively easily.

The value of the data collected in Techiman is that it provides a baseline of information on which further studies may build. To devise and target appropriate interventions that may reduce trading losses, it is obviously important to not only appreciate the severity of such losses but also their cause. Successive analyses designed to elucidate both the absolute physical and economic losses throughout the market chain will help to define the post-harvest technologies that may be introduced into the system and the resulting potential economic benefits.

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# Rapid dissemination of tropical root crop information via the Internet and other modes of communication

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Improvements in telecommunications technology in recent years allow quick distribution of information. This paper explores the problems and benefits of using electronic mail, electronic mailing lists, home pages on the Internet, facsimile, and other means for rapid dissemination of tropical root information and international information exchange by the International Society for Tropical Root Crops (ISTRC). Reference is made to the use of this technology by the Japanese Society of Root and Tuber Crops and other groups. An ISTRC web site has been created for member use at: <http://www.tiu.ac.jp/~bduell/ISTRC>.

Keywords: Information distribution; ISTRC; Roots; Tubers; Internet; Facsimile; E-mail

At present, there is a large gap among countries in the availability of telecommunication resources, and the gap continues to widen at a rapid pace (Anonymous, 1997). This disparity in resources adversely affects how information can be best distributed among widely-dispersed researchers, and the speed with which it can be disseminated. Proper utilization of Internet and other resources may allow for a more rapid exchange of tropical root crop information. The merits and demerits of new technology for information exchange are discussed by Branscomb (1991), Kay (1991), Ellsworth (1994), Baran (1995), Hafner (1995), Stoll (1995), Wallis (1995), Anonymous (1997), Bokanga (1997), Ernsberger and Sudip (1997), Hamilton and Miller (1997), Kahle (1997), Kuchment and Johnson (1997), Stefik (1997), and Williams (1997).

Article 2, Section 1 of the International Society for Tropical Root Crops (ISTRC) Constitution states:

"The objectives of the Society shall be to foster, stimulate, and support any type of activity leading to the general improvement of tropical root crop production and utilization."

Several examples are given in this section, including Item 5:

"Publish appropriate informative communications, such as newsletters, summaries of the status of particular crops, lists of research workers and their areas of specialization, proceedings of meetings, and other appropriate publications (Anonymous, 1992)."

Other examples of ISTRC activities in the Constitution appropriate for consideration in this paper include sponsorship and encouragement of events and activities that promote exchange and dispersal of information and materials.

It is proposed that the ISTRC Constitution gives the Society the mandate to expand its educational programme by utilizing new advances in telecommunications technology. New technology allows fresh opportunities for initiating innovative techniques for exchanging information to improve world tropical root crop production and utilization.

According to ISTRC Councillors' and members' energy and needs, the Society might consider new projects taking advantage of new telecommunication advances. At a minimum, new technology can be used to streamline the carrying out of current ISTRC activities.

This paper seeks to demonstrate some new avenues the ISTRC may explore for dissemination and exchange of root crop information.

## Materials and Methods

A search of literature and Internet resources was conducted in order to determine possible new courses of action the ISTRC might follow to broaden the scope of its mission: Improving world production and utilization of tropical root crops.

Information for this paper was gathered during 1997. Due to rapid changes in Internet technology, portions of discussion below about Internet resources, and especially Internet addresses, may be out of date by the time this study is published.



## Results and Discussion

The present situation of the ISTRC is that there is only one chance every three years for all members to gather for active exchange of information at a Symposium. However, for financial and other reasons, it seems to be difficult to gather all members at all Symposiums, especially since these are held throughout the world. Members tend to gather at Symposiums in regions closest to their own.

The ISTRC mailings are the current method of maintaining contact with members. However, receipt of issues of the *ISTRC Newsletter*, or announcements for upcoming Symposiums are not circulated as well as they should be.

The ISTRC Symposium proceedings can take up to three years to edit and send out. The longer it takes to publish Symposium papers the more outdated their findings become. It requires considerable expense for the ISTRC to print and mail these heavy tomes. By putting ISTRC publications on the Internet, the ISTRC can bypass printing delays, the expense of mailing, and the problem of some members failing to receive publications due to lost mailings or address changes. However, for the foreseeable future, the Society must continue also printing and sending out the paper versions of its publications since a significant number of members and potential members are without electronic (e)-mail or full Internet access.

The 1997 Trinidad ISTRC Symposium Organizing Committee Chairperson, Dr Lynda Wickham, Chairman, was the first to make extensive use of e-mail and, to a lesser extent, facsimile (fax) for speeding up the making of pre-conference arrangements for attendees. All other members were reached by mail. In certain cases, direct phone contact was also utilized. In this way, a hybrid of communication means was utilized to communicate with members as speedily as possible. There is still room for ISTRC to continue improving its services by taking further advantage of recent advances in telecommunication technology.

A recent development is that the ISTRC Councillor for Publications, Dr Mpoko Bokanga, established the ROOTCROPS electronic forum for ISTRC members in October 1997 (Bokanga, 1997). This service, which will direct members' postings to all other subscribers via e-mail, is designed for announcing "meetings, new publications, job offers, requests for collaboration on research," and other communications in line with ISTRC's goals.

Bokanga (1997) also proposed formation of an ISTRC web page. Such a homepage has been started by the author with ISTRC approval at: <http://www.tiu.ac.jp/~bduell/ISTRC/>.

An example of an organization similar to the ISTRC trying to have faster information distribution is the Japanese Society of Root and Tuber Crops (JSRTC). Founded in Spring 1997, the JSRTC uses fax as one medium of information

dissemination. The JSRTC members receive a monthly newsletter by fax, or by mail if without a fax. The JSRTC also maintains an Internet homepage (<http://www.jsai.or.jp/~jrt/>) for information exchange with both JSRTC members and non-members. Currently, all information distributed by JSRTC is in Japanese, however, information in English is scheduled to be added in the near future.

The JSRTC also maintains an e-mail mailing list to allow exchange of information among interested members. Currently, a primary use of this mailing list is for gathering answers to questions posed by viewers of the JSRTC Internet homepage. A JSRTC homepage manager fields these root and tuber questions, routes them to the JSRTC mailing list, then edits the received answers for placement in the JSRTC homepage's Frequently Asked Questions column.

Another organization similar to ISTRC that uses English as its medium of exchange is the Veg-Prod e-mail mailing list. Veg-Prod, run by the United States Department of Agriculture researchers, describes itself as a forum for open discussion of current developments in vegetable production technology. There is no membership fee to join the mailing list, and the list is open to experts, as well as to the general public, from any country. Both groups of users can pose questions to Veg-Prod in the hope of receiving suitable answers. Though Veg-Prod sponsors no events, many of its members use Veg-Prod as a medium for informing the public about events sponsored by local organizations. One can subscribe to the Veg-Prod mailing list by sending the command "subscribe" in the body of an e-mail to "veg-prod-request@reeusda.gov" for automatic enrollment.

Internet homepages are also used by some organizations and individuals to promote root and tuber crops. An ISTRC member, the International Potato Center (CIP), informs the public about itself and its research activities concerning potato, sweetpotato, and other roots and tubers of Andean origin, at:

<http://www.cgiar.org/cip/>

Sweetpotato Sampler contains a variety of information about sweetpotato, appealing to agricultural experts as well as the general public, and is located at:

[http://www4.linknet.net/s\\_potato](http://www4.linknet.net/s_potato)

The Sweetpotato Breeding Laboratory at Japan's National Agricultural Research Center maintains an English homepage at:

<http://ss.narc.affrc.go.jp/sakukai/sweetpe.html>

The Laboratory of Sweetpotato Breeding at Japan's Kyushu National Agricultural Research Station publishes the biannual *Sweetpotato Re-*

search Front Newsletter which is sent out by mail, but is also posted on the Internet at:

<http://ss.knaes.affrc.go.jp/sporf/sporf.html>

This author also maintains a very simple homepage in English introducing Japan-related sweetpotato information. The homepage is located at:

<http://www.tiu.ac.jp/~bduell/sp/>

The above is only a brief sampling of root-crop-related e-mail mailing lists and homepages.

With a steadily widening gap present among the world's countries concerning availability of advanced telecommunications resources, it is important to consider ways to more efficiently distribute information to ISTRC's widely dispersed members.

Internet access is reported to be available in all of Africa's capital cities. Even with Internet access, Internet accounts and phone charges are reported to be costly. Moreover, about 70% of Africa's population live outside the capitals which can make Internet access more difficult. There is hope that wireless or satellite links can be used to give even those rural areas access (Anonymous, 1997).

Due to insufficient access in many countries to telephones, sending an organization's e-mail or fax communications via telephone lines has drawbacks for undertaking efficient communication. It is reported that in India, with 112 people per telephone, there is a long wait to get telephone lines, and long waits for receiving repair of faulty phones. Cellular phones may help pave the way for improved telecommunications by bypassing the current dearth of telephone lines (Ernsberger and Sudip, 1997; Kuchment and Johnson, 1997).

Portland State University researchers have received a U.S. Department of Defense contract to research how to create a mobile computer network. This emerging technology has potential use by ISTRC researchers in the field for communicating with colleagues via the Internet. Such a network would also allow areas of the world without adequate phone lines to link into the Internet (Williams, 1997).

The above new technologies will make world communications more efficient, but may also leave many targeted users behind due to the expense of implementing and using the technology.

Various countries have made a variety of Internet access restrictions, or are moving toward implementing restrictions. The Electronic Frontier Foundation (<http://www.eff.org/>) maintains a homepage with information about current developments. Depending on the degree of restrictions placed on Internet access by different countries, certain regions may have more difficulties than others in utilizing ISTRC information resources via the Internet.

Even having one site in a country or region with Internet access can speed up that area's researchers' access time to Internet information. It would be useful to set up cells in areas with limited access. An Internet access point in the area, such as at a university, research institute, or government agency, could be designated as the point where area researchers could have e-mail sent or received, or where requests could be made to post or retrieve information from the Internet. Information sent between a designated Internet access point and that area's researchers could be sent back and forth by whatever means is fastest yet most economical, for example, e-mail, fax, telephone, letter, courier, or other method.

Learning to use a fax machine is relatively simple, but it is more involved using e-mail or web pages to communicate with colleagues and the general public. Use of the Internet by an organization to educate its members and share research information between two or more locations is still a relatively new undertaking.

Many ISTRC members' universities or research institutes already have networked computers, so there is no additional major cost, for distance-learning to take place between them and such learning can be initiated provided that learners and teachers are suitably trained. The ISTRC's membership of diverse background is spread worldwide, and should have much to offer each other by distance learning.

The ISTRC members at degree-granting institutions could offer online root-crops-related courses to ISTRC members or others. These classes could require a fee and be closed to non-registered students, a system similar to that operated by Marylhurst College (U.S.A.) (<http://www.marylhurst.edu/>), or could be open to auditors free of charge as done with some classes at University of West Florida (U.S.A.) (<http://www.vclass.uwf.edu/>). Colleges where ISTRC members teach could also invite ISTRC or other root specialists to offer courses online under the college's name.

Stoll (1995) suggests it is a fiction that the text-based environment of e-mail and Web pages is creating a Renaissance in reading and writing, that it will encourage a literate environment with well-thought out ideas. His experience is that the Internet has brought a preponderance of poorly-organized, poorly thought out self-expression. The ISTRC members should heed this advice and should first consider the simplest yet most effective technology to pursue new projects made potentially possible by new technology in keeping with its members' diverse range of available telecommunications resources.

Part of the worldwide sharing by ISTRC members could be to help members learn to navigate the disarray of the Internet to supplement current processes of literature searching, publication, and correspondence with colleagues. Hainer (1995) discusses how software servants

are being developed to help bring order to this information disarray.

The importance of archiving Internet information is discussed by Kahle (1997). In the excitement of using the Internet to provide information, important sources are being lost as authors continuously revise or abandon previous information pages. Researchers involved in joint projects would also do well to think early on how to archive posted Web materials before they are lost. Such archives can also document the evolution of a research project.

Initially, the most suitable information for ISTRC to archive are the abstracts from its 11 symposiums spanning 30 years. These abstracts are not currently easily accessible to members, most of whom do not own the full set of symposium proceedings. Were these abstracts archived on the ISTRC homepage, they would be quickly accessible to all members and the general public with full Internet access. Funding will be required to scan or type abstracts into electronic form for posting on the ISTRC homepage.

Full papers from the ISTRC symposium proceedings could be put online, but the ISTRC could lose potential income from reduced sales of proceedings. The labour and time needed to retype or scan papers into digital format could also be prohibitive.

Stefik (1997) deals with the importance of information security on the Internet using 'trusted systems', or terminals, printers, and other equipment set up to accept documents with different levels of security. In the case of researchers conducting a joint project on the Internet, there may be research or patent ideas to protect from being pilfered by the public at large for use in unwarranted ways. Researchers may opt for devising ways to prevent people unrelated to a joint project from viewing project materials online by using a password or other technique.

Branscomb (1991) discusses some legal issues relating to the Internet. Researchers conducting projects using the Internet would do well to consider two points Branscomb (1991) raises. Due to the open nature of the Internet, users need to be aware that any information placed on it can become accessible to all. Once general Web users access ISTRC information, there is little ISTRC can do to control how those users subsequently utilize their information.

Concerning possible unauthorized use of personal information a researcher may have on the Web, Branscomb (1991) points out that there are data collection services that harvest the Web for names, contact information, and any other pertinent information that may be sold to advertisers or others. Researchers with experience in using the Internet should be aware that this can happen when advising less knowledgeable colleagues on the information that should or should not be placed online, and

explaining to them the reasons for such considerations. Branscomb (1991) also touches upon copyright issues and the many legal grey areas related to the use of Web information which should be taken into account by researchers when planning Web content.

Kay (1991) explains that the Web is especially powerful for helping participants, motivated by a well-designed project, to collaborate with far-flung colleagues pooling information and sharing ideas. With ISTRC members working in all corners of the globe, there is great potential for members to pool data from local areas forming a world picture of root and tuber planting, diseases, harvest conditions, and other situations.

A concern related to the use of Internet-connected computers for improving the efficiency of ISTRC projects is whether such high-technology equipment is necessary or whether the same results could be realized by mailing documents and photos between far-flung colleagues or by simply sending faxes back and forth. Use of the fax mode of communication has advantages and disadvantages but will remain one useful alternative for reaching ISTRC members without e-mail or full Internet access.

Researchers wishing to collaborate with distant colleagues will need to carefully consider the level of technology to use for the exchange. The equipment each researcher has available for the exchange, the time needed for a researcher to learn how to use the chosen technology, and, likewise, the time needed to teach necessary skills to assistants for conducting such exchange also have to be taken into account.

A homepage on the Internet is a way to provide a quickly available record of a project, depending on the skill of researchers for constructing such a page, which would then be accessible by colleagues anywhere in the world. However, to be able to view such a homepage requires the proper equipment, full Internet access, and expertise in using the technology.

The organization sponsoring a homepage loses control of content by including links to member-made pages. In ISTRC's case, it will be advisable to at least initially maintain control of member-made material by having it sent to the Councillor for Publications for approval and posting on the ISTRC homepage. This approach, similar to that of refereeing entries to ISTRC's symposium proceedings, allows the maintenance of a high standard of content, and also allows control over which topics are presented.

The ISTRC homepage is expected to allow researchers to publish materials without any printing cost, and to distribute them rapidly via the Internet without any mailing fees. This can become especially convenient and cost-effective for researchers participating in joint projects to share data with colleagues in distant locations.

The recent rapid diffusion of new telecommunication technology gives the ISTRC an excellent opportunity to rethink how best to service the information needs of its widely-scattered membership and the general public. However, needs of researchers without reliable telephone, fax, e-mail, or full Internet access also need to be considered.

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# Diversification of cassava utilization in the Lake Zone of Tanzania: A case study

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The utilization of cassava in many regions of Tanzania is limited mainly to processing primary cassava products for flour and consumption of cooked fresh roots. During needs-assessment studies in the Lake Zone of Tanzania, women processors expressed their interest in increasing their range of cassava products, especially for income generation. A study was, therefore, implemented in three phases: a feasibility study, an acceptability study, and dissemination activities. Follow-up studies were undertaken to determine the level of uptake and refine the dissemination activities. The level of demand for the products warranted a dissemination phase during which training workshops were organized for over 30 women's groups from Mwanza and Mara regions. Various lessons were learnt during the study including, knowledge on the use of cassava flour was limited, information disseminated through groups was taken up on a sustainable basis, and certain products were preferred (cakes, doughnuts, and chinchin), so activities were focussed on these. Target groups for the dissemination of the technology were identified as cafe owners for doughnuts, market sellers for all products, and women's groups for products from which they could generate income. Future dissemination of the technology will focus on community development organizations to ensure cost effectiveness and sustainability.

Keywords: Cassava; Diversification; Needs assessment studies; Feasibility study; Women processors

Cassava is widely cultivated in Tanzania and it is an important food security crop. In coastal areas, and especially in Dar es Salaam, fresh roots are preferred by consumers. Processed products are important in the Lake and Southern Zones. In these areas, the major products include *makopa* (sun-dried pieces) and *udaga* (fermented dried pieces). Cassava utilization is, however, limited in terms of diversity of uses in comparison with many other parts of Africa (COSCA Tanzania, 1994).

This paper describes work undertaken to diversify the range of uses of cassava in the Lake Zone of Tanzania. The need for this intervention was identified during needs assessment studies undertaken in the Lake Zone of Tanzania (Thro, 1993; COSCA Tanzania, 1994). Based on the results of this study the following were undertaken: (i) determination of the current utilization practices for cassava in Lake Zone and identification of potential interventions; (ii) test of the acceptability of a range of different cassava products; and (iii) dissemina-

tion of appropriate information on cassava processing and (or) product preparation.

## Identification of the Opportunity for Product Diversification

A participatory needs-assessment study using the approach and methods of Kleih *et al.* (1997) was undertaken in Lake Zone, Tanzania, in October 1993. The study (Thro, 1993) was organized by the Cassava Biotechnology Network (CBN) in collaboration with the National Root and Tuber Crops Programme, the Tanzania Home Economics Association, and the Natural Resources Institute. The study mainly focussed on relating farmer needs to biotechnology research on cassava, but a number of other needs and opportunities were identified. In post-harvest, the desire of women to diversify their range of cassava products was included. The findings of this needs-assessment study agreed

with the interpretation of the analysis of data collected during the first phase of the Collaborative Study of Cassava in Africa (COSCA). In this study (COSCA Tanzania, 1994), it was proposed that in view of the limited range of products prepared from cassava (compared with other countries in Africa), efforts should be made to diversify cassava utilization. Such an approach would offer new marketing opportunities and so contribute to the developing market economy.

### Analysis of Current Cassava Utilization Practices

To further understand the issues arising from the CBN and COSCA studies, a feasibility study was undertaken in selected urban and rural areas in Mwanza and Mara regions of the Lake Zone. The study focussed on the major urban markets and selected rural villages within the marketing chain. Semi-structured group and individual interviews using the methodology of Kleih *et al.* (1997) were held with traders, restaurateurs, bakers, snack vendors, and farmers.

The feasibility study (Rwiza *et al.*, 1995) confirmed the importance of cassava as a main staple in rural and urban areas. Fresh roots and a sun-dried, fermented product (*udaga*) were the most commonly marketed forms of cassava. It was observed that women play a key role in the production, processing, and certain sectors of the cassava market chain. In the urban markets and rural areas, primary products were of paramount importance, whilst street vendors and cafe owners were involved in marketing a limited range of secondary products, namely *ugali* (flour and water paste) and occasionally *mbute* (boiled fermented pieces). The production of secondary products from cassava flour is limited, largely due to the lack of knowledge concerning alternative utilization practices and consumer preferences for other staples such as maize and rice.

The potential for cassava to substitute for other raw materials (such as wheat) in the preparation of certain products was evident. It was also suggested that preparation and marketing of products prepared from cassava flour could provide income-generating activities at both the rural and urban levels.

### Acceptability Study and Pilot Scale Dissemination Activity

Based on the results of the feasibility study and the niche markets, a pilot phase was initiated to investigate the acceptability of different cassava products. In light of the promising results of the acceptability study, the optimum mechanism for wider dissemination of the product preparation methods was implemented. Three training workshops were undertaken in September 1995 for 12 market sellers, 28 cafe own-

ers and vendors, and 35 households supplying major urban markets. The workshops were held in Mwanza urban (Pamba and Buhongwa), Mwanza rural (Nyang'holongo), Mara urban (Mazami), and Mara rural (Mkirira). A total of 75 women and men were introduced to the preparation of the following products from cassava flour: doughnuts, biscuits, chinchin, croquettes, and cakes. The products contained no wheat flour. The recipes used were initially developed by the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Simple product evaluation studies were carried out with consumers from the pilot areas to determine the acceptability of the products. An example of the data obtained is shown in Table 1. In general, products were considered to be highly acceptable by consumers, who were used to eating similar products prepared using wheat or maize flour.

A follow-up study was conducted in February 1996 (Kapinga *et al.*, 1996) to gain processors' perceptions of the introduced products and to examine the levels of uptake. A semi-structured interview approach (Kleih *et al.*, 1997) was used to collect data on these issues. Of the 75 people initially trained, 38 were located for interview (28 cafe owners and vendors, 5 market sellers, and 5 others). All of those who were contacted indicated that they were still preparing the products to a greater or lesser extent. The preferred products were doughnuts (produced by 32 out of 38 interviewed), chinchin (produced by 19 out of 38), and cakes (10 out of 38). Croquettes and biscuits were prepared by only a few people.

The most common product, doughnuts, was prepared once or twice a week by most of those trained, but a significant number (12 out of 32) produced them more frequently. Other commonly prepared products were typically prepared once or twice a week. For the most commonly prepared products (doughnuts, cakes, and chinchin), the most significant factors in their popularity were simple preparation procedures, short preparation times, and the acceptability of the marketed product. The most im-

Table 1 Acceptability of different cassava products in Lake Zone, Tanzania

Product type	Criteria (mean score for 44 consumers)			
	Taste	Shape	Colour	Overall acceptability
Doughnut	4.8 a	4.5 b	4.7 b	4.8 a
Chinchin	4.7 ab	4.5 b	4.6 b	4.5 b
Croquettes	3.5 c	4.1 b	4.2 c	3.8 c
Cakes	4.9 a	4.9 a	4.9 a	4.9 a
Biscuits	4.6 b	4.3 b	4.5 b	4.4 b

Products were scored on a scale of: 1 (very bad), 2 (bad), 3 (moderate), 4 (good), and 5 (very good); Mean scores with the same letters were not significantly different at the 5% level

portant reasons for not preparing the less common products were difficulty of preparation (following recipes), time required, and difficulties obtaining certain pieces of equipment such as cutters and baking facilities.

The majority of the sample of people surveyed (28 out of 38) were cafe owners or vendors, however, the products they prepared were mainly for home consumption. Cassava doughnuts and chinchin were marketed by almost 30% of those interviewed. Very few of the people interviewed sold cakes (2 of the 38 interviewed).

Nearly 50% of those interviewed stated that they had encountered some problems in preparing the products. The major problems and issues that they mentioned were need for more follow-up by trainers, lack of facilities for preparing some products (biscuits, croquettes, and sometimes cakes and chinchin), lack of raw materials (good quality cassava flour), and in some cases lack of funds. The lack of raw materials was specifically a problem in urban areas where people prefer to buy raw materials rather than process them themselves. Traditionally-prepared cassava flour was considered to be of poor quality and not suitable in terms of the taste, flavour, and texture for production of the secondary products prepared.

To determine the uptake and sustainability of the transfer of knowledge, the spontaneous dissemination through the society was monitored. Of the 38 interviewed, 23 had passed on at least one technique to at least one person (Table 2). The majority of those trained by those interviewed were in community organizations, specifically church and women's groups. The attachment to such community-based organizations assisted some processors in obtaining funds. The levels of income generated by those marketing the products were significant in terms of household income for the processors. The economic benefits that can be obtained from marketing secondary cassava products require further analysis.

Based on these observations on the pilot phase, a strategy for the wider dissemination of

the products was developed, the key elements of which were to: focus on only a small range of products (doughnuts, chinchin, and cakes); continue with the workshop-based format of dissemination; focus dissemination on women's groups; produce appropriate recipe booklets and other aids to dissemination; and build a means of technical support into the dissemination process.

### Wider Dissemination Activities

A further 115 women group-members (representing 30 groups) in Mwanza and Mara regions have so far been trained in the production of doughnuts, chinchin, and cakes. The groups were also trained in the processing of good quality cassava flour.

A follow-up study on these individuals was carried out approximately three months after the training workshops and a total of 67 people were interviewed. Only two of those contacted were not preparing the cassava products. As in the pilot study, doughnuts were the most common product. People produced the products for both home consumption and sale. Forty-six of the 67 trained had passed the knowledge on to others. It was estimated that a further 545 people had been trained. The majority of these were members of the same community-based groups (Table 3). This emphasizes the importance of targeting these groups for such dissemination activities.

In many of these products, cassava replaced wheat. Product acceptability was good and indications from some consumers were that it was difficult to differentiate between cassava- and wheat-based products. Preliminary indications are that the preparation and marketing of these products offer a significant income-generating opportunity for women. For those already processing such products, there is the potential to replace wheat with a less expensive raw material. Further work is required on the costs:benefits associated with the preparation and marketing of products made with wheat and cassava.

**Table 2** No. of people trained by those who were interviewed who had trained others in the pilot phase

Location	No. of those interviewed who trained others	No. of people trained by those interviewed			Total
		Relatives	Community groups	Friends and neighbours	
Mwanza — urban	10	27	10	12	49
Mwanza — rural	5	5	20	16	41
Mara — urban	3	0	10	8	18
Mara — rural	5	5	20	8	33
Total	23	37	60	44	141

**Table 3** Extent of secondary dissemination of knowledge concerning preparation of products from cassava flour for the wider dissemination phase

Location	No. of trainers <sup>1</sup> interviewed	No. of people trained by trainers			Total
		Relatives	Community group members	Others	
Tarime	14	31	114	20	165
Bunda	11	25	65	0	90
Musoma	15	18	167	83	268
Mwanza	6	7	15	0	22
Total	46	81	361	103	545

<sup>1</sup>Trainers were those trained by the project team who then passed information on to others

One clear issue from the follow-up studies was that there is a need to develop further, the processing of good quality cassava flour. Those preparing these products in urban areas require convenient raw materials that they can purchase directly off the shelf. Further development activities on flour processing should focus not only on the issues related to quality but also the incorporation of high-quality flour processing into the post-harvest system.

## Conclusions

It has been demonstrated that a logical step-wise approach to product diversification can be effective. The key stages used in this study were: identification of the initial need; confirmation of the validity of the need through a 'feasibility study' and cross-correlation with other secondary information; and an interactive pilot phase where detail was obtained on the factors that would facilitate sustainable uptake of the new knowledge.

In the case of product diversification in Lake Zone, there was clearly potential for flour substitution and certain new products. This was reflected in the high take-up rates in both the pilot and wider dissemination phases. The true measure of the sustainability of the processing of these products will be determined by the levels of long term use of the techniques and their secondary dissemination to other users. The economic benefits of processing these products from cassava flour requires quantification as does the potential impact on cassava producers.

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# Methods for examining the relationship between quality characteristics and economic value of marketed fresh sweetpotato

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Elizabeth Rwiza<sup>3</sup> and Andrew Westby<sup>2</sup>

In most urban markets in Tanzania, sweetpotato [*Ipomoea batatas* (L.)] is purchased in sacks or bamboo baskets and then sold to consumers in heaps at fixed prices. Heaps vary in total weight and quality. Two complementary methodological approaches for assessing the relationship between quality and economic value of fresh sweetpotato are described. The first approach used a range of participatory ranking and valuation exercises to gain an impression of the major quality issues, seasonality of marketing, and the impact of specific types of quality deterioration on retail value. This was supported by a statistical analysis of heaps of produce being retailed in the market. This second approach allowed the impact of different quality characteristics to be quantified. Application of these methods is demonstrated through analyses of the impact of different quality characteristics on retail value during low season marketing in Mwanza, Tanzania.

Keywords: Sweetpotato; Urban markets; Methodological approach; Participatory ranking; Valuation exercise; Tanzania

Although sweetpotato [*Ipomoea batatas* (L.)] is a traditional crop of subsistence farmers in Tanzania, the commodity is increasingly being marketed. The major production areas are Lake Zone (66% of national production), Southern Highlands (17%), and Western Zone (10%) (Anon., 1994). It is the third most important crop in terms of calorific value for rural and urban populations in Tanzania (Kavishe, 1993).

Little information is available on the marketing systems for sweetpotato. The perishability of the fresh tubers is accepted as an important constraint. Marketing systems for sweetpotato are poorly developed with high levels of root damage. Grading and storage are not common. In most urban markets, roots are purchased in sacks or bamboo baskets and retailed in heaps. Sacks usually contain ungraded roots and their price is negotiated on a daily basis depending on existing supply and demand conditions. Weighing scales are not normally available and, therefore, transactions are determined by volume rather than by weight. Heaps, consisting of 5–7 roots, are sold at fixed prices but they differ

in their weight depending on such factors as size, variety, and quality. In this study, for simplicity, size is assessed by weight with no account taken of shape. Damage is a complex variable to assess, but can mostly be covered by four categories: breaks, cuts, shrivelling, and weevil (*Cylas* spp.) damage.

This paper sets out two different, but complementary, methodological approaches for assessing the relationship between the quality and economic value of sweetpotato. One is based on participatory data collection involving both traders and consumers and the other uses a statistical analysis of marketed heaps of roots. By analysing the impact of the different types of damage on value of the produce, it should be possible to identify quality improvements that are most valued in the market.

## Methodology

### Participatory methods

Participatory methods rely on the active participation of market traders and consumers in the collection, analysis, and interpretation of data. It is possible to generate more detailed information than can be obtained using formal survey methods alone. Kleih *et al.* (1997) gives an overview of participatory approaches to needs identification in root and tuber crops post-harvest systems.

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### Elaboration of quality criteria

Knowledge of the important quality characteristics of sweetpotato is essential before starting to assess the relationships between quality characteristics and retail value. Previous studies have been undertaken in Tanzania by Kapinga *et al.* (1996). This information was supplemented with opinions gained from informal open discussions with consumers and traders in Mwanza Market. In this particular study, quality characteristics were divided into criteria associated with variety, damage, and size (Table 1). This type of information was very easy to collect by a small team (1-2 people) in less than one day.

### Sweetpotato marketing calendar

In order to gain an overview of the seasonality of the price quality relationships, a marketing calendar was prepared for each of the markets. The calendar was prepared through a participatory exercise with two market traders using a board and a set of counters such as beans. It was found that the use of two traders facilitated discussion and led to more self-correction of scorings. The involvement of more than two people led to situations that were difficult to manage. By trial and error, it was found that the best way to prepare the matrix was on a board with month on the horizontal axis and price, quantity, and quality on the vertical axis. Beans were used to represent relative amounts. Traders were asked to place the appropriate number of beans for each month based on their knowledge of the market. They were en-

couraged to discuss their decisions and were allowed to change the scores until they were happy with the whole picture. The process was enjoyable for the traders and relatively quick (taking only 20 min). From the discussions with the traders, additional information to explain the trends and relationships in the matrix was obtained. An example of a seasonal calendar matrix is given in Table 2. Some of the additional information collected is given below.

The quantity of sweetpotato traded is highest between May and July (Table 2) which is the main harvesting season. Prices are relatively low at this time of year. There is a second lower peak in quantity traded in December that coincides with the crop of sweetpotato from the paddy fields. Smaller quantities are traded in March and April (early harvest) and August and September (late harvest). Very little is traded in the remaining months since it is only available through in-ground storage and piece-meal harvesting. Produce quality is highest during the main harvest season when prices are low and quantities traded are high. Quality is lowest in September and October when roots have gone beyond their optimum harvest time and become watery. Prices fall to compensate for the poor quality at this time of year.

The participatory development of seasonal calendars not only gives an overview of the price and (or) quality relationship changes over the year, but also provides additional information (through the explanations given) on the traders' understanding why these occur. Such additional information would have been very difficult to obtain through formal questionnaire-based surveys. The example described above demonstrates that quality changes throughout the season, so this type of information is essential for a full understanding of the quality and (or) value relationships in urban markets.

### Participatory valuation of roots of different qualities

Although several approaches were evaluated for collecting participatory valuation data, only the most successful is reported here. The technique used involved creating heaps of sweetpotato of the same variety and weight (1.4 kg), but with different quality characteristics (good, with cuts, with breaks, and with weevil damage). The weight of roots selected (1.4 kg) was typical of

**Table 1** The quality characteristics of sweetpotato

Variety	Damage	Size
Taste	Breaks	Ease of handling
Starchiness and (or) texture	Cuts	Ease of peeling
Speed of cooking	Shrivelling	Speed of cooking
Skin colour and (or) appearance	Surface weevil Deep weevil Bruising Rotting	

Note: Information was obtained by reviewing previous Tanzanian literature and interviewing market traders and consumers

**Table 2** Sweetpotato calendar for Mwaloni Market, Mwanza, Tanzania

Quantity	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Quantity	*	**	***	****	*****	*****	*****	***	**	*	*	*****
Price	****	****	***	**	*	**	**	*	*	*	*****	****
Quality	*	**	***	****	*****	*****	*****	*			*****	*****

No. of asterisks indicate changes in variables from month to month as perceived by two key sweetpotato traders. Asterisks for quantity and quality give rough indications of changes, but cannot be used to measure absolute values. For prices, however, each asterisk represents approximately Tsh 1000 on the average wholesale price of a sack

a Tsh 200 heap. A sample of 5 traders and 15 customers were asked to rank the four heaps in terms of preference and then asked to value the heaps. For the second part of the exercise, Tsh 200 (the usual price) was assigned to the number one ranked heap and the individuals were asked to assign any one card labelled from Tsh 120 to Tsh 200 (in units of 10 Tsh) to each of the other heaps, bearing in mind their initial rankings.

An example of the data obtained is given in Table 3. Cut and broken roots were valued less than good roots (93% by traders and 85% by consumers of value of good roots for cut roots; 83% by traders and 87% by consumers of value of good roots for broken roots). Sweetpotato infested with *Cylas* spp. weevil was valued at 64% of the value of undamaged roots by traders (63% by consumers).

This approach to data collection gives a relatively rapid means of assessing the importance placed by consumers and traders on different types of damage and allows a value to be assigned to the different preferences. The technique was rapid, taking less than one day per market and required only one researcher.

### Statistical analysis of heaps of potatoes

The statistically-based approach to assessing the relationship between produce quality and value was based on measurements of representative samples of sweetpotato sales. A sale comprises roots in a heap on display plus additional ones that are given from behind the counter. These extra roots commonly comprise between 10 and 20% of the final weight of the heap and cannot be considered insignificant in data analysis. As the additions tend to be of below-average quality, this method is a way of selling poorer-quality produce whilst displaying the better roots. Heaps sell at fixed prices. There are three major price categories for heaps: small roots, Tsh 100; medium sized roots, Tsh 200; and large roots, Tsh 300.

Five sweetpotato traders in each market were selected at random. For each trader, three heaps of sweetpotato were chosen for each combination of variety and price. For example, if a trader was displaying three varieties in each

of the Tsh 100 and 200 price categories, 18 heaps would be sampled in total. Traders were asked to supply the usual "top-up" from behind the counter for each of the heaps. In order to facilitate subsequent statistical analysis, only heaps of the same variety were sampled. If necessary, traders were asked to arrange heaps accordingly. Each root in each heap was weighed and assessed for damage using the following four criteria: breaks, cuts, shrivelling, and weevil infestation. Scores of 0, 1, and 2 representing no, minor, and major damage, respectively, were used. To ensure maximum consistency of data, one member of the team weighed the roots and recorded the data while the other assessed levels of damage. A weighted mean score for each damage criterion was calculated based on the weight and score for each root in a heap.

Data were analysed using Statistical Package for Social Scientists (SPSS), version 4.01 which allows tabulation, regression, and other statistical interpretation of results. Selling price per kilogram was calculated for each sampled heap and used to represent 'value'. This variable was compared with various indicators of quality including variety, average potato size, and damage as represented by the scores for breaks, cuts, shrivelling, surface weevil, and deep weevil attack. Multiple regression analysis using a step-wise approach was used to produce best fit formulae for each of the variables assessed.

As an example of the use of the method, a survey was undertaken of the four major markets in Mwanza in April 1997 and a total of 184 heaps of roots were sampled. The three most common varieties, locally known as Polista, Njano, and Sinia comprised 92% of all roots sampled. There were marked differentials in average selling price per kilogram for each of these with Sinia selling at an average premium of 14% above the price of Polista, and Njano selling at a premium of 7% compared to Polista.

Root size had an effect on value. Sweetpotato in the medium-sized category, selling at Tsh 200 heap<sup>-1</sup> and weighing an average of 223 g each, was sold at a 12% premium (on a per kilogram basis) when compared

**Table 3** An example of market value assessment (Tsh heap<sup>-1</sup>) of sweetpotato with different quality criteria

Quality	Trader No.					Consumer No.														
	1	2	3	4	5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Good	200	200	200	200	200	200	200	200	200	200	150	200	200	200	190	200	200	200	200	200
With cuts	180	190	180	190	190	160	170	190	190	150	130	120	140	150	200	180	190	180	180	170
With breaks	150	180	140	180	180	190	180	180	180	130	200	150	180	170	180	170	170	170	160	150
With weevil damage	130	120	120	150	120	140	120	120	120	120	120	130	120	120	120	130	130	120	120	120

The trader mean (TM) and consumer mean (CM) for good quality sweetpotato was 200 (100%) and 196 (100% good), respectively; for sweetpotato with cuts, TM was 186 (93% good) and CM was 167 (85% good); for sweetpotato with breaks, TM was 166 (83% good) and CM was 171 (87% good); and for sweetpotato with weevil damage, TM was 128 (64% good) and CM was 123 (63% good)

to the smallest size category (selling at Tsh 100 heap<sup>-1</sup> and weighing an average of 109 g). Very large potatoes, selling for Tsh 300 heap<sup>-1</sup> and weighing an average of 392 g, sold at a price per kilogram very similar to the smallest size category. These results suggest that customers prefer medium-sized potatoes to very small or very large ones, possibly because very small ones are difficult to peel and give a higher proportion of waste and, maybe, because very large ones are difficult to handle.

Multiple regression analysis using a step-wise approach produced a 'best-fit' formula for Polista variety sweetpotato in Mwanza Central Market of:  $y = 144.16 - 8.17b - 9.99c$

where  $y$  = selling price per kilogram in Tanzania shilling;

$b$  = weighted average heap score for breaks damage; and

$c$  = weighted average heap score for cuts damage.

The constant term varies depending on the variety of sweetpotato and the market in which the sweetpotato is sold with a maximum of 168.74 and a minimum of 139.70. The equation does not contain terms for shrivelling or weevil damage. This is because relatively few sweetpotatoes with these types of damage were sampled.

Regression statistics for the data analysed are shown below in Table 4. Damage as measured by cuts and breaks, variety, and market location each have a significant impact on the price at which roots are sold. As an illustration of the use of the model, the effect of breaks in different markets on price is shown in Table 5.

The statistical analysis of the composition of heaps of roots provides quantitative data on the implication of various quality parameters on price. Both approaches produced similar levels of price reduction for broken roots (analysis of heaps, average 11.4% reduction; participatory approach, 13% for consumers and 17% for traders). Considerable resources are required for the statistically-based approach. In the example detailed above, three researchers

**Table 4** Accumulated analysis of variance for different variables affecting the quality of sweetpotato

Variable	Mean sum of squares	Variance ratio	F-statistic	Confidence level (%)
Variety	3414.5	10.71	<0.001	<99.9
Market	1265.4	3.97	0.009	00.1
Breaks score	1304.2	4.09	0.045	95.5
Cuts score	1309.4	4.11	0.044	95.6

$n = 184$

**Table 5** Estimated selling prices (Tsh kg<sup>-1</sup>) for broken and unbroken Polista sweetpotato in each market in Mwanza during April 1997

Market	No. of breaks	Bad breaks	Value of no. broken (%)
Central	144.16	127.82	88.7
Mwaloni	144.38	128.04	88.7
Kirumba	156.31	139.97	89.5
Zimbabwe	139.70	123.36	88.3
Mean			88.6

spent one week in the market collecting data and then a further one week analysing the data. Another limitation of the approach is that it is dependent upon a reasonable level of all types of damaged roots being available in the market. In the example given above, there were very few weevil-infested or shrivelled roots and so no analyses were possible with these variables.

## Discussion

The above data analyses illustrate how different methodologies, both participatory and statistically-based, can be used to investigate the same relationship, in this case that between the quality and economic value of sweetpotato.

There are advantages and disadvantages to the two types of approach evaluated. Statistical-based analysis of heaps allow data-gathering in a consistent and concise way, but it can be monotonous and time-consuming for both researchers and respondents. This can result in an element of carelessness when recording or giving data. Significant resources are required for field studies and access to a computer. It is also difficult to investigate opinions that are very sensitive or subjective in nature through such approaches. Data analysis is retrospective in nature and opportunities for the confirmation of observations or obtaining additional information are limited.

Participatory methods encourage a greater flexibility of data collection and can allow more avenues of experimentation not previously thought of, but can be difficult to apply consistently or produce data that are difficult to summarise. As can be seen from the examples in this paper, participatory methods were significantly faster than the corresponding statistically-based analytical approach. This has implications for human and financial resource allocation. It is sometimes necessary for policy decisions to have estimates of numerical relationships between variables and this is sometimes difficult to achieve through the use of participatory methods alone.

Compton *et al.* (1995) discuss the use of rapid survey methods for assessing storage losses of durable commodities and conclude that a range of tools should be used to meet the specific objectives of the study. Extensive random surveys (equivalent to statistically-based approach in this paper) were only recommended when strictly necessary because of the time and resources required and the amount of time required for data analysis.

Participatory exercises are the most appropriate for obtaining in-depth explanation of customer and trader opinions on an issue such as product quality. By involving participants in 'games' such as the construction of marketing calendars and valuation using monetary cards, interest is generated and people are more likely to give in-depth explanations of their preferences. More formal statistical methods are best where a large volume of numeric or simple verbal data that can be analysed in the office is required. The approach does, however, lack flexibility. It is possible to adopt both approaches to benefit from the advantages of each method and to compare the results of each as a cross check on accuracy. This was done during the development of the methodology in this study where the relationship between sweetpotato value and various damage criteria was investigated. The approaches could be used to study similar relationships for other commodities marketed in a similar manner.

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# Review of research in Papua New Guinea for sustainable production of taro (*Colocasia esculenta*)

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An overview of current research on taro [*Colocasia esculenta* (L.) Schott] and the prospects for taro improvement in Papua New Guinea are presented. Attempts are being made to address some of the major production constraints with a view to sustaining production. Major production constraints by biological agents include the Coleopteran insect pest *Papuana* spp. (taro beetle), and taro diseases, *Phytophthora colocasiae* and Alomae Bobone virus complex. The source of taro leaf blight and *P. colocasiae* resistance were identified from the national taro germplasm collection as well as from other countries and were assembled for targeted crosses. Selected genotypes with improved quality and resistance to diseases are being evaluated before distribution. A management strategy looking at the use of natural enemies, trap crops, barriers, seasonality, intercropping, and tolerant genotypes will be explored with a view to developing appropriate sustainable pest management practices. Research on the removal of other constraints to improved taro production such as soil nutrition, water management, and cropping systems (such as rotation, intercropping, plant spacing, and mixture of disease resistant and susceptible cultivars) is also reviewed.

Keywords: *Papuana* spp.; *Phytophthora colocasiae*; *Colocasia*; Taro; Crop improvement

Taro [*Colocasia esculenta* (L.) Schott] is an important food in Papua New Guinea (PNG). It is grown primarily for its edible corms and to a lesser extent for its foliage (Rangai, 1977). Taro is the third most important food crop after sweetpotato (*Ipomoea batatas*) and banana (*Musa* spp.) and is the second most important staple root crop after sweetpotato in PNG. The estimated annual production of taro in PNG is about 436 000 t from an area of 77 000 ha.

Research undertaken on taro by the Lowlands Farming Systems Research Team, Bubia Agricultural Research Centre, in collaboration with colleagues from other research institutions is described here. The factors that either limit or enhance taro production in PNG are also presented and technologies that enhance taro production are explored.

## Status of Research and Development of Taro

Taro is subject to many production constraints. These include taro beetle (*Papuana* spp.), taro leaf blight (*Phytophthora colocasiae* Racib), the

Alomae Bobone virus complex, inadequate planting material, soil nutritional disorders, weeds, abiotic stress such as low soil moisture, and inadequate market access.

## Taro beetle

Taro beetle is ranked as the greatest constraint, being moderate to severe in many areas where losses range from 0 to 77% (Arura *et al.*, 1987). The adult causes the damage to the crop. It borrows into the soil from where it bores into the corm attacking between the middle and the apex where it feeds. They have not been observed to attack the above-ground part of the exposed corm. This behaviour may be attributed to the high concentration of calcium oxalate near the top end of the corm, or escape from natural enemies, and (or) escape from the above-ground environmental conditions.

Adults have been observed to burrow deeper into the soil to about 30–40 cm below the soil surface and form cavities (Thistleton *et al.*, 1995). They feed on taro for about two months before the females seek suitable breeding sites to lay eggs. A study was initiated to determine the breeding habitat of taro beetle.

Numerous grass species were found to be hosts. Highest numbers of larvae were collected under Johnson grass (*Sorghum verticilliflorum*), Elephant grass (*Pennisetum purpureum*), kunai (*Imperata cylindrica*), and pitpit (*Phragmites karka*). High larva density was associated with decaying organic matter from flood deposits under these grass species (Sar and Niangu, 1995). These findings may prove valuable for control tactics. The association of soil type and vegetation to oviposition sites may be considered useful for forecasting where and when potential serious attacks of taro beetle may occur.

Another research study conducted at Buba Agriculture Research Centre demonstrated that the entomopathogenic fungus, *Metarhizium anisopliae*, the bacterium, *Bacillus popilliae* and the protozoa, *Vavraia*, can play an important role in regulating populations of taro beetle (Theunis et al., 1996). Laboratory and field trials are being conducted to ascertain the effectiveness of these natural agents on taro beetle mortality. Preliminary field trials indicated that isolates of *M. anisopliae* caused sufficient mortality after field application (Masamdu, pers. commun.). However, the initial effect was poor and very slow, requiring at least three weeks to effect more than 80% kill. Other pathogens investigated included nematodes, *Steinernema glaseri*, *S. carpocapsae*, and *S. feltiae*. These soil-borne pathogens inflict mortality on the larva. Over the long term, the ecological benefits may prove to be highly significant.

Various studies have been conducted on cultural control methods. Since taro beetle has a wide host range, Masamdu et al. (1990) investigated some of these hosts such as sweetpotato, banana, edible pitpit (*Saccharum edule*), and wood ash. Sar et al. (1991) investigated the potential of coconut husk, grass mulch, shade cloth, polythene film, and insect wire mesh as barriers to limit accessibility to taro.

Because of the very low infestation, these studies were unable to assess the effects of the treatments and further studies will be pursued. However, the study on the effects of barriers showed that mulch enhanced plant growth, thus, high yields were recorded and the high yields under mulch can compensate for loss caused by the taro beetle (Sar and Niangu, 1995). In the same study, immature stages of taro beetle were found under mulch. Based on this observation, a strategy could be developed to use mulch as a breeding site to attract females. Upon reaching the mulch, the females will come in contact with an insecticide or be infested with an effective entomopathogenic mortality agent. Trials are also in progress to confirm the use of *Rhododendron* spp. as a repellent against taro beetles. Leaves of *Rhododendron* are placed at the base of the taro planting material while planting as a control method against taro beetle in Gumine-Chimbu Province, PNG.

## Studies on taro leaf blight (TLB)

Taro leaf blight disease appears as small circular spots on the upper leaf surface and water-soaked on the underside. The spots can enlarge up to 10 cm in diameter and become irregular with a yellow margin (Kokoa, pers. commun.). Under favourable conditions there is rapid increase in the spread of the infection and the subsequent loss of the entire leaf. Leaf blight is the most destructive foliar pathogen of taro and is now endemic and widespread throughout PNG (Shaw, 1984; Muthappa, 1987; Kokoa, 1991). It has been reported that extensive damage to leaves during severe epidemics led to yield reductions by 50% or more (Parris, 1941; Johnson, 1960; Jackson et al., 1980; Cox and Kasimani, 1988; Vasquez, 1990). The ravages of taro by *P. colocasiae* in Bougainville is a good example. Taro was the staple crop 50 years ago, however, it is now replaced by the less favoured sweetpotato because *P. colocasiae* made taro production impossible (Clarke, 1978).

The national taro germplasm collection was screened for resistance to *P. colocasiae* in 1992. From a total of 433 accessions screened, 3 local varieties K333, K345, and Ainaben were rated highly resistant, 57 slightly resistant, and 373 highly susceptible (Kokoa and Darie, in press). These three resistant accessions with other donors from Solomon Islands and Thailand were used as sources of resistance for further selection and breeding.

A study was initiated to investigate the effect of intercropping or combinations of susceptible and resistant cultivars on the intensity and spread of *P. colocasiae* (Gunua, in press). Mixtures of susceptible and resistant taro cultivars in ratios from 100:0–30:70 (susceptible:resistant) were grown in 1995. A sample of 10 plants of the susceptible cultivar from each treatment was assessed. There was a decrease in leaf blight incidence with increase in proportion of resistant cultivars (Table 1). The treatment with 30% resistant plants sustained low infestation and concomitant higher yield.

Mixtures of resistant and susceptible cultivars slow the rate of development and spread of TLB relative to susceptible pure stands of taro.

**Table 1** The effect of intercropping susceptible and resistant taro cultivars on incidence of *Phytophthora colocasiae*

Treatment	% Susceptible variety	% Resistant variety	Yield (t ha <sup>-1</sup> )	Coefficient of disease index
A	100	0	3.1	22
B	60	40	3.2	18
C	50	50	3.3	17
D	40	60	3.7	15
E	30	70	4.8	13

Source: Gunua (in press)

According to Wolf and Barret (1979, 1980), cultivar mixtures can be easily exploited to provide a simple cheap means of controlling important airborne diseases and which will also insure against other diseases that occur sporadically. This study shows that control of TLB by cultivar mixture is possible and can result in substantial benefits. It also highlights the potential of resistant lines in the development of integrated disease management programmes even if the knowledge of the epidemiology of *P. colocasiae* is not completely elucidated.

### Breeding

In 1992, recurrent selection was used to improve taro through the selection of taro lines resistant to major diseases, with good yield and eating quality. In addition, crosses were made to select lines with other desirable traits such as adaptability to saline soil conditions and wet-lowland ecotypes, density tolerance, and early maturing.

The breeding programme is currently in its third cycle or generation. Combinations from the breeding programme were screened for response to TLB infection. All the combinations derived their resistance from three local cultivars and other parents from Philippines, Thailand, and Solomon Islands.

The best 10 performing hybrids were selected from 75 TLB-resistant lines from the first generation while 22 elite lines were selected from 500 lines in the second generation (Okpul *et al.*, 1997). Four resistant lines were selected in tests conducted to identify new accessions expressing tolerance to density while one line was selected for above-ground corm characters. The latter would be suitable against taro beetle since the pest avoids damage to above-ground corms. Because of the different agro-ecological zones in PNG, multi-locational trials will be conducted to select taro lines adapted to these agro-ecological zones.

### Taro Genetic Conservation

The national taro field genebank was assembled in 1985 in response to the need to conserve and utilize the taro diversity available in PNG. The principal role of the genebank is to collect, document, evaluate, multiply, and redistribute germplasm, from and to farmers and research collaborators. Originally 680 accessions were collected but these declined to 333 as a result of biotic and abiotic constraints. Passport data are available in both manual and computer forms.

The taro germplasm collection represents a largely untapped genetic resource of great potential value for improving taro. Three accessions resistant to *P. colocasiae* have been identified and used in the breeding programme.

Twelve accessions were selected for yield and good eating quality from which five accessions were further evaluated at four locations. There was high variability in yield among varieties and among sites (Table 2).

Efforts were made to maintain the 333 germplasm collection through an *in vitro* germplasm gene bank. Investigations have been conducted to establish minimal growth techniques for taro which will ultimately provide for the regeneration and long-term storage of the national taro collection. In addition, attempts will be made to look into micropropagation of meristem-cultured taro for production of pathogen-free planting material. In the absence of inherent virus in tissue cultured plantlets, early plant vigour will give potentially higher yields than from conventional taro planting materials from setts. Multiplication by conventional means is slow and the rapid multiplication of taro through tissue culture will address a major production constraint.

### Production Systems

Taro is cultivated in various crop mixtures by farmers relying on shifting cultivation and bush fallow systems for maintenance of soil fertility. Although such a system is stable where there is abundant land, as a result of population increase, fallow periods are greatly reduced, hence, the above system cannot support an increasing population.

The traditional method of conservation and utilization of fresh water resources for domestic and agricultural use is well documented. Irrigation is practised in locations such as Kabwum and Sialum (Morobe Province) and Wamira (Milne Bay Province) which involve the modification of water and soil conditions to create ecosystems which are favourable to the growth of particular crops, mainly taro (Spriggs, 1980). Another method is the use of traditional wetland or swamp agriculture which is found throughout PNG and elsewhere in the Pacific, for the growing of giant swamp taro

**Table 2** Yield of taro (Bubia) cultivars under four different environmental locations

Cultivars	Total fresh yield (t ha <sup>-1</sup> ) per location				Mean
	Bubia 1986	Unitech 1987	Worsera 1991	Ramu 1992	
BC003	2.11	4.49	2.99	9.70	4.83
BC026	3.36	6.22	2.96	nt	4.18
BC029	3.53	5.81	3.47	nt	4.27
BC030	5.35	8.25	2.01	14.20	7.45
BC121-1	1.49	7.85	1.39	21.10	7.96
BC030	1.87	1.37	nt	6.30	3.18

Source: Akus (unpubl.), Ososo *et al.* (1992)  
nt. Not tested at this site



*Cyrtosperma chamissonis*. Yields of taro under these different irrigation techniques are generally much higher than under dryland conditions. Taro yields under irrigation were 18.4–21.8 t ha<sup>-1</sup> yr<sup>-1</sup> and 37.6 t ha<sup>-1</sup> yr<sup>-1</sup> for Awa (Eastern Highlands), and Patep (Morobe), respectively. Dryland yields were lower than irrigated taro, producing only 12.5 t ha<sup>-1</sup> yr<sup>-1</sup> at Patep and 2.5–15 t ha<sup>-1</sup> yr<sup>-1</sup> elsewhere (Spriggs, 1980).

In the high-rainfall forest-covered foot hills of Upper Ramu District of Madang Province, a farming system is employed to initiate a rapid rate of succession within garden fallows. Selective felling of the vegetation on the garden site assists rapid regrowth. Thus, tree species which are known to regenerate quickly are pollarded whilst others are cut down and removed. Burning of vegetation is restricted while weeding is very selective. A very narrow range of crops is grown, the dominant crop being taro. There is a particular taro cultivar which has a characteristic degeneration of above-ground vegetative parts when mature, leaving only the tiny cormels to grow among the weed regrowth. The taro corms are left in the soil with little or no above-ground vegetative part and harvested progressively until a new site is cleared. Despite the very tiny size of the corms, normal size of taro plant is achieved when replanted. This system allows continuity in food supply and conservation of planting materials.

Various studies have been conducted to address soil nutritional problems and improve the status of taro in the farming system, and the potential of food legumes in crop rotation with taro and sweetpotato (Ivahupa *et al.*, 1991). Preliminary results from the first two cropping cycles showed some promise with 27% higher yield obtained from rotating taro with cowpea and wingbean. Based on the enhanced performance of taro intercropped with peanut in preliminary studies, further intercropping studies with peanut are planned.

Plant density trials have been conducted and reported by Risimeri *et al.* (in press). The common taro cultivar 'Numkoi' was planted at spacings of 1 m × 1 m, 1 m × 0.5 m, 0.5 m × 0.5 m, 1 m × 0.25 m, 0.5 m × 0.25 m, and 0.25 m × 0.25 m. The results (Table 3) showed that at a spacing of 1 m × 0.25 m with 40 000 plants ha<sup>-1</sup> produced a 66% yield increase.

Further work is required to determine the density required to achieve optimum economic and marketable corm yield.

### Food Technology — Storage and Utilization

Several findings from research and development in taro post-harvest physiology, storage, handling, and utilization at the Applied Science Department, University of Technology, have further

**Table 3** Effect of planting density on yield of taro

Spacing (m)	Plant population	Yield (t ha <sup>-1</sup> )	Marketable yield (t ha <sup>-1</sup> )
1 × 1	10 000	14.85	9.33
1 × 0.5	20 000	16.27	9.17
0.5 × 0.5	40 000	23.29*	15.88
1 × 0.25	40 000	24.64*	16.41
0.5 × 0.25	80 000	26.84*	15.21
0.25 × 0.25	160 000	33.55*	13.75

\*Significantly different from standard check ( $P < 0.05$ )

enhanced the work on taro improvement. A technique has been developed to reduce the oxalic acid content to utilize taro for french fries (Sopade, pers. commun.). The various processes evaluated included storing of fresh taro chips at low temperatures, heat treatment and soaking overnight in water, sodium chloride solutions, and calcium chloride solution. The chips were then dried before frying. Peeled taro can be stored in deep freeze without adverse effects on quality while the acidity of taro can be reduced by peeling and washing it in water. It was observed that the common Lae taro 'Numkoi' was found to be acceptable as fries.

There is potential for the use of taro for the manufacture of savouries such as chips. If the demand for fresh fries grows and if the price for taro chips is competitive, there would appear to be a substantial potential for taro. Moreover, it would maximise the use of taro damaged by taro beetle, hence, increase in the farm gate value of taro. With this as the precursor, it is envisaged that an integrated programme of taro production and utilization would make taro production more viable and attractive.

Other studies are being conducted to investigate taro canning, taro storage during transshipment, and appropriate packaging.

### Recommended Taro Improvement Priorities

To meet national needs and taking into consideration the apparent chances of achievements, the following are the priorities to which a taro improvement and production scheme should give emphasis: (1) introduce disease resistant lines with superior yield and good eating qualities targeted for high risk areas; (2) linkage between growers and retailers of taro should be established and contract growing of taro should be encouraged so that production and marketing are synchronized; (3) development of integrated pest management for taro, with minimum use of insecticides and the promotion of biological control; (4) strengthening of research on taro virus diseases; (5) socio-economic re-

search for understanding marketing and nutritional problems and assessing the impact of improved technologies, along with the constraints of technology transfer; and (6) development of taro-based cropping systems.

Demand for taro, both domestic and for export is high. Stability in production of good quality products, adequate transportation, and post-harvest handling and marketing of taro and its products, are required to further increase the economic importance of taro.

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# Production and utilization of tropical root crops in Trinidad and Tobago

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The root crops consumed in Trinidad and Tobago that are of significant economic importance are cassava, sweetpotato, yams, and dasheen. The Ministry of Agriculture, Land and Marine Resources provides education and training courses for farmers and research programmes in agronomy, crop protection, and post-harvest handling. Two significant aspects of the Government's policy that have continued to provide a strong basis for the sustainable production of root crops are the ongoing maintenance of its plant genetic resource base and the training programmes both in-house and in the field. Root crops are generally manually harvested in quantities that are saleable within two days in the markets minimizing the need for storage. Processed products include frozen cassava, chips, and farine which are consumed locally. Export and processing operations have fluctuated because of inconsistent supplies. However, there is a marketing thrust to stimulate production via increased utilization locally through the school-feeding programme. Root crops, therefore, will continue to significantly contribute to sustainable food security in the next millennium.

Keywords: Root crops; Sustainable production; Plant genetic resources; Processed products

The vision of the Ministry of Agriculture, Land and Marine Resources (MALMR) of Trinidad and Tobago as expressed in one of its publications (MALMR, 1993) is to develop a vibrant agricultural sector that is sustainable and competitive in domestic, regional, and international markets. Although the Government has acted as the prime motivator of the agricultural sector, Trinidad and Tobago still has a high food import bill, more than U.S. \$150 million annually and the economy still relies heavily on the non-agricultural sector. There is also the uncertainty by farmers to adopt new technology and, hence, existing systems of agriculture continue to predominate.

The major root and tuber crops grown are cassava, eddoes, sweetpotato, yam, and dasheen. Of these, the most widely cultivated are cassava and dasheen. The Central Statistical Office (CSO, 1994) reported that 433.4 ha were cultivated in cassava, 265.5 ha in dasheen, 49.6 ha in yam, 146.4 ha in eddoes, 50 ha in sweetpotato, and 5.2 ha in tannia. Two hundred and forty-seven hectares are grown in Nariva/Mayaro, 207.3 ha in Victoria, 101.1 ha in St. Patrick, 71 ha in St. Andrew/David, 56.8 ha in St George, and 42.8 ha in Caroni. The present production and utilization systems and the strategies which Government has adopted for sustainable development in root crops in Trinidad and Tobago are, therefore, considered.

## Soils and Cultivation Practices

Root crops produce high yields on sandy loams and silt soils (Edmond and Ammerman, 1971) producing adequate numbers of storage roots.

In Trinidad and Tobago, these soils are cultivated with vegetables that have a high market value, while the marginal soils, such as the Long Stretch Soil Series and heavy clay soils are cultivated with root crops that have a lower market value (Seesahai, 1995). The physical and chemical stress limitations of these marginal soils often result in reduced tuber yield. Problems are most evident in sweetpotato, where tubers are irregular in shape and size and often cracked with a rough skin (Seesahai, 1995). On the larger farms, the soil is normally cultivated using a brushcutter, disc plough, and rotavator. Only the sweetpotato farmers form banks before planting. Planting is done throughout the year, but cassava and yams are planted around May, just before the rainy season. Planting is labour-intensive. Holes are dug with a hand hoe and sets, slips, corms, or tubers are planted by hand. Cassava grown by small farmers are intercropped with corn and pigeon pea while sweetpotato, eddoe, and dasheen are grown mainly as a monocrop. Farmers obtain their planting material from the previous crop, from a neighbour's farm, or from the MALMR.

## Fertilization

Fertilization is done two to four weeks after planting using 200 to 300 kg ha<sup>-1</sup> 12:12:17:2, 13:13:21, or calnitro. Some farmers in the sugar cane belt use urea (Ramnanan *et al.*, 1992). Most cassava, eddoe, and yam farmers apply fertilizer as a spot placement on the surface of the mounds. In the case of sweetpotato, fertilizer is placed on the ridges as a band

application and covered by hand moulding. Cow and chicken manure may be incorporated before or during growth of these crops, e.g., in yams, manures are placed inside the planting hole.

## Diseases

Several diseases have been recognized in Trinidad and Tobago on cassava, yam, and sweetpotato (Rajnauth, 1989). The most serious disease problem in cassava, i.e., cassava bacterial blight caused by *Xanthomonas campestris* pv. *manihotis* was reported in 1977. Cultural practices, varietal resistance, sanitation, and farmer education, have been adopted for control of this disease and have significantly reduced losses. Anthracnose, caused by the fungus *Colletotrichum gloeosporioides* is the most important yam disease. The main methods adopted for the control of anthracnose involves the spraying of fungicides, e.g., benomyl (Rajnauth and Pegus, 1987) and the treatment of planting material as well as the introduction of tolerant varieties such as Belep and Kinabayo. The sweetpotato stem borer, *Megastes grandalis* is the most important pest of sweetpotato. Cultivar tolerance due to the narrow leaf margins was found in a local cultivar, chicken foot.

## Harvesting

Harvesting is labour-intensive. A few farmers use a single tyne side lifter to lift sweetpotato and some cassava from the more friable soil types in the dry season. Punctures from fork, cutlasses, and the mechanical side lifter during harvesting and breakages during separation of individual tubers or during bagging have contributed to mechanical damages. Such damage has been estimated at an average of 10% for sweetpotato and cassava from surveys of such operations (Postharvest Unit, unpubl. data). Farmers stagger their harvest, lifting quantities that can be sold within two to three days.

Sorting is most commonly practised for sweetpotato and the aroids to separate larger marketable tubers from the smaller, damaged ones that are sold at a cheaper price. There is no organised curing activity. Root crops are generally packed into polypropylene sacks and transported from the fields on the head and shoulders of handlers along field paths to vehicles. Handlers and traders maximize on space in the vehicles by stacking bags of produce on each other. Produce is transported to market within 6–12 h minimizing the need for storage. There is a thriving harvest of the young succulent leaves of the aroid dasheen for use as a leafy vegetable.

## Research and Extension Activities

### General

The MALMR (1996) has outlined research and extension activities in the identification, selection, adaptation, and development of appropriate technology to enhance yield. Agronomic evaluation trials have been conducted on several cassava, sweetpotato, and yam cultivars. Multi-disciplinary programmes have been developed incorporating agronomic studies, pest and disease control, storage, and utilization.

### Varietal selections and supply of planting material

Systems have been developed to maintain a continuous supply of planting material from season to season. For example, new cassava cultivars, Mcol 1469, Mmex59, and Mbra12 possessing character traits of high yields and resistance to cassava bacterial blight have also been introduced in the farming community. Indigenous cultivars of the Oriental type showing high tolerance to anthracnose have also been introduced to the farming community. The MALMR has also promoted the use of indigenous cultivars although they are low-yielding, because of their adaptation to wide environmental conditions and their tolerance to major pests, e.g., the 'chicken foot' cultivar of sweetpotato is tolerant to *Megastes grandalis* infestation. Production of clean planting material has also been accomplished using tissue culture techniques.

### Soil management

Generally, farmers do not perceive soil management and soil fertility to be an important problem in root crop production even though the soils on which these crops are grown are generally impoverished. The MALMR is currently developing a soil management advisory service for farmers in root crop production areas. Advice is being given on the fertilizer requirement and the use of organic soil amendments to maintain soil structure and improve tuber quality.

### Crop protection

The MALMR operates a crop protection diagnostic laboratory which assists in the diagnosis of pest and diseases. Research and extension officers make on-site visits and recommendations for the control of these organisms.

In root crop production, new cultivars of yams and cassava have been introduced which are tolerant to anthracnose and cassava bacterial blight, respectively. These introductions have resulted in a reduction in the use of agricultural pesticides.

## Education and training

Extension staff have been trained in root crop production and there are field days and seminars for farmers in selected counties for the dissemination of this information. Courses are also conducted at the Farmers Training Centre biannually or annually. The MALMR has also been the prime motivator for public awareness programmes via the media as well as the production and publication of bulletins and factsheets. Hence, there is always an on-going interaction between researchers, extension officers, and farmers updating technological advances to enhance the quantity and quality of root crops. Seesahai (1994) and Roberts (1984) have investigated key areas of constraints to root crop production locally, e.g., storage of yams and use of organic amendments in sweetpotato production.

## Plant Genetic Resource Management

Both the MALMR and The University of the West Indies have supported the acquisition and conservation of root crop germplasm both *ex situ* in field collections and genebanks and *in vitro* culture (MALMR, 1995). Links have been established by the Republic of Trinidad and Tobago with the Food and Agriculture Organization (FAO) in a national crop germplasm conservation and improvement activities. The FAO (1997) has recently established a computerized regional germplasm database which facilitates the exchange of morphological information on varieties of germplasm from the English-speaking Caribbean and Suriname. Various international organizations such as Centro Internacional de Agricultura Tropical (CIAT) and Inter-American Institute for Cooperation in Agriculture (IICA) have provided root crop germplasm from which agronomic studies have resulted in cultivars being released to farmers.

## Marketing

Root crops are sold locally at retail markets and road-side stalls at ambient conditions without packaging. At supermarkets, varying quantities of all roots and tubers are available under air-conditioned temperatures and are rarely packaged. Purchasers are skeptical of the storage life and cooking quality of cassava and dasheen at certain periods of the year and purchasing is reduced at such times. Demand for root crops peaks during the Lenten and Divali cultural periods in urban areas. There is a year-round demand for dasheen leaves and demand sometimes outstrips supply during the dry season.

The Government is encouraging and promoting the greater use of root crops locally,

through the School Nutrition Programme and the Tourism and Industrial Development Company. The ultimate goal is to extend the export market from ethnic communities to mainstream metropolitan markets.

Large quantities of dasheen leaves are exported mainly to the United Kingdom, Canada, and the United States of America. Larger quantities of eddoes, dasheen, and sweetpotato are exported than the other tubers, but in all cases quantities fluctuate from year to year (Table 1).

## Processing of root crops

A few attempts have been made to process dasheen leaves but processing was not sustained. Presently a sweetpotato snack chip is being produced when raw material is available (Hassanali, pers. commun.). Cassava is the favoured raw material for all other processed products. Cassava is produced into farine (grated, dried granules), flour, cassava, bread, frozen cassava, and plain and flavoured snack chips. Tobago produces farine from roots that are considered inedible. The products are consumed locally and any export of processed products is minuscule.

Processors have small- to medium-scale semi-automatic operations (Bharath, Hamid, Hassanali, Ramtahal, Singh, pers. commun.). These processors have indicated that collectively they utilize 153 000 kg of cassava per annum in Trinidad and 10 000 kg in Tobago but all have capacity for increased production. Irregular raw material supply have contributed to the closure of some enterprises and this continues to plague the industry. This inconsistent supply is as a result of price competition from fresh produce sales at the local market, limited availability of raw material during the August to October period, and inadequate production levels nationally.

Tubers are processed within 48 h to limit the development of vascular streaking. Refrigeration prior to processing of flour, farine, or chips is not considered a viable storage option.

**Table 1** Quantities of root crops exported from Trinidad and Tobago 1994-96

Commodity	Quantity (kg) exported fresh or dried by natural means		
	1994	1995	1996
Cassava	414	360	632
Sweetpotatoes	88 286	2 033	267
Dasheen	1 107	2 786	10 509
Eddoes	27 884	34 589	2 505
Tannia	—	—	27
Yams	882	2 939	1 189
Dasheen leaves	287 775	112 404*	18 148*

Source: \*CSO (1994-96)  
\*MALMR (1997)

Processors prefer a variety that is harvested mature with a circumference of 10–15 cm. It should be easy to peel, non-woody, and with guaranteed cooking quality. Cultivars Mmex 59 and 'Maracas black stick' are the preferred locally available cultivars in Trinidad, while cultivar 'Butter stick' is more popular in Tobago. While the market for processed products is not saturated, processors have felt that apart from cassava bread and chips, there is not much more room for expansion of the local market.

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# Improving the impact of post-harvest research and development on root and tuber crops: The needs-assessment approach

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Resources for research and development activities are limited and, therefore, it is important to accurately target research work and technical interventions to the true needs and opportunities of farmers, processors, or traders. This paper details the use of needs-assessment techniques to assess the constraints and opportunities in post-harvest systems. Two examples of needs-assessment studies and subsequent adaptive research and technology dissemination activities, are presented to demonstrate the use of needs assessment in the planning and implementation phases of the project cycle. The examples are taken from work in Tanzania and Uganda. Needs-assessment techniques have the potential to improve the relevance of post-harvest research and development and are recommended as part of a participatory approach to prioritising research, technology generation, and diffusion. The methods do, however, have their weaknesses and shortcomings and these are discussed.

Keywords: Root crops; Needs assessment; Post-harvest systems; Research and Development; Shortcomings; Planning and implementation

Research and development activities in the post-harvest area are complex in nature because of the interaction of technical constraints with the social and economic contexts of primary producers, processors, marketing systems, and consumers. When interventions are made, there needs to be a clear understanding of these complexities if they are to have impact.

Informal needs assessment (NA) is a term used to describe a range of qualitative diagnostic methods such as rapid rural appraisal (RRA) and participatory rural appraisal (PRA) (Cropley and Gilling, 1993). Their essence is that they facilitate scientists to allow farmers to participate in the formulation of the research agenda. A common criticism of previous post-harvest research, and indeed agricultural research in general, is that technical innovation has been high, but adoption has been poor. The use of NA can improve this situation by actively involving beneficiaries in the key phases of the project or research and development cycle in which priorities for research are set, or in which technology choices are made. By ensuring the relevance of

research and subsequent technical interventions, the prospects for adoption and, therefore, impact, are greatly improved.

In the early 1980s, the RRA approach to initial project preparation was developed. The RRA was defined (Conway, 1986) as:

"a systematic, but semi-structured activity carried out in the field by a multi-disciplinary team designed to acquire information rapidly on, and hypotheses about, rural life."

Essentially, RRA is an approach which relies on semi-structured interviews using checklists. While recognising the need to understand problems and constraints in the context of prevailing socio-economic conditions, RRA tended to be extractive with analysis of constraints and research priorities undertaken by the team of "experts". The PRA evolved from RRA approaches with an emphasis being given to interactive (or participatory) methods of problem diagnosis and approaches to resolving them. Chambers (1992) gives a detailed account of the way in which methods have evolved. An important concept of PRA is that rural communities have a contribution to make to the process of identifying and prioritising their constraints and aspirations, and to the planning and implementation of ways of solving constraints or achieving development objectives. The NA uses elements of both RRA and PRA in a diagnostic fashion to prioritise technical research or to assist in technical

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choice. Overviews of participatory research approaches are given by Chambers (1992) and Chambers and Gildyal (1985). Informal survey methods are not the only approaches available for determining constraints and setting the research priorities. Site visits by researchers and formal questionnaires are alternative approaches.

### Why Use Needs Assessment?

As indicated above, the technical competence of research and technical innovation has been high, but adoption has in many cases not met expectations. One reason for this is researcher biases concerning the actual needs of farmers, traders, and consumers. This has arisen due to inherent structural weakness in the institutional organization of agricultural research practice. The resulting physical and professional separation of scientists from farmers has made it difficult for the agendas and needs of the latter to be given adequate consideration. The notion of farmers collaborating with scientists in the process of agricultural research and development (farmer participatory research) emerged originally in the late 1970s due to a dissatisfaction with institutional models of agricultural research in operation at the time. In these models, the research process was centralised and relied on an extension system to disseminate "proven" findings to farmers (Hall and Clark, 1995). The consequence of the separation of technology generation and its extension, are that not only is adoption inhibited by an unreceptive socio-economic context, but also that the technologies do not reflect the views and needs of the users who are the ultimate clients (Hall and Clark, 1995).

The relatively poor impact of technological interventions indicates that agricultural and (or) post-harvest problems should not be seen as wholly technical phenomena. Two of the main issues for post-harvest research, are: (i) problem diagnosis. Technologies are often developed to solve problems that researchers assume meet the needs of beneficiaries; and (ii) context of application. When providing solutions to agricultural problems, it is often not appreciated that technologies not only need to work well but that they must also be compatible with the resources of farmers, their cultural preferences, and social and physical systems. The reason therefore why NA should be undertaken in post-harvest research and development, is so that researchers can understand the constraints facing farmers, processors, and consumers and the context in which these occur.

### Needs Assessment Methodologies

It is not the aim of this paper to provide a description of NA methodologies. This is adequately covered by Theis and Grady (1991)

and Kleih *et al.* (1997) for root and tuber crops post-harvest systems. The tools should, however, only be used as means to facilitate a two-way dialogue between scientists and farmers, processors, traders, and consumers. As in all scientific research, hypothesis formulation and testing are central and take place through an iterative process of discussion and explanation.

### Examples of Research or Development Activities Developed from NA Studies

The NA approach has been tested and adapted in post-harvest systems of root and tuber crops by the Natural Resources Institute (NRI) in collaboration with national programmes in Ghana, Tanzania, and Uganda. Adaptive research and technology transfer activities have been developed as a result. Two examples of these are briefly outlined below to demonstrate how NA studies have been used to influence the research agenda.

#### Sweetpotato storage in northern Uganda

During NA studies in northern Uganda (Hall *et al.*, 1997), it became clear that the livelihood system in the area relied on subsistence food production as a means of obtaining physical access to food (although sweetpotato was sold for a relatively short period of the year). This suggested that the key factors which needed to be understood were the relative importance of different crops in food production and consumption, when these different types of food became available, and where gaps in food availability appeared. It was also well known that a significant change had occurred in the food system resulting from the reduction in cassava production due to Africa Cassava Mosaic Disease. It was important to understand the ways in which farmers had coped with modifications in the post-harvest systems of the remaining crops, and the constraints which had arisen because of this.

Seasonal calendars were used to assess the relative importance of different food crops and their availability. Secondary data were also used to examine crop production patterns and this highlighted the decline in cassava and the increase in sweetpotato production. Coping strategies were investigated using scoring and ranking matrices to examine the post-harvest characteristics of the replacement crop (sweetpotato). In this way, it was possible to identify and weigh the most important post-harvest issues and relate them to the constraints faced by farmers. Semi-structured interviews with groups and individuals were used to investigate the importance of the post-harvest constraints identified, as well as to validate what appeared to be the main researchable constraints.

Using the methods discussed above it was possible to make the following conclusions con-



cerning the sweetpotato post-harvest system and its constraints. A traditional method of preservation is practised in which sweetpotato roots are sliced, dried in the sun, and then stored. This practice was linked to the availability of fresh cassava to provide food towards the end of the dry season. This was during the period when dried sweetpotato was no longer available. With the virtual disappearance of cassava, dried sweetpotato was required to provide the major source of food for an extended period (up to six months rather than three). During this additional storage period (months 4–6), insect infestation reached unacceptable levels. The ability, therefore, for dried sweetpotato to provide food for the desired period was threatened.

The NA survey, by gaining a clear understanding of the food and farming systems and their constraints as perceived by the farmer, was able to precisely identify the major constraint and make recommendations for further research. The storage of sweetpotato roots was considered as an option for prolonging the period when fresh roots are available. Stored roots could be subsequently dried and stored if necessary. This would reduce the total period that dried roots would need to be stored. As a consequence, serious infestation of stored roots would not take place in the crucial period of food scarcity when no other foods are available.

In-ground storage of fresh roots is a traditional technique used in the area, but unharvested roots are subject to worsening attack by weevils as the dry season progresses (Hall *et al.*, 1997). A number of other traditional storage methods are described in the literature (Woolfe, 1992). The main methods are: pits, clamps (or mounds), and indoor storage (e.g., in huts, buildings, or other stores). Pit and clamp storage methods were tested on-station in Uganda and were found to be successful. It was decided to adaptively test these methods with the farmers in order to identify those which best suited their needs and circumstances.

It was anticipated that the provision of simple storage facilities for fresh sweetpotato roots would: (i) provide an opportunity for subsistence farmers to dry roots later in the dry season thereby avoiding in-ground storage; (ii) lessen the constraint which arises from the short shelf life of dried slices which is a limiting factor in the current food security system; and (iii) provide an opportunity for the farmer to stagger the sale of roots and by doing so, benefit from higher market prices later in the season.

Initial on-farm work clearly demonstrated that roots can be stored in pits below ground and clamps above ground for up to three months in the dry season under ambient conditions and in some cases roots were stored up to five months (Hall *et al.*, 1997). What is more important, is that the research has shown that this is a useful practice for farmers to under-

take. The technology chosen did not allow farmers to achieve direct economic benefits from higher prices for their roots during the particular season under study. It has, however, greatly improved their food security situation during the critical period towards the end of the dry season by prolonging the period during which fresh roots are available.

This technology is low-cost and fills a need in the food system. With the continued decline of cassava production in the semi-arid zones of Uganda, this technology has the potential to provide an extremely valuable aid to household food security. Consequently, dissemination of this concept and the technology itself should be seen as a priority food security intervention in these districts.

### The need for fresh cassava storage in Tanzania

A NA study (Ndunguru *et al.*, 1994) was used to identify problems and opportunities in the marketing chains for fresh cassava roots entering Dar es Salaam from villages in Pwani and Tanga Regions. The main techniques used were market-chain analysis and semi-structured interviewing. The major constraints identified within the system were associated with delays in the marketing chain causing physical and economic losses. Time delays were an important factor in determining the level of price discounting. In some stages in the marketing chain, economic losses were greater than 90% of initial value. It became clear during the study that the main players in the market chain did not perceive quality or reduced shelf life as important issues. They were purely concerned with economic losses associated with cassava marketing. This point of view was taken into account when developing a strategy for disseminating an appropriate technology.

A process of adaptive technology transfer was initiated using elements of a low-cost cassava storage technology developed in South America by Centro Internacional de Agricultura Tropical (CIAT) and the NRI. The various elements of this storage technology showed significant beneficial effects relative to traditional marketing practices. Observations also indicated that some categories of small and damaged roots which are normally discarded as being unusable by the country buyer can be stored successfully for a week under Tanzanian conditions. This is important as small roots and cassava pieces of even poor internal quality are sought by low-income consumers (Ndunguru *et al.*, 1995).

Trials conducted in markets in Dar es Salaam and villages in Pwani Region that supply the markets demonstrated that low-cost cassava storage technology can maintain the quality and freshness of cassava for 7–10 days, compared to 1–2 days using conventional techniques. Sup-

porting economic studies (data not shown) demonstrated the economic benefits of adoption of the technology. Market commission agents and representatives of village governments agreed that the technology should be disseminated throughout the market chain with initial emphasis being placed at the level of farmers and country buyers.

A participatory approach was used to develop a dissemination strategy for the fresh cassava storage technology. The approach proved useful as it involved key players from the market chain in identifying the best approach for dissemination, development of suitable supporting materials (leaflets and posters), and in carrying out training activities with minimal support from project personnel. It was also possible to ensure that a clear link was established in the minds of potential beneficiaries between the technology and improved income from cassava marketing.

This case study demonstrated how the use of a participatory approach has led to the adaptation of a known post-harvest technique to the demands of the marketing system in Tanzania, and the direct involvement of potential beneficiaries in the design and implementation of the dissemination process.

## Conclusion

The use of NA as part of the project cycle offers the potential to improve the impact achieved by research and development activities in the post-harvest sector. Care is needed in the application of these tools as the method can potentially have a number of methodological and institutional shortcomings. Many of the methodological issues can be overcome through experience and training. The incorporation of the NA approach into national programmes is the best approach to addressing the institutional limitations.

The examples highlighted in this paper demonstrate the use of the NA approach at the start and as part of a participatory approach to research and technology transfer, and can help to match technical solutions to problems faced by potential beneficiaries, and thus increase the prospect of successful adoption of the technologies in the field. The examples detailed above do not in themselves provide definitive proof of the success of the NA approach because there was no direct comparison with other approaches. This said, it is difficult to visualise how, for example, formal survey methods would have been able to analyse the complex situation

detailed in Uganda and to recommend a technology that would have immediate benefit.

## Acknowledgement

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## Poster

# Mechanized direct planting system for sweetpotato

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Sweetpotato is generally planted using rooted vine cuttings. However, some cultivars of sweetpotato (for example J-Red with high carotene content) can be grown from seed tuber. Direct planting reduced costs and labour requirements because nursery beds and seedling facilities were not required. The planting machine was a self-propelled semi-automatic, walking machine, and consisted of a feeder and planting beak mounted on a two-wheel-drive chassis. Optimum direct planting conditions were 25–99 g weight seed tuber, whole or 1/2 tubers, cross-shaped mulch hole, and vertical epidermis up or horizontal planting position. Seed tubers were fed into the eight feed cups and a beak cut the mulch. Planting speed was restricted by human feeding efficiency, which was 0.19–0.33 m s<sup>-1</sup> with a 33-cm inter-row spacing of mulching ridge. Good control of the planted seed tuber position was achieved.

Keywords: Direct planting; Sweetpotato; Planter; Mechanization

Sweetpotato is generally planted using rooted vine cuttings. However, some cultivars developed at the Laboratory of Sweetpotato Breeding, Kyushu National Agricultural Experiment Station in Japan can be grown from seed tubers and produce normal yields. These cultivars are used for processing. These new cultivars sprout and new sweetpotato grows independently from the seed tuber. Other sweetpotato cultivars grow as part of their own planted seed tuber. Direct planting is able to reduce costs and labour requirements, because no nursery beds and no raising facilities are required. In Japan, use of sweetpotato is changing from starch to processing, therefore, it is important to reduce production costs and increase productivity. The specific objectives of this research were to determine the optimum seed tuber weight and cutting method for direct planting; shape of mulch hole; the planting position of seed tuber; and measurement of the feed accuracy and planting speed of a prototype direct planter.

### Materials and Methods

In 1995, five sweetpotato cultivars were planted and their yields were compared. In 1996, two selected cultivars were used for direct planting:

J-Red (Kyushu 120) (high carotene content) and Ayamurasaki (high anthocyanin content).

Sweetpotatoes used in processing, usually, must weigh at least 150 g. Seed tubers fell into several weight groups: 10–24 g, 25–49 g, 50–99 g, and 100–199 g for J-Red and 25–49 g, 50–99 g, and 100–199 g for Ayamurasaki. Many cutting methods were also used: whole, 1/2, 1/4, and 1/8 for J-Red and whole, 1/2, 1/4, and cross 1/4 for Ayamurasaki. Each seed tuber was planted on 2 May 1996 and for comparison, cut vines were planted on 20 May 1996. The yields for both groups were measured from 15–22 October 1996.

Cutting a seed tuber can reduce the amount of seed needed. Whole sweetpotato will sprout from only one end but if cut in half width-wise, it will sprout from both uncut ends. Weight distribution of harvested sweetpotato was investigated to ensure adequate supply for the following year. Plants were spaced 75 cm (inter-row) × 33 cm (interplant) and the planting depth was 1–2 cm. Fertilizer (N:P:K) was applied at the rate of 24:72:72 kg ha<sup>-1</sup>, respectively. Manure was applied at 12 t ha<sup>-1</sup>.

Seed tubers 100–199 g were cut into quarters, planted in different shaped mulch holes, and the yields measured. Circular and cross-shaped holes 80 and 120 mm in size were used.

Seed tubers 50–99 g cut into halves were planted vertically, horizontally, and epidermis in the up or down position. Similarly, 100–199 g seed tubers cut into quarters were planted and the yields were measured.

Seed tubers planted by prototype direct planting machine. The planting machine was a self-propelled semi-automatic walking machine, consisting of a feeder and planting beak mounted on a two-wheel-drive chassis. The feeder has eight cups which revolve intermittently and seed tubers are fed into the cups by human hand. The planting part consisted of a beak-type planting finger which cut the mulch. Seed tuber planting accuracy was measured. Operators fed the seed tubers to try and insure a constant position, for example, vertical or horizontal, or epidermis up or down. After planting, the distribution of seed tuber positions in the soil was immediately measured. Planting speed and feed rate of seed tuber also were measured and compared.

### Results and Discussion

In J-Red, the yield for cut seedlings was 38.9 t ha<sup>-1</sup>, which was similar to the yields obtained from whole tubers (50–99 g) and 1/2 cut tubers (25–49 g) which produced 39.8 and 32.5 t ha<sup>-1</sup>, respectively. For Ayamurasaki, the yield for cut seedlings was 23.4 t ha<sup>-1</sup>, and this was similar to 1/2 cut tubers (100–199 g) which produced 21.1 t ha<sup>-1</sup>. In J-Red, the optimum seed tuber weight was 25–99 g and cutting method was whole or cut in half. Ayamurasaki was not effective for direct planting, because many seed tubers were needed to achieve the same yields as cut seedlings (Table 1).

Sweetpotatoes weighing less than 100 g, which were too small for processing, were <16% of the harvest from 48 plants. Thirty-five sweetpotatoes weighing between 25–49 g were cut in halves to produce 70 seed tubers for the next season.

Cross-shaped holes of length 80 mm and 120 mm produced much higher yields of 32.8 and 31.6 kg 10 ac<sup>-1</sup>, respectively, than circular holes of diameter 80 mm and 120 mm which produced yields of 31.9 and 26.2 kg 10 ac<sup>-1</sup>, respectively, for J-Red, using 1/2 cut seed tubers weighing 100–199 g. The smaller size of mulch hole also produced a higher yield, as the soil temperature around the seed tuber was higher. However, if the mulch hole was too small, the bud of sweetpotato touched the mulch and was killed which decreased the germination rate. Therefore, an improved planter beak is necessary for making the cross-shaped mulch hole.

Seed tuber 50–99 g cut into halves, planted horizontally or vertically with epidermis up produced higher yields (28.1 kg 10 ac<sup>-1</sup>) than seed tubers planted vertically with epidermis down (25.7 kg 10 ac<sup>-1</sup>) indicating the need to control the seed tuber position.

When operators fed the seed tubers in the vertical and epidermis-up position, 63% of the planted seed tuber were vertical and epidermis up, 28.8% horizontal, and 8.2% were vertical and epidermis down. Planting positions for this planter were very good for high yield. Planting speed was restricted by human feeding efficiency; it was from 0.19–0.33 m s<sup>-1</sup> with a 33-cm interplant spacing of mulch ridge, twice that of a cut seedlings planter. When operators fed the seed tuber vertically with epidermis up, the planting speed was from 0.19–0.21 m s<sup>-1</sup>.

**Table 1** Effect of different seed tuber weight and cutting method on yield (t ha<sup>-1</sup>)

Cultivars	Mulch colour	Seed tuber weight (g)	Cutting method				
			Whole	1/2	1/4	1/8	Cross 1/4
J-Red	Clear	10–24	27.9 (89)				
		25–49	30.7 (96)	32.5 (95)			
		50–99	39.8 (96)	28.4 (89)	23.9 (79)		
		100–199		31.4 (95)	29.3 (93)	25.3 (86)	
J-Red	Black	25–49		26.5 (86)			
		50–99		33.5 (95)	16.6 (77)		
		100–199			27.1 (82)	23.5 (64)	
Ayamurasaki	Clear	25–49	13.1 (82)	21.1 (100)			
		50–99	18.5 (93)	18.6 (86)			21.4 (73)
		100–199		26.3 (96)	16.1 (55)		24.1 (80)

Planting date : Seed tuber (direct) 2 May 1996; Seedling 20 May 1996  
 Mulch hole shape : Circle d: 120 mm  
 Seed tuber position : Horizontal, epidermis up (cut)  
 Cutting method cross 1/4 : Cut widthwise and then into quarters  
 Values in parentheses are germination percentages

The prototype planter for direct planting was quite good, but the seed tuber soil coverings were less than desired.

### **Conclusion**

Optimum direct planting conditions were 25-99 g weight seed tuber, whole, or 1/2 cut,

cross-shaped mulch hole, vertical, and epidermis up or horizontal planting position. When these conditions are met, the yield of direct planting is greater than, or equal to, yields from cut seedlings. Yield tests have been carried out since 1995, and more persistent testing was needed to establish the optimum weight and cutting method of seed tuber. The next step is the development of a seed tuber cutting machine.

## Poster

# Chemical and hot-water treatments to improve the survival of yam minisetts

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Tubers of white yam (*Dioscorea rotundata*) and water yam (*D. alata*) were cut to produce minisetts each weighing about 25 g. They were treated by either dipping in a fungicide (Benomyl, thiabendazole, or iprodione) solution for 1 h, or immersing in water at 50°C for 5, 10, or 20 min. They were allowed to cure overnight and then planted according to a randomized complete block design at several sites in Nigeria, and assessed regularly to determine the effect of the treatments on minisettt survival, plot yield, and anthracnose disease development. The hot water treatment for 20 min at 50°C reduced the survival of *D. alata* at all sites. Treatment for more than 5 min killed *D. rotundata*. The results indicated that the fungicide were generally better at increasing the survival rate of both *Dioscorea* species than the hot water treatments.

Keywords: Yam minisettt; Hot water; Fungicides; *Dioscorea* spp.; Survival; Yield; Nigeria

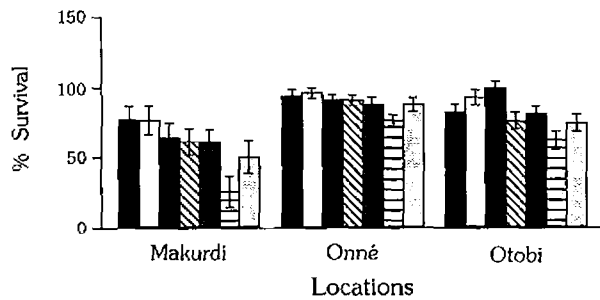
Most yams of the genus *Dioscorea* are vegetatively propagated through the planting of small 'seed' tubers or pieces of tuber (setts). To increase the multiplication rate for yams, the National Root Crops Research Institute (NRCRI) and the International Institute for Tropical Agriculture (IITA), Nigeria, developed the minisettt technique whereby smaller setts could be used. Different micro-organisms (fungi, bacteria, and nematodes), some saprophytic and others plant-parasitic, can colonize the periderm, cortex, and meristematic layers of yam tubers and the surrounding soil. Some of these pathogenic organisms can also cause disease on the yam vines and leaves (e.g., anthracnose), and their contamination of seed yams or setts often result in poor growth of the plant and allows carry-over of the pathogens from one season to the next. The exposed surfaces that result from cutting tubers into setts provide ready access for pathogens to enter, especially those already present in the periderm and cortex.

Yams are usually grown by resource-poor farmers. Yam setts are probably most vulnerable to pathogen attack at planting, and treatments at this stage to protect the crop against pathogens are likely to be most effective and efficient (only small amounts of material to treat). Heat

has been used to eliminate pathogens in other systems, and is more readily available (and environmentally benign) than most chemicals. The objective of this study was to test different fungicides and hot-water treatments for their efficacy at increasing the survival rate of yam minisetts by eliminating or reducing pathogen contamination before planting.

## Materials and Methods

Tubers of white yam (*D. rotundata*) and water yam (*D. alata*) were cut to produce minisetts each weighing about 25 g. The minisetts were treated by either dipping in a fungicide solution for 1 h, or immersing in water at 50°C for 5, 10, or 20 min. After treatment, the minisetts were allowed to cure overnight in a well-ventilated and dry area, and then planted according to a randomized complete block design with 8 × 4 minisetts per plot and four replicate plots per treatment. The trials were replicated in different areas of Nigeria, and plots were assessed on a regular basis to determine what effect each treatment had on survival of the minisetts, the yield from each plot, and the development of anthracnose disease on the foliage.



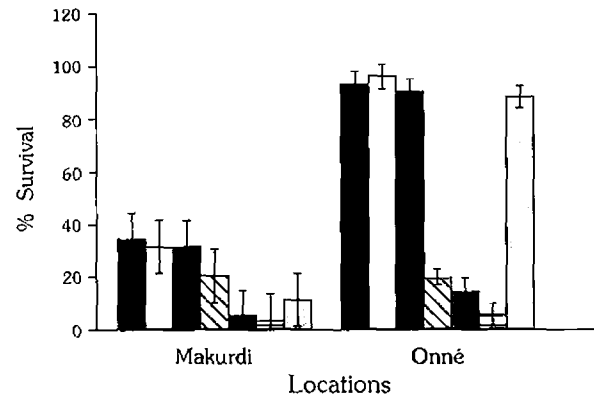
**Figure 1** Comparison of survival of water yam minisetts treated with different chemicals or different hot water immersion periods at different sites in Nigeria; (Treatments from left to right are: Fungicide: Benomyl, thiabendazole, iprodione; and heat treatment: 5 min at 50°C, 10 min at 50°C, 20 min at 50°C; Control)

**Results**

Hot water treatment for 20 min at 50°C reduced the survival of water yam minisetts at all sites (Figure 1). Treatment at 50°C for more than 5 min killed 'Amula', the white yam cultivar used (Figure 2). The fungicides were generally better at increasing the survival rate of either water or white yam minisetts than the hot water treatments (Figures 1 and 2). Survival of both water and white yam minisetts was generally good at Onné and no treatment gave a significant improvement (Figures 1 and 2). Survival of white yam minisetts at Makurdi was poor in the season of the trial probably because the rains arrived late (Figure 2). Fungicide treatment of the minisetts had no significant effect at delaying the onset or development of anthracnose on the vines (data not shown).

**Discussion and Conclusions**

The fungicide treatments were the most effective, probably because they had both curative



**Figure 2** Comparison of survival of white yam (*D. rotundata*) minisetts treated with different chemicals or different hot water immersion periods at different sites in Nigeria; (Treatments from left to right are: Fungicide: benomyl, thiabendazole, iprodione; and heat treatment: 5 min at 50°C, 10 min at 50°C, 20 min at 50°C; Control)

and protective activity. However, they are probably out of the reach of most yam growers. Hot-water treatment of up to 10 min at 50°C was effective at improving the survival of the water yam minisetts, probably eliminating most of the pathogens present in the setts, but not protecting against re-infection from the soil. Different yam cultivars and species appear to differ in their high-temperature tolerance, and, thus, the optimum temperature and time will have to be determined for each if the system is to be promoted to farmers. Probably the only way it would be possible to have hot water treatment widely used in Africa, would be if it could be arranged on a community scale, since water temperature control is critical and difficult to achieve in small setups, and it is more fuel-efficient to heat a large tank than a small one.

## Poster

# The rapid detection of *Colletotrichum gloeosporioides* in yam tubers using ELISA

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The Enzyme Linked Immuno-Sorbent Assay (ELISA) diagnostic protocol was capable of specifically detecting superficial infections of *Colletotrichum gloeosporioides* in periderm tissue of artificially-inoculated yam tubers, but not the deep-seated natural infection in tuber meristem tissue and, thus, may be used to determine if 'deadskin' is always associated with this pathogen.

Keywords: Yam tubers; *Colletotrichum gloeosporioides*; Detection; ELISA

Strains of the fungus, *Colletotrichum gloeosporioides*, not only cause the typical lesions on yam vines known as anthracnose, but have also been shown to colonise the periderm and meristematic regions of yam tubers where they can cause 'deadskin' symptoms. Since yams are almost entirely vegetatively propagated from tubers, tubers with 'deadskin' are a likely source of infection for anthracnose epidemics in Barbados, and other areas where anthracnose is a problem.

Conventional methods for diagnosing *C. gloeosporioides* infections in tubers are based on isolating, culturing, and identifying the infecting fungi. This is labour-intensive, time-consuming, and often not very reliable because of contamination of cultures by other saprophytic and pathogenic organisms. An Enzyme Linked Immuno-Sorbent Assay (ELISA) method for specifically detecting *C. gloeosporioides* directly from yam tuber material could give an accurate diagnosis in a few hours. The objective was to determine the suitability of ELISA in the direct detection of *C. gloeosporioides* in yam tuber tissue. The work described here is part of a larger 'Yam Diseases Project' funded by the U.K. Department For International Development (DFID) primarily to investigate the nature and impact of diseases of *Dioscorea* spp.

### Materials and Methods

*Dioscorea alata* (water yam) var. White Lisbon tubers were inoculated with *C. gloeosporioides*

by dipping in a suspension of conidia. After 'deadskin' symptoms had developed, tuber periderm and meristem tissues were excised and macerated. The tissue preparations were coated on to microtitre plate wells, and an ELISA test was performed using monoclonal antibodies active against *Colletotrichum* spp.

### Results

The mean ELISA absorbance values for periderm and meristem tissue from yam tubers inoculated with *C. gloeosporioides* were compared with similar tissues from uninoculated control tubers (Figure 1). The monoclonal antibody used appears specific for *Colletotrichum*

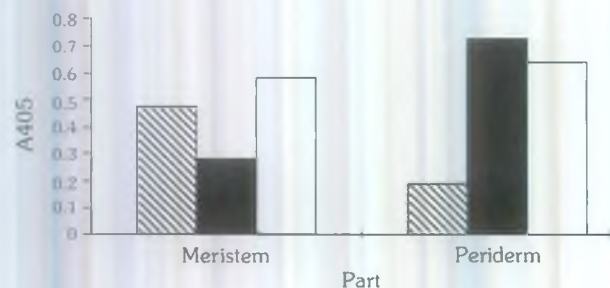


Figure 1 Mean ELISA absorbance values for different tissues of yam tubers inoculated with either *C. gloeosporioides* from Ghana (□) or *C. gloeosporioides* from Barbados (■), and control (▨)



spp. and did not react with pure cultures of other fungi isolated from yam (data not shown). Periderm tissue of yam tubers inoculated with *C. gloeosporioides* gave significantly higher absorbance values than the controls ( $P < 0.05$ ). Meristem tissue had high background absorbance values, and values for inoculated yams were not significantly greater than the controls. The two strains of *C. gloeosporioides* tested (G, from Ghana var. Seidu-bile, B, from Barbados) gave similar results in periderm tissue.

## Discussion and Conclusion

These preliminary results indicate that the inoculation method used probably induced only superficial and not deep-seated infection of tubers. It is probably necessary to change the ELISA format or use a different antibody (or cross-absorb this antibody) to reduce the high background reaction and allow the test to be used to detect deep-seated, natural infections in tuber meristem tissue.

## Poster

# Leaf harvesting effects on leaf retention and pest and disease incidence of cassava (*Manihot esculenta* Crantz)

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Two field experiments were conducted in the 1996-97 crop season to investigate the effect of leaf harvest frequency on leaf retention and pest and disease incidence on cassava genotypes, at two sites in the derived (Ibadan) and southern Guinea savannah (Mokwa) zones of Nigeria. Four leaf harvest frequencies (one-, two-, and three-monthly intervals, and no harvest) were imposed on each genotype. The effects of genotype, harvest frequency, and their interaction were highly significant for the number of leaves retained at the peak dry season at both sites. Generally, as the intensity of leaf harvest increased, the number of leaves retained decreased. Genotype and frequency of leaf harvest significantly affected African cassava mosaic virus disease (ACMVD) at Mokwa with the intensity increasing as harvest frequency increased. For cassava bacterial blight (CBB) harvest frequency effect was significant. Monthly and two-monthly frequencies had the highest CBB infection. Effects of genotype and harvest frequency were highly significant for cassava green mite (CGM). Generally, as leaf harvest intensity decreased, CGM damage increased, with no-leaf harvest incurring the highest damage and the monthly harvest treatment the lowest.

Keywords: *Manihot esculenta* Crantz; Genotype × environment interactions; Leafy vegetable; Root yield

Cassava is an important root crop in the humid and subhumid agro-ecological zones in sub-Saharan Africa, and is grown for its leaves and tuberous roots in both the upland and lowland ecologies (Lahai *et al.*, 1997). Cassava leaves are consumed in almost all countries of the cassava belt in Africa, from Senegal to Mozambique. They are a major component of the diet and constitute a very significant source of dietary protein, minerals, and vitamins (Bokanga, 1994).

The roots contain only 10-20 g kg<sup>-1</sup> protein, but the leaves are rich in protein, Ca, Fe, and vitamins, comparable to other green leaves regarded as good protein sources (Lancaster and Brooks, 1983; Ekanayake *et al.*, 1997). They contain up to 70 and 267-399 g kg<sup>-1</sup> protein on fresh and dry weight basis, respectively (Tupynamba and Vieira, 1979).

There is virtually no association of cyanide intoxication with the consumption of cassava leaf meals, even though cyanogenic potential is

5-20 times greater than that of roots (Bokanga, 1994; Githunguri *et al.*, 1996). This is because cassava leaves have the ability to rapidly lose cyanogens during processing, due to the presence of a high concentration of the enzyme linamarase (over 200 times greater than in the roots), and which is capable of breaking down linamarin and lotaustralin (Bokanga, 1994; Ekanayake and Bokanga, 1995).

Cassava has a longer growth period (one year or more), unlike the other annual vegetable crops which take less than six months to mature (Simwambana *et al.*, 1992). As a result, cassava leaf harvest can be spread over a year or more, producing higher leaf yields during the rainy season, when the other vegetables may not perform well due to foliar pathogenic problems. However, it is argued that leaf harvesting can increase pest and disease problems on cassava as well as other physiological stresses such as leaf retention during drought periods, but relatively few studies have been carried out in this direction. This paper presents the initial results on leaf harvesting effects on leaf retention during the peak of the dry season and pest and disease pressure on cassava in two agro-ecological zones in Nigeria.

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## Materials and Methods

Two field trials were conducted during the 1996–97 crop season at Ibadan, Mokwa in Nigeria, using 11 and 12 cassava genotypes (one local landrace and 10 or 11 improved IITA clones as checks) genotypes, respectively. Ibadan (7°30' N, 3°54' E) representing the forest-savannah transition zone of West Africa has an annual rainfall and potential evapo-transpiration (PET) of 1270–1524/1261–1374 mm, seven months rainy season, and maximum/minimum temperatures of 28–36/18–23°C. Mokwa (9°35' N, 5°11' E) represents the southern Guinea savannah zone of West Africa and has an annual rainfall and PET of 762–1016/1592 mm, six months rainy season, and maximum/minimum temperatures of 27–35/12–23°C. Soil characteristics for the sites are, Ibadan: Oxic Paleustalf (pH = 6.4, organic C = 1.3%, total N = 0.13%, available P = 20.7 ppm Bray 1, and K = 1.9 cmol kg<sup>-1</sup> soil), and Mokwa: Alfisol (pH = 5.8, organic C = 0.5%, total N = 0.05%, available P = 5.1 ppm Bray 1, and K = 0.21 cmol kg<sup>-1</sup> soil).

The land was ploughed, harrowed, and ridged at 1-m intervals. Stakes 0.25–0.30 m long and taken from mature plants were planted 1 m apart on the ridges, giving a plant population of 10 000 plants ha<sup>-1</sup>. Planting was done on 18 May and 18 June 1996 at Ibadan and Mokwa, respectively. Four leaf harvesting frequencies, namely, no leaf harvest (0), monthly (1), two-monthly (2), and three-monthly (3) intervals, commencing three months after planting were imposed on each genotype in a split-plot design, arranged in three randomized complete blocks. The genotypes were the main plots and the leaf harvesting frequencies the subplots. The area of each main plot was 24 m × 10 m and that of the subplot was 10 m × 6 m. Leaf harvesting was carried out between 800 and 1200 h by removing the shoot tips just below the third fully expanded leaf.

Leaf retention during the dry 'harmattan' season was monitored by counting the number of leaves remaining on four plants per leaf harvesting frequency (Ekanayake, 1996). The rating for reactions to African cassava mosaic virus disease (ACMVD), cassava bacterial blight (CBB), and cassava green spider mite (*Mononychellus tanajoa* Bondar) (CGM), was based on five classes: class 1, no infection or attack visible; class 2, mild infection or attack (1–20%); class 3, moderate infection or attack (20–60%); class 4, severe infection or attack (60–80%); and class 5, very severe infection or attack (above 80%). Records of disease development were taken one month after the commencement of leaf harvesting and then at six months after planting for both ACMVD and CBB. Scoring for CGM was at the end of the wet season (November–December).

The data were analysed for each location using mixed model analysis of the Statistical

Analysis System for Microsoft Windows, Release 6.10 (SAS Institute, 1991), with genotype and leaf harvesting frequency as fixed effects and replication as random effect.

## Results and Discussion

### Leaf retention at peak dry season

Significant variations in the number of leaves retained at the peak of the dry season among cassava genotypes were noted at both field sites, although different sets of genotypes were used (Table 1). At Ibadan, TMS 30572 retained the highest number of leaves followed by TMS 91 02322, TMS 4(2)1425, and TMS 92/0057, respectively. TMS 91/02324, TMS 91/02327, and Isunikankiyan (local check) had the lowest leaf number. At Mokwa, TMS 50395 and TMS 91934 were the highest leaf retainers, followed by TMS 30572, TMS 81/01635, TMS 87/0018-28, and TMS 84/00316, while TMS 81/0003-1 and TMS 4(2)1425 had the lowest number. One strategy to maintain acceptable yields during prolonged droughts is to maintain a photosynthetically active leaf area duration indirectly monitored as stay-green ability and leaf retention (Ekanayake *et al.*, 1996). Thus, acceptable leaf retention is important for both the final root yield as well as frequent leaf harvesting. It was noted that the two genotypes TMS 30572 and TMS 4(2)1425 common to both sites had similar numbers of leaves at both sites. This is not surprising as cassava performance at the two sites, although in two different vegetation zones, are comparable (IITA, 1993a).

Leaf harvesting frequency significantly ( $P < 0.001$ ) reduced leaf number at both sites (Table 1). The genotype × harvest frequency interaction effect was also highly significant at each site, indicating differential leaf retention abilities of the genotypes when subjected to these leaf harvesting frequencies. For TMS 91/02322 and TMS 92/0427 (Ibadan) and TME 1 (Mokwa), the monthly leaf harvest treatment had the highest number of leaves. In the case of TMS 91934 (Mokwa), the three-monthly harvesting treatment gave the lowest leaf number.

The genotypes (TME 1, TMS 91934, TMS 50395, TMS 91/02322, and TMS 92/0427) which maintained high leaf numbers at the monthly leaf harvest frequency, were found to regenerate shoots faster and also retained most of their older leaves after frequent leaf harvesting. These genotypes were also easier to harvest because the shoot tips could break more easily than others. Thus, these genotypes might be more tolerant to frequent shoot removal than others. TMS 4(2)1425, TMS 30001, TMS 81/00110, and TMS 87/0018-28 lost most of their older leaves, and new shoots formed slowly when subjected to monthly leaf harvest-

**Table 1** Leaf retention (no. plant<sup>-1</sup> at the peak of dry season for various cassava genotypes as affected by leaf harvest frequency at two sites in Nigeria, 1996-97)

Genotype (Gen)	Ibadan					Mokwa					
	Leaf harvest frequency (LHF)				Gen mean	Genotype	Leaf harvest frequency (LHF)				Gen mean
	0	1	2	3			0	1	2	3	
TMS 30572	117	73	75	101	92	TMS 30572	117	63	81	105	92
TMS 4(2)1425	63	22	32	51	42	TMS 4(2)1425	62	10	35	52	39
TMS 82/00058	43	32	25	33	33	TMS 30001	97	23	30	77	57
TMS 91/02322	54	65	44	54	54	TMS 50395	182	111	91	165	137
TMS 91/02324	15	14	20	22	18	TMS 81/0003-1	12	20	33	48	28
TMS 91/02327	18	20	18	20	19	TMS 81/00110	76	26	71	85	65
TMS 92/0057	56	38	37	37	42	TMS 81/01635	118	76	77	95	92
TMS 92/0326	37	33	32	33	34	TMS 82/00661	88	53	54	110	76
TMS 92/0398	26	31	29	35	30	TMS 84/00316	72	76	108	59	79
TMS 92/0427	28	60	18	22	32	TMS 87/0018-28	131	50	75	69	81
Isunikankiyan	23	18	21	22	21	TMS 91934	137	128	137	90	123
						TME 1	50	116	58	35	65
LHF mean	44	37	32	39			95	63	71	83	
S.E.D.											
Gen means			1.9						4.0		
LHF means			0.9						2.3		
Gen × LHF means											
within Gen				3.0						8.0	
between Gen				3.2						8.0	
Fixed effects (F value)											
Gen			255.7****						64.5****		
LHF			55.7****						21.8****		
Gen × LHF			24.8****								

S.E.D., Standard error of the mean difference for comparing various means  
\*\*\*\*, denotes significance at  $P < 0.0001$  level

ing, and are likely to be less suitable for frequent leaf harvesting. To maintain high levels of productivity of both leaves and tuberous roots, fast regeneration of shoots and high leaf retention after shoot removal are desirable during drought stresses, which in most cassava-growing areas are as long as, and sometimes longer than, the rainy periods.

### Disease and pest pressures

Genotypes reacted differently to attack by ACMVD at Mokwa (Table 2). TMS 30572, TMS 30001, TMS 84/00316, and TMS 81/0003-1 showed no observable ACMVD symptoms, while TMS 50395, TMS 81/00110, TMS 81/00661, TMS 81/01635, and TMS 87/0018-28 expressed very slight symptoms when shoots were removed. For the genotypes with slight symptoms, only the regenerated young leaves were infected, which recovered with time. Hahn *et al.* (1989) indicated two types of resistance to ACMVD, namely resistance to vector infection (physical barrier) and resistance to spread within the resistant plant

(anti-viral factors). Thus, it appears that genotypes which showed slight or no sign of infection even when shoots were removed may be resistant to spread of the casual geminivirus within the plant. Both sites have a high incidence of the vector whitefly (*Bemisia* spp.). TMS 4(2)1425 and TME 1 were mildly affected, but TMS 91934 with moderate symptoms was the most susceptible to ACMVD. The susceptibility of TMS 91934 to ACMVD has previously been noted (IITA, 1993a). There were also highly significant differences among the leaf harvesting frequencies for ACMVD, with the intensity of infection increasing as the frequency of shoot removal increased. The monthly leaf harvesting frequency was the most susceptible to the disease and the no-leaf harvest the least susceptible. This is not surprising, as leaf harvesting inflicts injury on the plant which reduces resistance to vector infection, and makes it easier for the whitefly transmission of the geminivirus to the plant. However, ACMVD infection was generally low in the genotypes. This is to be expected as most of the geno-

**Table 2** African cassava mosaic virus disease (ACMVD) and cassava bacterial blight (CBB) scores of 12 cassava genotypes as influenced by leaf harvest frequency at Mokwa, Nigeria, 1996-97

Genotype (Gen)	ACMVD					CBB				
	Leaf harvest frequency (LHF)				Gen mean	Leaf harvest frequency (LHF)				Gen mean
	0	1	2	3		0	1	2	3	
TMS 30001	1.0	1.0	1.0	1.0	1.0	2.0	2.7	2.7	2.0	2.3
TMS 30572	1.0	1.0	1.0	1.0	1.0	2.0	2.0	2.0	2.0	2.0
TMS 4(2)1425	1.3	1.7	1.7	1.7	1.6	2.3	1.7	1.7	1.7	1.8
TMS 50395	1.0	1.3	1.3	1.0	1.2	1.7	2.0	2.0	1.7	1.8
TMS 81/0003-1	1.0	1.0	1.0	1.0	1.0	1.7	2.0	2.3	1.7	1.9
TMS 81/00110	1.0	1.3	1.3	1.0	1.2	2.0	2.7	2.3	2.0	2.3
TMS 81/01635	1.0	1.3	1.3	1.3	1.3	1.7	2.7	2.7	1.7	2.2
TMS 82/00661	1.0	1.3	1.3	1.0	1.2	1.3	1.7	1.7	1.7	1.6
TMS 84/00316	1.0	1.0	1.0	1.0	1.0	1.7	2.0	2.7	1.7	1.8
TMS 87/0018-28	1.0	1.3	1.0	1.0	1.1	2.0	2.0	2.0	2.0	2.0
TMS 91934	2.0	3.0	2.7	2.3	2.5	2.0	2.3	2.0	2.3	2.3
TME 1	1.3	2.3	2.0	1.7	1.8	2.0	2.3	2.3	2.0	2.2
LHF mean	1.1	1.5	1.4	1.3		1.9	2.2	2.2	1.9	
S.E.D.										
Gen means			0.2					0.3		
LHF means			0.1					0.1		
Gen × LHF means										
within Gen			0.3					0.3		
between Gen			0.3					0.4		
Fixed effects (F value)										
Gen			14.0***					1.32 ns		
LHF			7.88***					7.12***		
Gen × LHF			0.88 ns					1.02 ns		

S.E.D., Standard error of the mean difference for comparing various means  
 ns, \*\*\*, \*\*\*\*, denote non-significance and significance at  $P < 0.001$  and  $P < 0.0001$ , respectively

types tested, were improved genotypes with good resistance to the disease.

Genotype and genotype × leaf harvest frequency effects were not significant for CBB, but the leaf harvesting frequency effect was highly significant (Table 2). The no-leaf harvest and three-monthly leaf harvesting frequencies had similar CBB scores and were significantly lower than those for monthly and two-monthly frequencies which were also similar. This suggests that injuries inflicted on the plants during leaf harvesting reduces the physical barrier to infection, with chances of natural inoculation with the casual organism (*Xanthomonas campestris* pv. *manihotis*) increasing as shoot tips are frequently removed. Results showed that disease severity was low for all the genotype tested (a mean score of 2.1). This can be partly explained by the fact that most of the genotypes are improved genotypes with less susceptibility to CBB. However, TME 1 (landrace) which has been reported to give higher CBB scores than most of the improved genotypes in this study

(ITTA, 1993a) reacted in a similar fashion to the others. There is a possibility that the weather in 1996 in Mokwa was not favourable for the development of the disease beyond the leaf spot symptom (a disease severity score of 2.0). Similar results have been reported earlier (IITA, 1993b). Due to variations in weather condition it was suggested that an annual screening for CBB be conducted for a period of at least five years. During this time, favourable conditions for the development of the disease would have been encountered for more meaningful conclusions to be made (IITA, 1993b).

Cassava green mite has become one of the most destructive pests of the African cassava belt since its discovery in Uganda in 1972 (Hahn *et al.*, 1989; IITA, 1993b). In the present study, there were significant variations among genotypes for their reactions to attack by CGM (Table 3). TME 1 gave the lowest score of virtually no damage symptoms (score of 1.1), followed by TMS 91934, TMS 4

**Table 3** Cassava green spider mite (CGM) scores of 12 cassava genotypes as influenced by leaf harvest frequency at Mokwa, Nigeria, 1991-97

Genotype (Gen)	CGM				Gen mean
	Leaf harvest frequency (LHF)				
	0	1	2	3	
TMS 30001	5.0	3.0	3.0	4.7	3.9
TMS 30572	4.0	2.0	2.7	3.3	3.0
TMS 4(2)1425	3.0	2.3	2.7	3.0	2.8
TMS 50395	4.0	3.0	3.3	4.0	3.6
TMS 81/0003-1	4.0	3.0	3.3	3.7	3.5
TMS 81/00110	4.0	2.3	2.7	3.7	3.2
TMS 81/01635	4.0	2.0	2.0	3.0	2.8
TMS 82/00661	4.0	2.7	2.7	3.3	3.2
TMS 84/00316	4.0	3.0	3.0	3.7	3.4
TMS 87/0018-28	5.0	3.3	3.3	4.7	4.1
TMS 91934	3.3	1.7	2.3	3.0	2.6
TME 1	1.3	1.0	1.0	1.0	1.1
LHF mean	3.8	2.4	2.7	3.4	
S.E.D.					
Gen means			0.7		
LHF means			0.2		
Gen x LHF means					
within Gen			0.5		
between Gen			0.8		
Fixed effects (F value)					
Gen			3.03**		
LHF			38.53****		
Gen x LHF			0.65 ns		

S.E.D., Standard error of the mean difference for comparing various means  
 ns, \*\*, \*\*\*\*, denote non-significance and significance at  $P < 0.01$  and  $P < 0.0001$ , respectively

(2)1425, and TMS 81/01635. The highest scores were registered for TMS 87/0018-28, TMS 30001, TMS 50395, and TMS 81/0003-1. The high level of resistance of TME 1 and the less susceptibility of TMS 91934 and TMS 4(2)1425 as well as the susceptibility of TMS 30001 and TMS 50395 confirmed the results of previous CGM screening studies using these genotypes (IITA, 1993a, b). Pubescence of the young top leaves was one of the important factors for resistance to CGM (Hahn *et al.*, 1989; IITA, 1993b) in addition to tolerance, non-preference, and to a limited extent, the antibiosis, in some genotypes (IITA, 1993b).

There were also highly significant differences among the various leaf harvesting frequencies, but the interaction between genotype and leaf harvest frequency was not significant (Table 3). Generally, as the intensity of leaf harvesting increased, there was a decrease in CGM damage, with a shift from severe to moderate symptoms with frequent leaf harvesting in many susceptible

genotypes. Cassava green mite prefers young succulent leaves and attacks them, causing them to remain small and undeveloped; it also causes defoliation which progresses downward from the shoots (Hahn *et al.*, 1989; IITA, 1993b). The method of leaf harvesting employed in the present study which involved breaking the shoot tip including three young fully expanded leaves, deprived CGM of the young succulent shoots leading to low preference for those treatments in which the shoots were removed than those with intact shoots. The low attraction increased with increasing frequency of shoot removal. Thus, shoot removal confers some degree of non-preference mechanism on cassava against CGM. Results showed that the CGM pressure was high, confirming the result of several multilocational trials which indicated a high CGM pressure in Mokwa (IITA, 1993a, b).

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## Poster

# Growth and flowering behaviour of four cassava genotypes in two soil types and locations in southern Nigeria

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Flower initiation in some plant species is associated with the physical and chemical soil characteristics such as the availability of certain mineral elements and soil moisture status. The objective of this study was to examine the effects of soil physicochemical traits on the differential flowering and growth habits of cassava (*Manihot esculenta* Crantz). Three trials were established at two locations, Ibadan and Ubiaja in southern Nigeria, on two soil types, sandy loam (Ferric Luvisols, Ibadan) and loamy sand (Dystric Nitisol, Ubiaja) soils at different planting times. Four genotypes, TME1 and TME2 (local landraces and shy flowering) and TMS 30555 and TMS 91934 (improved and profusely flowering) were compared. Plant growth, days to branching, branching events, days to flowering, and flowering events were better for all genotypes in Ubiaja soil than in Ibadan soil. However, soil type alone did not explain the differential flowering behaviour of shy and profuse flowering genotypes. Flowering behaviour supported the multifactorial flower induction theory where soil characteristics were, but one, of the interacting modulating climatic factors determining flowering.

Keywords: *Manihot esculenta* Crantz; Branching; Flowering events; Nitrogen; Potassium

The onset of the reproductive phase in cassava (*Manihot esculenta* Crantz) is indicated by branching (Ekanayake *et al.*, 1997) even though not all genotypes produce inflorescences at the first branching level. Since cassava is a perennial crop plant, both vegetative and reproductive growth phases occur simultaneously and over years until harvested. Available information on the influence of the physical and chemical soil status on flower initiation or branching induction in cassava is limited. According to Howeler (1985), K deficiency may markedly reduce vertical growth, but lead to continued branching, producing a prostrate cassava plant. Kraus (1925) found that if C:N ratio in the plant was moderately high, flowering would be promoted, whereas a low ratio (high N) favoured vegetative growth. Different species respond differently to flower initiation under various soil conditions (Kramer and Kozlowski, 1979).

Shy flowering cassava landraces, TME1 and

TME2, have been observed to flower consistently in Ubiaja, Nigeria, but not at other locations, i.e., IITA, Ibadan, Nigeria. A comparison of soil samples collected from these two sites indicated various differences in soil mineral contents and physical properties. Ubiaja soil is sandy but under high organic matter levels, retains more soil water. Due to the differences in soil physicochemical characteristics of the two sites and rainfall patterns but with minimal difference in photoperiods, it may be hypothesized that the differential flowering habits at these two sites is due more to the soil factors than climatic factors. Therefore, the objective of this study was to examine the effect of two soil types at two field sites located in the lowland humid and derived savanna agro-ecozones of Nigeria, on flower induction and production, and growth habits of two shy flowering cassava landraces and two high-flowering and improved cassava clones.



## Materials and Methods

### Plant materials

The four cassava cultivars used in the study included two improved clones: TMS 30555 (early branching) and TMS 91934 (medium branching and flowering) and two landraces, TME1 and TME2 (late branching and shy flowering types which flower at Ubiaja but do not flower at Ibadan).

### Experimental sites, field designs, and management

The experiment was conducted at two sites, IITA, Ibadan, in the derived savannah agro-ecozone at 7°3' N latitude, 3°54' longitude, 243 m above sea level (asl) and Ubiaja in the lowland humid agro-ecozone at 6°39' N latitude, 6°20' E longitude and 170 m asl, using large plastic bags. A split-plot design was used, with soil type as the main plot and cultivars as sub-plots, with four replications.

Three field experiments conducted at these two sites are referred to as follows: Ibadan, December planting (Expt 1); Ibadan, April planting (Expt 2); and Ubiaja, April planting (Expt 3). About 11 t of Ubiaja (Dystric Nitisol) and Ibadan [Ferric Luvisols Ibadan series, (Moorman *et al.*, 1975)] top soil (30 cm depth) were reciprocally exchanged in the two sites. At the site, the two types of soil were separately filled in large black plastic bags (160 kg capacity) and spaced 1 m apart in the field. To reinforce the container, four bags inside each other were used. Two drainage holes 30–40 cm above ground, were perforated around the bottom of the bag. At Ibadan, the experiment was planted twice, first on 9 December 1991, during the dry season and secondly on 17 April 1992, concurrently with that at Ubiaja (15 April 1992) at the onset of the rainy season.

The planting materials were all collected only from those plants that had flowered during the previous season at Ubiaja, for uniformity and flower induction ability. Two mature stem cuttings of about 20 cm long were selected from healthy plants and initially planted vertically in each bag. After plant establishment, they were thinned to one plant per bag. Bags were supplied with presprouted stem cuttings to compensate for any failures in order to have a complete plant population in each bag at that time. At IITA, Expt 1 (December planting) was irrigated during the dry season at three-day intervals to field capacity.

### Soil and plant measurements

Three replicates of composite soil samples at 0–30 cm depth were collected from both sites and physico-chemical traits analysed at the IITA Analytical Services Laboratory. During the dry season, soil moisture retention was monitored at Ibadan

by using a soil moisture tensiometer located at 30-cm depth (Model 2710, Soil Moisture Equipment Corp., Santa Barbara, CA 93105, U.S.A.).

Plant growth data collected included the following parameters. Plant establishment was monitored at 2 weeks after planting (WAP). Branching and plant height records were taken monthly (Ekanayake, 1996). Date of 50% first branching (50% of the plants produced first level branches) and 50% flowering (50% of the plants producing male or both male and female flowers) were noted. Numbers of flowers formed per flowering event were also counted. Flowering event is defined as that of the occurrence of reproductive structures at a given branching level (Ekanayake *et al.*, 1997). Branching event is defined as the occurrence of forking which may or may not be reproductive branching. Dry weight of the whole plant was determined by chopping individual plant parts and oven-drying at 100°C to a constant weight.

The weather data for Ibadan were obtained from the IITA central weather station. Weather data for Ubiaja were collected at the farm using a LI-1200 automatic minimum weather data set recorder (LI-Cor Inc., 4421, Superior St, P.O. Box 4425, Lincoln, NE 68504, U.S.A.).

All data were submitted to split-plot analysis of variance for each experiment, and means and standard errors were calculated where appropriate (SAS Institute Inc., 1990).

## Results and Discussion

### Weather and soil characteristics

Total rainfall during the one-year life cycle of plants of Expt 1 at Ibadan was 1174.9 mm, in addition to the irrigation supplied (about 10–12 L container<sup>-1</sup>) before the onset of the rainy season. Average minimum and maximum temperatures were 21.4 and 31.4°C, respectively. Minimum and maximum relative humidity were 50.7 and 93.1%, respectively, with an average daily solar radiation of 14.6 MJ m<sup>-2</sup>. Plants of Expt 2 in Ibadan had a total rainfall of 1115.8 mm, 21.6 and 30.2°C minimum and maximum temperature, respectively, 60.1 and 95.9% minimum and maximum relative humidity, respectively, and a daily average of 13.8 MJ m<sup>-2</sup> solar radiation. At Ubiaja, Expt 3, plants received a total rainfall of 1861 mm (40% higher rainfall budget) during the year. Average minimum and maximum temperatures for the period were 20.3 and 30.7°C, respectively, and the average relative humidity over the same period ranged from 68.8 to 90.2% with an average daily solar radiation of 13.3 MJ m<sup>-2</sup>.

The physicochemical characteristics of the two types of top soil used are presented in Table 1. Soil water potentials taken during the dry season (November–February) at Ibadan site for Ubiaja soil was always higher than that of

**Table 1** Physicochemical properties of Ibadan and Ubiaja soils used in the three experiments

Parameter	Ibadan soil	Ubiaja soil
<b>Chemical properties</b>		
pH	6.4	6.0
Organic C (%)	1.14	1.09
Total N (%)	0.167	0.131
Available P (Bray-1, ppm)	5.9	7.1
NH <sub>4</sub> OAC exchangeable cations (meq 100 g <sup>-1</sup> )		
Ca	6.50	2.16
Mg	1.24	0.81
Mn	0.80	0.64
K	0.64	0.20
Na	0.22	0.14
Total acidity (meq 100 g <sup>-1</sup> )	0.29	0.70
ECEC (meq 100 g <sup>-1</sup> )	9.69	4.66
<b>Physical properties</b>		
Sand (%)	75	87
Silt (%)	13	6
Clay (%)	12	7
Texture	Sandy loam	Loamy sand

Ibadan soil by 0.01 to 0.03 MPa. Diurnal mean soil temperatures measured at a 30-cm depth were not significantly different between the two soils at both sites.

Soil water availability for plant growth depends on soil moisture content, soil moisture retention characteristics, soil hydraulic conductivity, and rooting zone and depth (Ekanayake *et al.*, 1989). Ubiaja soil is loamy sand with a high organic matter content compared with Ibadan soil (sandy loam). Loamy sandy soils have better percolation rate and drainage compared to sandy loam soils of Ibadan which tend to have more run-off and dry faster than loamy sand. As a result of the high organic matter in Ubiaja soil, which acts as a mulch, soil moisture retention was higher than in Ibadan soil. This was further improved in Ubiaja (745 mm more) by the high annual rainfall as compared with Ibadan (average rainfall of 1145.5 mm for Expts 1 and 2).

**Plant height**

The trends in plant heights of individual genotypes and treatments for Expts 1 and 3 showed that plants grew taller in all treatments from one to eight months after planting (MAP) (data not shown). At Ibadan, plants in Ibadan soil were significantly ( $P < 0.05$ ) taller than in Ubiaja soil at 1 MAP (Expt 1). However, the plants grown in Ubiaja soils were significantly

( $P < 0.05$ ) taller than those in Ibadan soil (Expt 2). At Ubiaja (Expt 3), mean soil types were significant ( $P < 0.05$ ) only at 2 MAP. Plants in Ibadan soil were significantly ( $P < 0.05$ ) taller than those in Ubiaja soil. From 2 MAP and throughout the plant growth, soil type mean differences were not significant.

Genotype mean differences for plant height in all three experiments were highly significant ( $P < 0.01$ ) throughout the growth period. Type of soil x genotype interaction mean differences for plant height in Expt 1 were significant ( $P < 0.01$ ) at 1, 7, and 8 MAP. During these intervals, TME1 plants were shorter in Ubiaja soils than TMS 30555 in Ibadan soil. In Expt 2, interaction mean differences were significant ( $P < 0.05$ ) only at 2 MAP. The two experiments simultaneously planted in April (Expts 2 and 3) showed poor plant growth at Ibadan compared to Ubiaja.

**Number of days to 50% first branching and branching events**

Time to 50% first branching in these genotypes was not influenced by the type of soil used in both sites (Table 2). On average in Ibadan,

**Table 2** Effect of the type of soil and location on the mean number of days to 50% first branching and number of branches during the crop season of four cassava genotypes (Ibadan and Ubiaja, Nigeria)

Genotype	No. of days to 50% first branching				No. of branches			
	Ibadan		Ubiaja		Ibadan		Ubiaja	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
<b>Expt 1 at Ibadan (December planting)</b>								
TMS 30555	43.0	49.3	46.2	16.7	6.8	5.5	6.2	4
TMS 21934	56.8	59.8	58.3		5.0	5.3	5.2	
TME1	45.0	108.0	76.5		0.8	1.0	0.9	
TME2	158.3	224.0	191.2		2.0	1.5	1.8	
Mean	75.8	110.3			3.7	3.3		
S.E.	11.8				0.3			
<b>Expt 2 at Ibadan (April planting)</b>								
TMS 30555	41.8	40.5	41.2	3.6	3.0	3.0	3.0	0.06
TMS 21934	60.5	52.8	56.7		3.0	3.0	3.0	
TME1	58.5	59.5	59.0		1.0	1.0	1.0	
TME2	120.8	124.0	122.4		1.0	1.3	1.2	
Mean	70.4	69.2			2.0	2.1		
S.E.	2.6				0.04			
<b>Expt 3 at Ubiaja (April planting)</b>								
TMS 30555	41.5	41.5	41.5	0.5	5.3	5.5	5.4	0.4
TMS 21934	50.0	50.0	50.5		6.3	5.5	5.9	
TME1	76.0	76.0	76.0		2.3	2.5	2.4	
TME2	101.0	101.0	101.0		2.3	2.8	2.6	
Mean	67.1	67.1			4.1	4.1		
S.E.	0.4				0.3			

S.E. is Standard error

plants in Expt 2 reached 50% first branching earlier (70 days) than in Expt 1 (93 days). Genotypic mean differences for 50% first branching were significant ( $P < 0.01$ ) for all experiments, in both locations. In both soils, TMS 30555 branched earlier than TME2. Type of soil  $\times$  genotypic interaction mean differences were not significant for both soil types in both sites.

The type of soil did not affect the number of branching events during the season for all soil types at both sites (Table 2). However, genotype means for number of branching events were highly significant ( $P < 0.01$ ), for all experiments in both locations. In general, TMS 30555 or TMS 91934 had a higher number of branching events than the landraces, TME1 and TME2. Type of soil  $\times$  cultivar interactions were not significant at all instances. Plants in Ubiaja (Expt 3) had a higher number of branching events than at Ibadan (Expt 2) although planted during the same time. This may be additional evidence of greater moisture stress and biotic stresses at Ibadan, where plants in Ibadan (Expt 2) suffered severe die-back due to cassava bacterial blight (CBB) and where plant growth and vigour was generally poor compared with Ubiaja. Cassava bacterial blight contributed to lack of flowering in TME1 plants despite branching in Ubiaja soil (Expt 3).

### Number of days to first flowering and flowering events

The numbers of days to first flowering is shown in Table 3. TMS 30555 and TMS 91934 branched for the first time at the same time in January, 43 days after planting (DAP) in both soil types (Expt 1). At the end of January (53 DAP) two plants of TME1 branched for the first time in Ibadan soil with two male flowers, which aborted shortly after. Later in June (176 DAP), TME2 also branched for the first time in Ibadan soil and formed a bunch of flower buds which also aborted before opening. TMS 30555 branched and flowered at the same time in both locations [34 DAP (Expt 2) and 35 DAP (Expt 3)]. Flowering of TMS 91934 was delayed till October (124 DAP) in both soils in Expt 2 and a few male flowers were observed from the third branching level. TME1 in Ibadan soil (Expt 3) first flowered at 117 DAP from the third branching level, and TME2, 193 DAP from the third branching level in Ibadan soil. TME1 and TME2 did not branch in Expt 2. Although flowering in TME1 and TME2 was recorded early at Ibadan (Expt 1), flower abortion was high. Flowering was poor in Ibadan (Expt 2) compared with Ubiaja (Expt 3).

### Mean total number of flowers per flowering event

The type of soil did not affect the number of flowering events during the season for both soil types (Table 3). TMS 30555 had more flower-

**Table 3** Effect of the type of soil and location on the mean number of days to first flowering and flowering events of four cassava genotypes (Ibadan and Ubiaja, Nigeria)

Genotype	No. of days at first flowering			Flowering events		
	Soil type			Soil type		
	Ibadan	Ubiaja	Mean	Ibadan	Ubiaja	Mean
<b>Expt 1 at Ibadan (December planting)</b>						
TMS 30555	43	43	43	5	4	4.5
TMS 91934	43	43	43	1	3	2
TME1	53	—	53	0	0	0
TME2	176	176	176	0	0	0
Mean	57	57		3.5	3.5	
<b>Expt 2 at Ibadan (April planting)</b>						
TMS 30555	34	34	34	3	3	3
TMS 91934	124	124	124	0	1	1
TME1	—	—	—	0	0	0
TME2	—	—	—	0	0	0
Mean	52	72		3	2	
<b>Expt 3 at Ubiaja (April planting)</b>						
TMS 30555	35	35	35	5	6	5.5
TMS 91934	38	38	38	6	6	6.0
TME1	117	138	130	1	†	1.0
TME2	93	173	177	1	2	1.5
Mean	65	93		3.3	4.7	
S.E.	8					

—, denotes that flowering did not occur throughout the study period

†, Plants were severely damaged by cassava bacterial blight (CBB)

S.E. is standard error

ing events than other genotypes at both sites. TMS 30555 and TMS 91934 had more flowering events than TME1 and TME2 in Ubiaja. Generally, more flowering events occurred in Ubiaja than in Ibadan.

The total number of flowers produced on Ubiaja soil was higher than that of Ibadan soil in all experiments, but the soil type means for total number of flowers per flowering event were not significant (Table 4). TMS 91934 produced a significantly ( $P < 0.01$ ) higher number of flowers per flowering event than other genotypes. Type of soil  $\times$  cultivars interaction mean differences were not significant for the three experiments. The total number of flowers per flowering event decreased from December to April planting. April planting in Ibadan had a lower number of flowers compared with the April planting in Ubiaja, but the number of flowers per flowering event was higher in Ibadan (Expt 1) than Ubiaja (Expt 3). Generally, the number of branching events declined from December to April planting at Ibadan, illustrating the photoperiodic effect on branching and subsequently on flowering.

**Table 4** Effect of the type of soil and location on the mean total number of flowers per flowering event of four cassava genotypes (Ibadan and Ubiaja, Nigeria)

Genotype	Soil type			S.E.
	Ibadan	Ubiaja	Mean	
<b>Expt 1 at Ibadan (December planting)</b>				
TMS 30555	249.1	395.0	322.14	106.8
TMS 91934	264.3	573.4	418.9	
Genotype mean	256.7	484.2		
S.E.	75.5			
<b>Expt 2 at Ibadan (April planting)</b>				
TMS 30555	105.0	181.1	143.1	11.7
TMS 91934	0.8	21.9	11.4	
Genotype mean	52.9	101.5		
S.E.	8.3			
<b>Expt 3 at Ubiaja (April planting)</b>				
TMS 30555	209.4	155.9	182.7	30.5
TMS 91934	228.3	160.8	194.6	
TME1 <sup>1</sup>	37.5	101.4	69.5	
TME2 <sup>2</sup>	10.0	91.3	50.7	
Genotype mean	121.3	127.4		
S.E.	21.6			

<sup>1</sup>TME1 and TME2 did not flower at the sites at the December and April planting  
S.E. is standard error

Cultivars TME1 and TME2 (Expt 1) had floral initiation on the first branching at Ibadan contrary to the reported lack of flowering of these landraces in Ibadan. All such flowers, however, aborted prior to fruiting despite irrigation during the dry period. Kramer and Kozlowski (1979) expressed greater difficulties to generalize the effect of plant water deficit on reproductive growth than on vegetative growth due partly to differences in timing of reproductive growth among species and cultivars. The effect of water balance on reproductive growth is also influenced by the plant's capacity for alternative bearing and other internal growth factors such as mineral supply, hormone balance, and biomass partitioning for vegetative organs versus reproductive organ development. Severe internal water deficits may inhibit any one of the phases of reproductive growth, such as floral initiation which is suppressed or inhibited by severe water deficit (Kramer and Kozlowski, 1979; Ekanayake *et al.*, 1989) and by high levels of endogenous abscisic acid (Bernier *et al.*, 1981; Bernier, 1988).

The role of K in the induction of branching (Howeler, 1985) and subsequent flowering needs to be reassessed. Ubiaja soils had less K than Ibadan soil, but were not deficient in K. A re-

sponse to K was obtained in Nigerian soils with 0.14 and 0.16 cmol of exchangeable K<sup>+</sup> kg<sup>-1</sup> in Oxisols (Obigbesan, 1973) and at 0.11 cmol K<sup>+</sup> kg<sup>-1</sup> (Ngongi *et al.*, 1976). Soil exchangeable K<sup>+</sup> levels are not always good indicators of growth responses, due to differences in soil texture, Ca, and Mg contents, K-supplying power of soil, genotypic differences, pest and disease incidences, and drought (Howeler, 1985). A negative correlation between leaf K content and CBB severity has been reported while K fertilization at moderate levels was also shown to reduce severity of CBB incidence (Odurukwe and Arene, 1980). But in Ubiaja (lower K) soils, CBB incidence was less severe. According to Havelange and Bernier (1993) K and Mg are not supplied in greater amounts to the buds of flowering-induced plants of *Sinapis alba*, while an increased supply of Ca to the buds appears as a secondary effect of induction.

Phosphorus deficiency is suspected of causing a shift in biomass partitioning by limiting shoot growth more than the root growth (Cock *et al.*, 1979). Cassava grown in low P (2.49 mg g<sup>-1</sup> soil; critical P level is 4 mg g<sup>-1</sup>; Howeler and Cadavid, 1990) and low K (0.16 meq 100 g<sup>-1</sup> soil; critical K level is 0.17 meq 100 g<sup>-1</sup> soil), was shown to compete and be responsive to P application particularly in the formation of aerial apices and reproductive organs such as flowers and fruits (Pellet and El-Sharkawy, 1993).

Soil C:N ratio in Ubiaja soils (8.32) was higher than Ibadan soils (6.83), in the top 30-cm depth. High C:N ratio in Ubiaja soils was also due to lower N levels. A high C:N ratio promoting flowering has been noted as early as 1925 (Kraus, 1925).

At Ubiaja, TME1 and TME2 flowered in both types of soil and when grown in Ibadan soil under Ubiaja conditions, showing that the consistent flowering of TME1 and TME2 in Ubiaja is not only due to soil chemical composition, but perhaps more related to physical properties such as water distribution in the soil profile in particular, in addition to the climatic factors. Climatic factors such as shading, photoperiod, and temperature modified the flowering behaviour (Simwambana *et al.*, 1995).

#### Total dry matter (DM) per plant

The total DM data are given in Table 5. Plants in Ubiaja soils accumulated significantly ( $P < 0.05$ ) higher total dry weight than in Ibadan soil (Expt 2). TME2 accumulated significantly ( $P < 0.01$ ) higher total DM per plant than TMS 30555 at Ubiaja. TME2 in Ubiaja soil had a higher total DM per plant than TME1 in Ibadan soil (Expt 2). Generally, the plants grown in Ubiaja soil in Expt 2 accumulated more DM per plant than in Ibadan soil. TME2 in particular developed bigger plants in Ubiaja soil than in Ibadan soil. TME2 also

**Table 5** Effect of the type of soil and location on total plant dry weight (g plant<sup>-1</sup>) at 10 MAP for four cassava genotypes established in April at Ibadan and Ubiaja

Genotype	Expt 2 (Ibadan)				Expt 3 (Ubiaja)			
	Soil type		Soil type		Soil type		Soil type	
	Ibadan	Ubiaja	Mean	S.E.	Ibadan	Ubiaja	Mean	S.E.
TMS 30555	6556	808	732	56	1018	693	856	205
TMS 91934	564	788	676		1345	780	1062	
TME1	517	792	654		1233	1336	1284	
TME2	677	968	823		1726	2165	1946	
Mean	603	839			1331	1243		
S.E.	40				145			

S.E. is Standard error

developed bigger plants than other genotypes in both locations.

In conclusion, there was no evidence to support the hypothesis that soil type alone influenced the induction of flowering of landraces at the favourable site. The better flowering performance of all genotypes in Ubiaja soil and at Ubiaja in both soil types, could be due to a combination of ecological factors which were more suitable for flower induction and development than at the IITA site. The principal desirable features were higher rainfall amount and better distribution in combination with soil factors such as better drainage and high organic matter. These factors helped to mitigate a short season drought stress of the plants and associated alterations in flowering behaviour. The present observations also support the occurrence of multi-factorial floral induction control for cassava. Under short day tropical conditions, soil traits for both the chemical and physical factors altered the stronger modulating climatic factors of flower induction such as photoperiod and temperature. Therefore, further testing is required to clarify the site specificity in the flowering patterns of the various cassava genotypes.

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# Microbial linamarases for the detoxification of vegetable products

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Fifty-eight strains of *Penicillium*, known to produce interesting concentrations of pectolytic activities, were screened for linamarase and amygdalase activity in culture broths. Seven strains were active at a level of interest for further investigations. *Penicillium aurantiogriseum* (P35) was selected for optimization of enzyme production. Likewise, 75 strains of food-grade organisms (lactic acid bacteria, yeasts, and filamentous fungi) were investigated for their ability to degrade amygdalin. Among 16 effective degraders *Mucor circinelloides* (M40) proved to also degrade linamarin effectively. The enzymes from *P. aurantiogriseum* (called PGI and PGII) were characterized with respect to molecular weight, temperature and H optimum and stability, substrate affinities, and inhibitors. The molecular weights were estimated to be approximately 247 and 147 kDa, respectively. Both enzymes showed pH optimum at 6.0; the optimum temperature (60°C) of PGII was higher than that of PGI (55°C). A wide range of cyanogenic glycosides were hydrolyzed by both of these enzymes. The apparent  $K_m$  values for prunasin, amygdalin, and linamarin of PGI were approximately 0.43, 0.47, and 2.41 mM, respectively, and those of PGII were 0.13, 0.11, and 2.32 mM, respectively. Thus, filamentous fungi, [among which are *P. aurantiogriseum* (P35) and *M. circinelloides* (M40)] are candidates for the production of  $\beta$ -glycosidases, and for the detoxification of food and feeds that contain toxic glycosides, such as linamarin in cassava.

Keywords: Cassava; Detoxification; Enzymes; Food; Feed; Glycosidases; Glycosides; Toxins

The number of secondary constituents known in fungi, plants, and animals is high and increasing. Twenty-six main biosynthetic groups were subdivided into 107 subgroups, and many contained more than 1000 known structures (Luckner, 1990). The alkaloids have been in focus for over 150 years. In 1950, about 2000 of these substances were recognized. By 1970, the number had increased to about 4000 and 20 years later to 10 000 (Raffauf, 1996).

Secondary compounds can act as defense substances against microbial and viral attack, and against browsing animals. The defence against herbivores can act in several ways. Compounds may be toxic, have anti-nutritional (digestibility-reducing) effects, or have an unpleasant taste or odour.

Several of these groups of constituents are known as toxic, anti-nutritional, or palatability-reducing compounds in foods or feeds. A number of different glycosides, causing physiological effects (toxins) or reduced use of nutrients (anti-nutritional compounds), are known from the

plant kingdom, as are a number of bitter tasting glycosides, which reduce palatability. While a few of these glycosides have been demonstrated to be protective to the plant, most have only been identified as possessing one of the above-mentioned properties when considering domestic animals or humans as consumers.

Several toxic glycosides (including various saponins and cyanogenic glycosides) are known to be bitter tasting. Hence, the term bitter, as opposed to sweet, has traditionally been used to designate naturally-occurring or selected groups within a plant species, that contain high amounts of the toxic substance. The groups may be divided by variety, form, or cultivar. Examples of species (crops) for which such a division has been used, are seeds of *Prunus dulcis* (and other *Prunus* spp.), as well as roots of *Manihot esculenta* (cassava). Both of these contain the cyanogenic glycosides, amygdalin and linamarin, respectively (Seigler, 1991).

A number of detoxification methods are used in traditional preparation of food from vegetables containing toxic glycosides. Industrially, only

the debittering of citrus juice (Lea, 1991), and the detoxification of oil press cakes from jojoba (Abbott *et al.*, 1991) and linum (flax) (Wanasundara *et al.*, 1993) have been studied. Unfortunately, these studies have been performed without any linkage to one another, in spite of the fact that the problem in all cases is the removal of glycosides.

The aims of the studies reviewed here were to identify micro-organisms with a promising potential for the production of  $\beta$ -glycosidases, to study the substrate specificities and other characteristics (pH and temperature range and enzyme stability), to investigate the optimum conditions for enzyme production, and to perform studies on the detoxification of various food and feed products.

## Materials and Methods

### Micro-organisms and growth conditions

Fifty-eight strains of *Penicillium* (Brimer *et al.*, 1994) were screened for the presence of linamarase and amygdalase activities in growth supernatants. Basal culture medium and growth conditions were as previously described (Brimer *et al.*, 1994; Petruccioli *et al.*, unpubl.). Seventy-five strains of food-grade micro-organisms (Brimer *et al.*, 1993) were screened semi-quantitatively for their ability to degrade (*in vivo*) amygdalin.

### Chemical and biochemical assays

The screening of culture broths was conducted by a semi-quantitative method as described by Brimer *et al.* (1994), while investigations of micro-organisms for the degradation of amygdalin *in vivo* were performed after the method of Brimer *et al.* (1993). The absolute  $\beta$ -glycosidase activity, of supernatants, fractions, and purified enzymes, was determined by assaying for the hydrocyanic acid (HCN) released from the different cyanogenic glycosides (substrates). This was done using a modification of the method of Essers *et al.* (1993) detailed by Brimer *et al.* (1997). Investigations of the mechanism of degradation of amygdalin (one- or two-step reaction) were made by means of thin layer chromatography (TLC) analysis (for cyanogens) of time-stopped incubations of growth supernatant/purified enzymes with the substrate (Brimer *et al.*, 1996, 1997). The  $K_m$  values were based on analysis of such incubations by high performance liquid chromatography (HPLC) (Petruccioli *et al.*, unpubl.).

### Purification and characterization of enzymes

After concentration, the culture broths were purified by two steps of column chromatography both monitored by the assay for amygdalase

activity (Brimer *et al.*, 1997). Molecular weights were estimated from the volumes of elution in gel permeation chromatography (Brimer *et al.*, 1997; Petruccioli *et al.*, unpubl.).

## Results and Discussion

Seven of the *Penicillium* strains, investigated for the presence of linamarase and amygdalase in the culture broth, were found to be active at a level of interest for further investigations. These were: *P. aurantiogriseum* P35 (formerly *P. turbatum* DBVPG 8565), *P. aurantiogriseum* (UCD 2-57-6), *P. crustosum* (UCD 2-23-19), *P. expansum* (UCD 2-53-2), *P. paxilli* (NRRL 2008), *P. piceum* (DBVPG 8561), and *P. oxalicum* (DBVPG 8550) (Brimer *et al.*, 1994). Grown on the same medium, the absolute activities of amygdalase and linamarase varied between the strains, as did the relation between the amygdalase and the linamarase activity (Table 1). *Penicillium aurantiogriseum* (P35) was selected for further optimization of growth and enzyme production. The optimal culture medium for the production of  $\beta$ -glycosidase activity (measured as the linamarase and amygdalase activity of the supernatant) was (g L<sup>-1</sup> water): pectin, 10.0; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 8.0; KH<sub>2</sub>PO<sub>4</sub>, 8.0; Na<sub>2</sub>HPO<sub>4</sub>, 2.8; MgSO<sub>4</sub>·7 H<sub>2</sub>O, 0.5; and yeast extract, 4.0. On this medium, the levels of enzyme activity were 50.5 and 9.4 mU mL<sup>-1</sup> for amygdalase and linamarase, respectively. Amygdalase activity was defined as the overall reaction to release the cyanohydrin (mandelonitrile) from this  $\beta$ -bis-glucoside, irrespective of the mechanism (Petruccioli *et al.*, unpubl.).

Of the 75 micro-organisms (lactic acid bacteria, yeasts, and filamentous fungi), screened for their ability to degrade (*in vivo*) amygdalin, 16 were found to be highly active (Brimer *et al.*, 1993). The culture broth from *Mucor circinelloides* (M40) was found to effectively hydrolyse both amygdalin and linamarin (Table 1).

The crude enzymes (broths) from both micro-organisms hydrolysed amygdalin with the release of prunasin as an intermediate product, i.e., by a two-step (sequential) mechanism (Brimer *et al.*, 1996). Separation of the proteins present in the growth supernatant, as monitored by assays for amygdalase and linamarase activity, respectively, revealed the presence of two  $\beta$ -glycosidases in the case of *P. aurantiogriseum* and one in the case of *M. circinelloides* (Brimer *et al.*, 1997). These were further purified (Brimer *et al.*, 1997). All three glycosidases could hydrolyse both linamarin and amygdalin. Amygdalin was in all cases hydrolysed by the sequential mechanism (Brimer *et al.*, 1997). The two enzymes from *P. aurantiogriseum* were characterized with respect to molecular weight, temperature and pH optimum and stability, substrate affinities, and inhibitors of the enzymatic activity (Petruccioli *et al.*, unpubl.).

**Table 1** Amygdalase (AM) and linamarase (LI) production and growth of selected strains<sup>†</sup>

<i>Penicillium</i> strains	AM (mU mL <sup>-1</sup> )	LI (mU mL <sup>-1</sup> )	Growth (g L <sup>-1</sup> )
<i>P. crustosum</i> UCD 2-23-19	9.6±0.8	1.53±0.12	4.92±0.45
<i>P. paxilli</i> NRRL 2008	17.1±2.5	2.13±0.69	3.86±0.37
<i>P. piceum</i> DBVPG 8561	23.5±1.0	2.88±0.27	3.87±0.17
<i>P. aurantiogriseum</i> P35	26.2±1.4	6.09±0.60	3.44±0.58
<i>P. aurantiogriseum</i> UCD 2-57-6	21.2±0.7	4.74±0.36	4.15±0.19
<i>P. expansum</i> UCD 2-53-2	8.7±0.8	3.81±0.30	3.71±0.73
<i>P. oxalicum</i> DBVPG 8550	28.4±2.0	6.16±0.88	2.96±0.65

<sup>†</sup>Amygdalase and linamarase activities at time of maximal enzyme production, biomass at 192 h of fermentation  
Values represent means of two repetitions ± standard deviations

The two β-glycosidases were called PGI and PGII. Their molecular weights were estimated to be approximately 247 and 147 kDa, respectively. Both enzymes showed the same optimum pH (6.0). The optimum temperature (60°C) of PGII was higher than that of PGI (55°C). A wide range of cyanogenic glycosides (glucosides and β-bis-glucosides with very differing aliphatic as well as aromatic aglycones) were hydrolysed by both enzymes. These included amygdalin, cardiospermin sulphate, dhurrin, epivolkenin, gynocardin, linamarin, lucumin, passiflorin, prunasin, sambunigrin, taxiphyllin, and tetraphyllin B (Petruccioli *et al.*, unpubl.). The  $K_m$  values for prunasin, amygdalin, and linamarin of PGI were approximately 0.43, 0.47, and 2.41 mM, respectively, and those of PGII were 0.13, 0.11, and 2.32 mM, respectively (Petruccioli *et al.*, unpubl.).

Preliminary investigations, using from 0.1 to 2.0 enzymatic units for every 350 g of grated cassava root, have shown that addition of growth supernatant from *M. circinelloides* (M40), considerably speeds up the hydrolysis of glucosides during the production of gari. Further investigations are in progress. In conclusion, several filamentous fungi [among which are

*M. circinelloides* (M40) and several *Penicillium* spp.] are good candidates for the production of β-glycosidases and for the detoxification of food and feed containing toxic glycosides, e.g., linamarin and lotaustralin in cassava. The wide range of structurally different cyanogenic glycosides cleaved by the enzymes of *P. aurantiogriseum* (P35), indicates that other glycosidic toxins probably will be degraded as well. Research along this line is in progress.

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# Correlation between cyanogenic glucoside content and taste of fresh cassava roots

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Two roots were collected from each of 246 plants of the 10 most commonly-grown cassava cultivars in a farming community in Nkhata Bay District, Malawi. Each of the 492 roots was split longitudinally, and cyanogenic glucoside levels were determined in one half of each root and the degree of bitterness in taste was graded for the other half, by a trained taste panel of 12 persons. The mean taste scores obtained by the taste panel for each root correlated with the glucoside levels ( $r = 0.77$ ), and even stronger with the log values of glucoside levels in each root ( $r = 0.87$ ). The mean levels of glucosides for all roots from each cultivar correlated much more strongly with the average taste score of all roots from each cultivar ( $r = 0.98$ ). It seems plausible that the bitter taste in cassava roots may be due to the presence of the cyanogenic glucosides and the results confirm farmers' statement that toxicity can be predicted by tasting fresh cassava roots.

Keywords: Cassava tubers; Bitterness; Taste; Cyanogenic glucosides; Linamarin; Correlation; Malawi

Cassava (*Manihot esculenta* Crantz) is cyanogenic and produces hydrocyanic acid (HCN). The two cyanogenic glucosides, linamarin and lotaustralin are found in the ratio of 9:1 (Nartey, 1968). The cassava plants also contain an enzyme which can break down cyanogenic glucosides but is located in different compartments of the plant cell (Mpkong *et al.*, 1990). The destruction of the plant cell brings the cyanogenic glucosides into contact with an endogenous enzyme, linamarase, and the resulting enzymic hydrolysis of the glucosides releases the toxic HCN via the intermediate, cyanohydrin. This process of releasing cyanide is known as cyanogenesis (Nartey, 1968). However, toxic effects of cassava are very rare in relation to the wide consumption of cassava products made from roots with high amounts of cyanogenic glucosides (Rosling, 1996), the reason being that processing methods, such as soaking fol-

lowed by drying, have proven to reduce the toxic cyanogen compounds to negligible levels (Dufour, 1989; Hahn, 1989). It is known from studies of cassava lines grown at agricultural research stations that the degree of bitterness of cassava roots is positively correlated with the amount of linamarin in the fresh roots (Sunderesan *et al.*, 1987). The taste and cyanogenic potential of fresh cassava roots are highly variable and the association of these two characteristics remains disputable (Coursey, 1973). The cyanogenic glucoside, linamarin, contributes largely to the bitterness of the root parenchyma and bitterness is not affected by the sweet taste of sugar but suppressed by the presence of citrates and malates (King and Bradbury, 1995). This may be responsible for the poor correlation between taste and content of cyanogenic glucosides. The variation in the glucoside levels in cassava roots is also very large, such that roots from the same plant contain different levels (Rosling, 1996). For example, cassava roots from 20 cultivars from Solomon Islands, Fiji, and Papua New Guinea contained 2.1–652 mg HCN equivalent (eq.)  $\text{kg}^{-1}$  fresh weight (Bradbury and Holloway, 1988) while 28 cultivars from Fiji gave 14.0–121.0 mg HCN eq.  $\text{kg}^{-1}$  fresh weight (Aalbersberg and Limalevu, 1991). The large variation in cyanogenic glucosides of cassava roots is due to environmental factors such as rainfall, soil composition, time of harvest, and moisture levels in the soil (Bokanga *et al.*, 1994). The character and strength of that correlation has not been studied in roots from cultivars grown by communities nor compared to farmers classification

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of cultivars into bitter and non-bitter types (Rosling, 1996). It is reported from many cassava farming systems that cassava varieties are grouped into two categories designated as bitter or sweet (or cool). The classification is based on whether roots must be processed before consumption or not. In contrast to roots from bitter cultivars, those from non-bitter varieties are often consumed fresh or following direct boiling. Although both non-bitter and bitter cultivars are available in most cassava-growing areas, it has been observed that many cassava-growing communities prefer to grow cassava cultivars with bitter roots for their staple food production (Rosling *et al.*, 1993). This seems to be because the bitterness and toxicity of roots protect against theft and reduce animal spoilage (Rosling, 1996).

In Nkhata-Bay District in northern Malawi, farmers prefer bitter cultivars to produce roots for soaking, drying, and pounding into the flour used to make the dumpling-like porridge eaten as staple food. The farming population also cultivate smaller amounts of cool cultivars and the non-bitter roots from these cultivars are eaten fresh as a snack or boiled for breakfast. The farmers also state that they could predict toxicity by tasting the tip of peeled roots. The aim of this study was, therefore, to determine if the cyanogenic glucoside levels correlated with the taste of the roots as measured by a well-trained taste panel.

## Materials and Methods

### Study area and sampling of cassava roots

Nkhata-Bay district in northern Malawi lies along the shore of Lake Malawi at 474 m above sea level (asl). The population of the district is about 140 000 and mainly composed of small-scale farmers (Malawi Government, 1991). Cassava occupies 70% of the cultivated land.

In the present study conducted in September 1996, roots from the 10 most commonly grown cultivars were sampled (Table 1) in the fields of 29 farming households. The farmers' fields were consecutively selected from the list of farming households kept by the extension staff. Two plants of each cultivar which were ready for harvesting were identified by the farmer in every field and two roots were sampled from each plant. A total of four roots were obtained from each cultivar grown in every field visited. During the field work, two sets of four plants were excluded as the farmers' classification of the type of cultivar did not coincide with that of the agricultural specialists. In both instances, the mis-classification was done by a male head of a household without a woman.

Exactly 492 roots from 246 plants of the 10 cultivars were, thus, sampled over an 11-

**Table 1** Mean levels of cyanogenic glucosides and mean taste score for the 10 most commonly grown cassava cultivars in Chintcheche area of Nkhata-Bay District, Malawi

Type of cultivar	No. of roots	Mean glucoside level (mg HCN eq. kg <sup>-1</sup> fresh weight)		Mean taste score	
		±95% CI	Range	±95% CI	Range
<b>Cool cultivars</b>					
Mbundumali	48	25±5	(2-79)	1.5±0.1	(1.0-3.0)
Chimphuno	40	30±9	(1-123)	1.6±0.1	(1.0-2.6)
Nyachikunde	44	31±8	(6-114)	1.8±0.2	(1.0-3.5)
Total	132	29±4	(1-123)	1.6±0.1	(1.0-3.5)
<b>Bitter cultivars</b>					
Koloweki	52	114±14	(34-263)	3.1±0.2	(1.1-4.6)
Nyahrarawa	48	117±17	(35-279)	2.9±0.2	(1.7-4.4)
Deperwe	52	120±14	(39-283)	3.1±0.2	(1.2-4.9)
Ngwenyani	52	158±28	(58-559)	3.4±0.2	(2.0-4.9)
Nyankhata	52	160±24	(22-397)	3.4±0.2	(1.3-4.9)
Gomani	56	161±23	(38-419)	3.7±0.2	(1.7-4.9)
Nyamakozo	48	242±38	(86-661)	4.2±0.2	(2.8-5.8)
Total	360	153±10	(22-661)	3.4±0.1	(1.1-5.0)

<sup>1</sup>Taste score 1 (very cool) to 5 (very bitter)

day period, corresponding to a range of 20-28 plants and 40-56 roots from each cultivar. The roots were harvested between 700-1200 h each day, placed in marked paper bags, transported with care to the laboratory, and immediately peeled and washed in tap-water on arrival at 1300 h. Thereafter, each root was split longitudinally with a sharp knife and during the same afternoon, one half was immediately used for evaluation by the taste panel and the other processed for chemical analysis.

### Taste analysis

A 12-person taste panel selected through standard procedures (Watts *et al.*, 1989) was trained to grade the taste of pieces of the fresh cassava roots. A grading scale was developed based on farmers grading of the bitterness into five steps from very cool (1), cool (2), intermediate (3), bitter (4), and very bitter (5). Each half root was cut in pieces of 2-3 cm size and each member individually graded the taste of each and a taste score was calculated for each root as the mean taste score given by the 12-member panel.

### Chemical analysis

Linamarin (L-9131) and linamarase (39117-2R) were purchased from Sigma, St Louis, MO 63178, U.S.A. and BDH, Poole, BH15 1TD, U.K., respectively. Thin layer chromatography (TLC) sheets Polygram ionex 25 SB AC(806023) were bought from Macherey-Nagel, Duren, 5160, Germany. All other chemicals used in this study were of analytical quality

from Merck, Darmstadt, 64293, Germany. For the chemical analysis, each half root was cut with a sharp knife into 1-cm sized cubes; the cubes were mixed, and 49.5–50.5 g were weighed into 380-mL plastic cups and mixed with 160 mL 0.1M ortho-phosphoric acid. The mixture was homogenised for 75 s on a KVL-IPU Konstruktionsturick blender and thereafter placed in a refrigerator for 30 min. Aliquots (10  $\mu$ L and 100  $\mu$ L) of the supernatant were pipetted into separate tubes containing 1000  $\mu$ L pH 6 phosphate buffer placed in a plastic incubation block (Brimer, 1994). A total of 48 tubes with samples and 6 tubes for a standard curve (10–120 nmol of linamarin) were placed in the plastic incubation block. Thereafter, 100  $\mu$ L of linamarase (2.0 EU) was added to each tube and the block was tightly covered with a picrate-impregnated TLC sheet as earlier reported (Saka *et al.*, 1997). The blocks were left at ambient temperature for 20 h, the picrate sheet was removed, and the colour intensity of the spot that had developed on the picrate sheet above each tube was determined with a Nycocard<sup>®</sup> reflectometer (Brimer, 1994). The corresponding amount of formed HCN (in nmol) was calculated against the standard curve.

Moisture content of each root was determined by oven-drying weighed amounts of cubes from the root. The levels of glycosides in the fresh roots were calculated (in mg HCN eq.  $\text{kg}^{-1}$  fresh weight) as described by O'Brien *et al.* (1991) which includes sample moisture. The calculation for the improved solid state method (Saka *et al.*, 1997) is:

Cyanogenic glucosides (mg HCN eq.  $\text{kg}^{-1}$  fresh weight) =  $(27.02 \times N \times V') / (S \times W)$   
 where: N = nmol of HCN as calculated from the calibration curve;

S = volume of sample aliquot in  $\mu$ L;

W = weight of fresh sample in g;

V' (extraction volume adjusted to include the sample moisture) =  $V + (M \times W) / 100$ ;

V = 160  $\text{cm}^3$  0.132M  $\text{H}_3\text{PO}_4$ .

M = moisture in per cent and W as defined as above.

The factor 27.02 is the molecular mass (in g) of HCN. Whenever the reading of the 100- $\mu$ L sample was below that of 120  $\mu$ mol on the standard curve, it was used for the calculation, otherwise the reading for the 10  $\mu$ L aliquot was used.

To establish the reproducibility and reliability of the chemical analysis and taste data, control experiments were carried out. During each day, one root was randomly selected and divided into four equal longitudinal portions to obtain double determinations for both mean taste score and the cyanogenic glucosides. The 11 double estimations of taste yielded a correlation coefficient

(*r*) of 0.95 and those of chemical determination gave 0.99.

The data were subjected to statistical analysis as described elsewhere (Graantz, 1987).

## Results

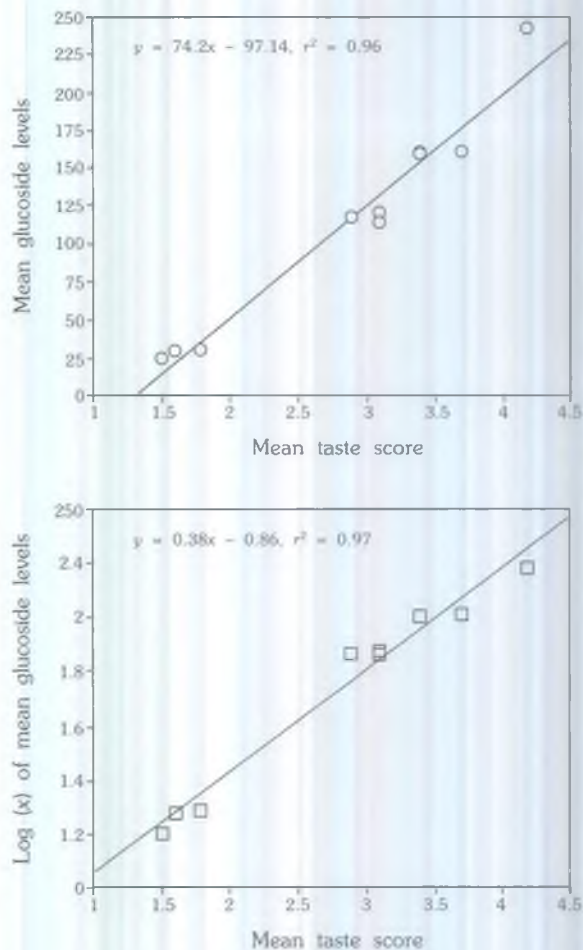
The levels of cyanogenic glucosides in the 492 roots which ranged from 1 to 661 mg HCN eq.  $\text{kg}^{-1}$  fresh weight and the range of taste scores are also provided in Table 1.

The mean levels of cyanogenic glucosides and mean taste scores for the roots from each of the 10 cultivars were also calculated and are presented in Table 1. The mean glucoside and mean taste scores were not significantly different in all three cool cultivars ( $P < 0.05$ ). Both variables for all the seven bitter cultivars were more than three- and two-fold higher. The mean moisture in the bitter roots,  $57 \pm 4\%$ , was similar to the cool roots,  $56 \pm 3\%$ .

The taste score for each root was positively correlated ( $r = 0.77$ ) with the glucoside levels and the correlation ( $r = 0.87$ ) between taste score and the logarithm of glucoside levels was even stronger (data not shown). The use of mean taste score and average glucoside level for each of the 10 cultivars provided a much higher and significant correlation ( $r = 0.98$ ) (Figure 1). The correlation between taste and glucoside level was highly significant ( $P < 0.001$ ).

## Discussion

The present work confirms earlier findings of a positive correlation between the bitter taste and glucoside levels in cassava roots (Sunderesan *et al.*, 1987). Several studies elsewhere indicate that some correlation exists between bitterness and cyanide potential (Sinha and Nair, 1968). The bitterness is due to bitter substances, largely cyanogenic glucosides (King and Bradbury, 1995). Although taste grading by a taste panel is arbitrary when expressed on a linear scale, the double determination of both glucoside levels and taste score gave *r* values of 0.99 and 0.95, respectively. Thus, the methods used in this study seem reliable and quantitative. Further studies directed to delineate the true nature of the relation are underway. All the roots that were graded as very cool by all members of the panel had less than 20 mg HCN eq.  $\text{kg}^{-1}$ , and of those roots graded as very bitter by everyone, none had a glucoside level below 200 mg HCN eq.  $\text{kg}^{-1}$  (Figure 1). Of the roots that had a taste score of 2.0 or less (thus cool to very cool) only one root had a glucoside level slightly higher than 100 mg HCN eq.  $\text{kg}^{-1}$ . In contrast, among the roots with a taste score of 4.0 or more (i.e., bitter to very bitter) only one had a glucoside value slightly below that limit. This shows that farm-



**Figure 1** Correlation between mean taste scores and average glucoside levels of the 10 cassava cultivars

ers may predict toxicity from the taste of the roots. A recent study by King and Bradbury (1995) has shown that the bitter taste of linamarin accounts for most of the bitterness of the parenchyma of cassava roots. The present results seem to support strongly that the bitter taste of cassava root is caused predominantly by the level of glucoside. Development of cassava roots without citric and maleic acids would probably enhance the degree of the correlation between taste and bitterness due to cyanogenic glucosides. Citrates and malates are taste modifiers and mask the bitterness due to cyanogenic glucosides (King and Bradbury, 1995). These two chemicals were not measured in this study but may have influenced the quantitative relationship between taste and the mean levels of glucosides among the 10 cultivars studied. However, the mean glucoside levels were similar in the three cool cultivars but were more than three times higher in the bitter cultivars. This seems to show that bitter and cool cultivars have two distinct ranges of cyanogenic glucosides (Figure 1) which are consistent with the results of the authors' earlier interview with farmers.

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# Contents of $\beta$ -carotene and $\alpha$ -tocopherol of sweetpotato cultivars newly developed for processing purposes

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Contents of  $\beta$ -carotene and  $\alpha$ -tocopherol in storage roots of sweetpotato cultivars newly developed for processing purposes were examined. These two compounds were simultaneously determined by high performance liquid chromatography (HPLC) using an ODS column, an isocratic methanol mobile phase, and both a UV-visible and a spectrofluorometric detector. Under normal cultivation conditions without mulching, Kyushu 122 had the highest content of  $\beta$ -carotene (147 mg kg<sup>-1</sup> on a fresh weight basis) among the orange-fleshed cultivars tested, and Tanegashimamurasaki had the highest content of  $\alpha$ -tocopherol (16.5 mg kg<sup>-1</sup> on a fresh weight basis) among the purple-fleshed cultivars. The  $\alpha$ -tocopherol content of the purple-fleshed cultivars on a fresh weight basis was higher than that of the orange-fleshed ones, while Koganesengan, a yellow-fleshed cultivar, was the richest in  $\alpha$ -tocopherol content of all the cultivars tested. A similar trend was observed in cultivars grown under vinyl mulching and heavy fertilizer dressing.

Keywords: Sweetpotato;  $\beta$ -carotene;  $\alpha$ -tocopherol; HPLC

New cultivars of sweetpotato [*Ipomoea batatas* (L.)] with orange or purple flesh were recently developed for processing into powder, juice, and pigments (Yoshinaga and Yamakawa, 1996). Several sweetpotato cultivars with orange flesh were reported to contain  $\beta$ -carotene as their main pigment (Bushway, 1986; Takahata *et al.*, 1993), and cultivars with purple flesh were shown to have anthocyanins (Zulin *et al.*, 1992).

An epidemiological study on dietary intake and human health indicates that foods containing carotenes and tocopherols may provide protection against several kinds of diseases (Kohlmeier and Hastings, 1995). It was also reported that consumption of a diet containing antioxidants such as  $\beta$ -carotene, tocopherols, and ascorbic acid may reduce the plasma level of lipid peroxide (Singh *et al.*, 1995). However, previous reports on foods containing these compounds have been focussed mainly on vegetables, fruits, legumes, and plant oils. Additionally, a report on tocopherols of several foods described tocopherol content of sweetpotato roots but did not mention the names of the cultivars (Ichikawa and Tomioka, 1984). Therefore, the contents of  $\beta$ -carotene and  $\alpha$ -tocopherol of roots of newly developed sweetpotato cultivars, especially those with orange and purple flesh, were investigated using high performance liquid chromatography (HPLC).

## Materials and Methods

### Reagents

Alpha- and  $\beta$ -carotenes were purchased from Sigma (St Louis, MO). Alpha-,  $\beta$ -,  $\gamma$ -,  $\delta$ -tocopherols, and other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). All solvents used were HPLC grade.

### Sample preparation

Sweetpotato cultivars [Kyukei 174, Kyukei 184, Kyushu 119, Ayamurasaki, and Tanegashimamurasaki (purple flesh), Kyushu 114, J-Red, Kyushu 122, and Benihayato (orange flesh), and Koganesengan (yellow flesh)] were grown in a field of the Kyushu National Agricultural Experiment Station (131°1' E, 31°45' N) at Miyakonojo, Miyazaki, Japan, in 1996. Under normal cultivation conditions without mulching, the materials were transplanted on 12 May and harvested on 3 October. Compound fertilizer at 600 kg ha<sup>-1</sup> containing 6% N, 8% P, and 12% K was applied before transplanting, and each cultivar was grown in two plots. In another field with vinyl mulching and heavy fertilizer dressing, the materials were transplanted on 20 April and harvested on 31 October. The same compound fertilizer at 1200 kg ha<sup>-1</sup> was applied before transplanting, and each cultivar was grown in three plots. One day af-

ter harvesting, six roots of each cultivar from each plot were washed and cut into sticks. A part of the cut materials was oven-dried to determine dry matter content and the remaining part was lyophilized. The lyophilized materials were ground by a mill and the resulting powder was stored at  $-20^{\circ}\text{C}$  until analysis.

### The HPLC system

The HPLC system consisted of a Model LC-10AT pump, a Model SIL-10AXL autoinjector, a Model CTO-10AC column oven, a Model SPD-M10AVP photodiode array UV-VIS, and a Model RF-10A spectrofluorometric detector (Shimadzu, Kyoto, Japan). The latter detector was connected to the outlet of the former. The system was controlled by a CLASS-LC10 workstation (Shimadzu). The column was a YMC-Pack ODS-A A-302 (150 mm  $\times$  4.6 mm i.d., 5- $\mu\text{m}$  particles; YMC, Kyoto, Japan). The temperature of the column oven was set at  $40^{\circ}\text{C}$ . The mobile phase was methanol and the flow rate was  $2\text{ mL min}^{-1}$ . Beta-carotene was detected by the photodiode array detector and determined at 460 nm. Alpha-tocopherol was measured by the spectrofluorometric detector set at 295 nm excitation and 325 nm emission.

### Procedure

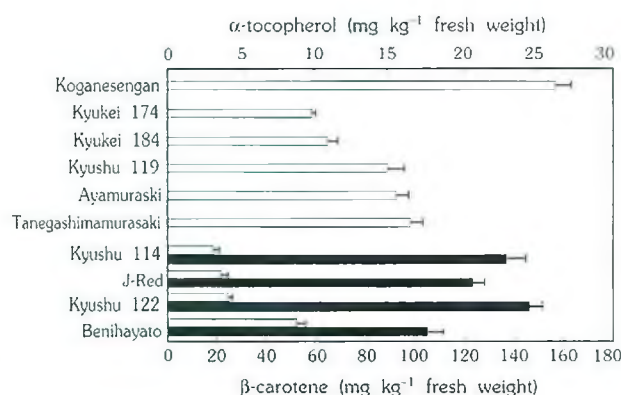
One millilitre of ethanol containing 0.1% butylated hydroxytoluene (BHT) was added to 50 mg of sweetpotato root powder in a centrifuge tube and mixed by a vortex mixer. To this mixture, 3 mL of *n*-hexane containing 0.05% BHT was added followed by further mixing. After addition of 1 mL of deionized water and mixing, the mixture was centrifuged at 2000 rpm for 5 min. A 2-mL aliquot of the upper layer of the supernatant was taken and 3 mL of *n*-hexane containing 0.05% BHT was added to the rest. After reextracting, 2 mL of the upper layer was taken and the combined extract was evaporated under a stream of nitrogen gas. The residue was redissolved in 1 mL of tetrahydrofuran containing 0.05% BHT and filtered through a membrane filter (DISMIC-13HP, pore size: 0.2  $\mu\text{m}$ ; ADVANTEC, Tokyo, Japan). A 10- $\mu\text{L}$  portion of the filtrate was injected into the chromatograph and eluted as described above.

### Results and Discussion

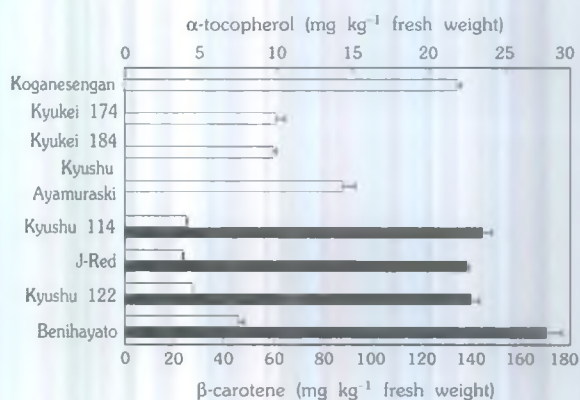
It was reported that retinol,  $\alpha$ -tocopherol, lycopene, and  $\alpha$ - and  $\beta$ -carotenes in plasma can be determined simultaneously by an HPLC method using an ODS column and an isocratic methanol mobile phase (Milne and Botnen, 1986). This method has the advantage in that it can simultaneously determine several compounds in a single run, and it is convenient because no complex solvent systems are used. Therefore, in this study, the determination of

$\beta$ -carotene and  $\alpha$ -tocopherol contents of sweetpotato roots was carried out by use of an ODS column and of an isocratic methanol mobile phase. In the HPLC system equipped with only a UV-visible detector, standards of  $\alpha$ - and  $\beta$ -carotene were separated ( $t_{\text{R}} = 14.0$  and 14.9 min, respectively), but on chromatograms for sweetpotato samples, many peaks were observed in the region where tocopherols were expected to be eluted (data not shown). Under the HPLC conditions used, standards of  $\alpha$ - and  $\delta$ -tocopherols were eluted separately at 3.5 and 2.7 min, respectively, while  $\beta$ - and  $\gamma$ -tocopherols had the same retention time of 3.1 min (data not shown). Because the use of a spectrofluorometric detector enabled specific detection of tocopherols, both a UV-visible detector and a spectrofluorometric one were used for measurement of  $\beta$ -carotene and  $\alpha$ -tocopherol, respectively.

Contents of  $\beta$ -carotene and  $\alpha$ -tocopherol of roots grown under non-mulching conditions are shown in Figure 1. Kyukei 174, Kyukei 184, Kyushu 119, Ayamurasaki, and Tanegashimamurasaki are cultivars with purple flesh colour. Kyushu 114, J-Red, Kyushu 122, and Benihayato have orange flesh. Koganesengan has yellow flesh, and is one of the popular cultivars in the southern area of Kyushu in Japan. Among the cultivars tested, Kyushu 122 was found to have the highest content of  $\beta$ -carotene ( $147\text{ mg kg}^{-1}$  on a fresh weight basis). Alpha-carotene was not detected in all of the cultivars. Under the HPLC conditions used here,  $\beta$ - and  $\gamma$ -tocopherols were not separated. However, for all samples analysed, the peak area of  $\beta$ - (or  $\gamma$ ) tocopherol was less than one-tenth that of  $\alpha$ -tocopherol, while that of  $\delta$ -tocopherol was not determinable. Among the purple-fleshed cultivars tested, Tanegashimamurasaki had the highest content of  $\alpha$ -tocopherol ( $16.5\text{ mg kg}^{-1}$  on a fresh weight basis). Among the orange-fleshed cultivars, Benihayato was shown to have the highest content of  $\alpha$ -tocopherol



**Figure 1** Contents of  $\beta$ -carotene and  $\alpha$ -tocopherol of roots of sweetpotato cultivars grown under conditions without mulch in 1996. Bars represent mean  $\pm$  SD of data from three plots;  $\square$ ,  $\alpha$ -tocopherol,  $\blacksquare$ ,  $\beta$ -carotene



**Figure 2** Contents of  $\beta$ -carotene and  $\alpha$ -tocopherol of roots of sweetpotato cultivars grown under conditions with vinyl mulching and heavy fertilizer dressing in 1996

Bars represent mean  $\pm$  SD of data from two plots;  $\square$ ,  $\alpha$ -tocopherol,  $\blacksquare$ ,  $\beta$ -carotene

( $8.7 \text{ mg kg}^{-1}$  on a fresh weight basis). It was found that the purple-fleshed cultivars tested had no detectable content of  $\beta$ -carotene but were richer in  $\alpha$ -tocopherol than the orange-fleshed ones. It was also shown that Koganesengan having no detectable content of  $\beta$ -carotene had a higher level of  $\alpha$ -tocopherol content ( $26.3 \text{ mg kg}^{-1}$  on a fresh weight basis) than the purple-fleshed cultivars. The contents of  $\beta$ -carotene and  $\alpha$ -tocopherol of sweetpotato roots grown under conditions with mulching and heavy fertilizer dressing are shown in Figure 2. Under these conditions, it was also found that the purple-fleshed cultivars had no detectable content of  $\beta$ -carotene, but were richer in  $\alpha$ -tocopherol than the orange-fleshed ones. These data suggest that purple-fleshed sweetpotato cultivars may have possible biological activity such as

antioxidation due to  $\alpha$ -tocopherol rather than to  $\beta$ -carotene. The HPLC method using two types of detectors, UV-visible and spectrofluorometric, enabled the simultaneous determination of  $\alpha$ -tocopherol and  $\beta$ -carotene in sweetpotato roots, and is, therefore, advantageous in reducing analysis time.

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# Biochemical comparison in storage: Stress response between sweetpotato and cassava

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During storage after harvest, both sweetpotato (SP) and cassava (CV) showed different stress responses. When wounded, SP cured injury by forming a lignin layer at the cells very close to the surface, while CV suffered from physiological deterioration (PD), followed by microbial deterioration (MD). In wounded SP tissue, many enzymes such as phenylalanine ammonia-lyase (PAL), acid invertase, peroxidase (PERO), and polyphenol oxidase (PPO) were induced. Polyphenols such as chlorogenic acid (CA) and isoCA were produced and respiratory rates of tissue and mitochondria (Mt) were increased, including CN-insensitive respiration. When penetrated by some fungi such as *Ceratocystis fimbriata* or by larvae of some weevils such as *Cylas formicarius*, these changes appeared more vigorously, and additional ones occurred. There were productions of coumarins (umbelliferone), about 30 sesquiterpenes (ipomeamarone), ethylene, and cell death and discoloration by the penetration. Storage proteins were degraded and converted to other proteins. In wounded CV tissue, various changes involving PD were caused principally in the same way as in infected SP tissue. Both roots suffered from chilling injury, CV being more sensitive in the cold than SP.

Keywords: Sweetpotato; Cassava; Storage; Wounding; Infection; Physiological deterioration; Microbial deterioration; Phytoalexin

The roots of both sweetpotato (SP) and cassava (CV) may easily be wounded during harvest and transportation. Cassava roots in particular are normally wounded when harvested, and so-called vascular streaking, or vascular discoloration, occurs in the roots. The cause of the deterioration and the protection against it were investigated by many researchers (Rickard, 1985; Plumbley and Rickard, 1991; Beeching *et al.*, 1994). The deterioration is called primary deterioration (PD). Some days after the occurrence of PD, microbial infection occurs, and the tissue is damaged. This is termed MD or secondary deterioration. The cause of PD in CV, and the process at the biochemical level were previously investigated (Uritani and Reyes, 1984; Uritani *et al.*, 1994). The biochemical changes of SP in response to wounding and fungal infection were also investigated (Uritani, 1978, 1985). This paper discusses the differences and similarities between SP and CV in their biochemical reactions caused in response to wounding, and fungal or insect invasion.

## Materials and Methods

Sweetpotato cv. Norin No. 1 and cv. Kokei No. 14 roots were stored at 10–12°C until used.

## Tissue wounding

Sweetpotato was cut perpendicularly at a thickness of 3–20 mm and incubated at 25–30°C and 85–99% relative humidity (RH) for a few days.

## Tissue infection

Wounded tissue was inoculated with a spore suspension of *Ceratocystis fimbriata* (Ellis et Halsted) Elliott (a typical pathogenic fungus) and incubated as described above. Cassava cv. Golden Yellow and cv. Hawaiian 5 just after harvest were cut in the thickness of 10–50 mm and incubated at 24–26°C to 30–31°C and 75–78% to 90–92% RH for some days.

The experimental methods in biochemistry were used according to Uritani (1985), Uritani and Reyes, (1984), and Uritani *et al.* (1994).

## Results

### Cytological changes in wounded and infected SP and CV

In wounded SP tissue, the 2–3 cell layers adjacent to the cut surface began to be lignified during 2–3 days of incubation. Formation of lignin layer progressed during incubation for a

longer time. Some days after lignin layer formation, suberin-containing cells appeared. Infection occurred sometimes on wounded tissue. When SP tissue was attacked by *C. fimbriata*, the mycelia penetrated into the inner tissue to a depth of 2–3 mm during 3–5 days, followed by a brownish discoloration in the infected region, then the infection was inhibited because of the production of the phytoalexins. Lignin and suberin layers were then formed.

In wounded CV tissue, PD and vascular discoloration occurred only in the outer part of secondary xylem, called B-part (Uritani and Reyes, 1984), at first in xylem parenchymatous cells, then in xylem vessels. As described later, a bluish fluorescence appeared and some enzyme activities occurred preceding vascular discoloration not only in the B-part, but also in the A-part, i.e., the part between cortex and B-part, and in the C-part, i.e., the part inward from B-part. Primary deterioration appeared on the surface and in the inward tissue of B-part, after about three days of incubation. Under the ambient conditions described above, lignin was not or hardly formed in the wounded surface of the CV tissue, thus differing from the case of SP. Lignin was found in PD-suffering xylem only as one of the constituents. After PD occurrence, CV tissue suffered from MD by some pathogenic fungi such as *Botryodiplodia theobromae* Pat., since any physical barriers such as lignin layer and periderm were not formed.

## Biochemical changes in wounded and infected SP and CV

### Changes in enzyme activities

In response to wounding of SP, many enzymes were induced. The enzymes first activated belonged to those in the phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL) and

*trans*-cinnamic acid 4-hydroxylase. Phenylalanine ammonia-lyase activity reached the maximum within 24 h after wounding, then gradually decreased. Phenylalanine ammonia-lyase activity was enhanced a little more in infected tissue, than in the wounded tissue. Acid invertase was also activated very soon after wounding.

In wounded CV tissue, PAL activity was enhanced in a similar way. The time taken to reach the maximum was later in CV than SP and the maximum activity was about the same as reported by Tanaka *et al.* (1983) and Uritani and Reyes (1984) (Figure 1). Also, acid invertase activity was induced in wounded CV tissue.

Peroxidase activity in wounded CV tissue increased continuously from 30 to 60 h with a 24 h lag, like PERO and polyphenol oxidase (PPO) in wounded SP tissue, but was 0.4–0.7 times smaller than in SP tissue at the 24 h incubation period. Polyphenol oxidase activity was present in fresh tissue and increased during incubation, but weak when  $\pm$  catechin, the CV main polyphenol, was used as substrate.

The activities of the three enzymes, PAL, PERO, and PPO increased both before and after the occurrence of discoloration in all of the A, B, and C parts of CV tissues wounded naturally and artificially, without a clear difference among the parts.

### Changes in respiration

Respiratory rates in both wound SP tissue and in its Mt were activated, and reached the maximum after about 20 h, which was maintained for a while. Some respiratory enzyme activities in Mt increased as did the number of Mt per cell. In accordance with the increase in the pentose phosphate shunt (PPS) in wounded tissue, glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities increased, and reached the maximum around 12 h after wounding, and the levels were sustained for 96 h or more. In

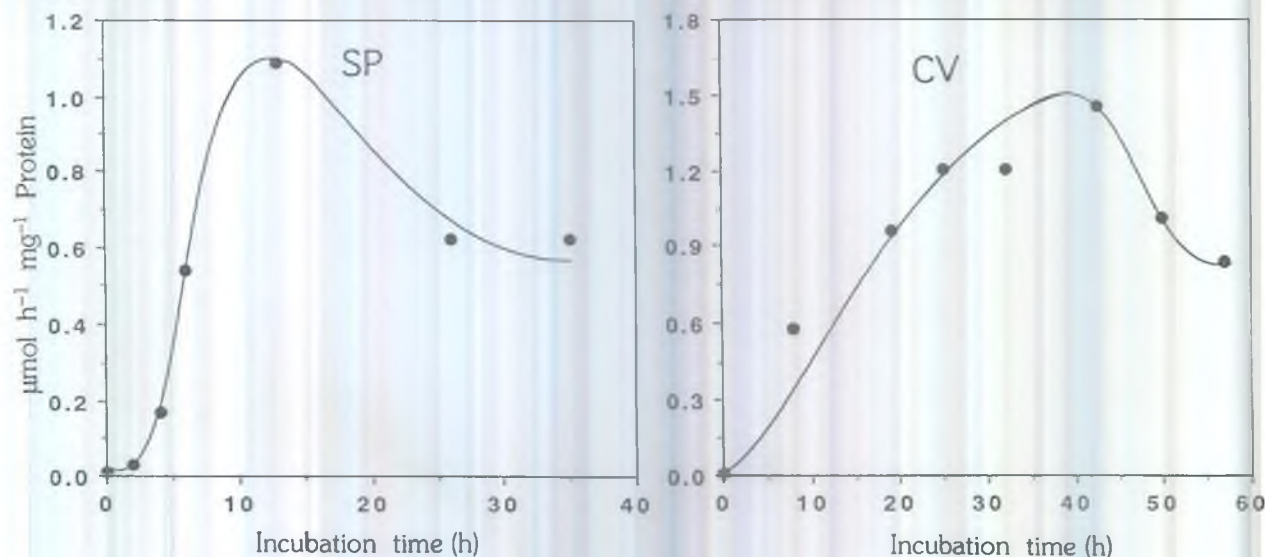


Figure 1 Changes in PAL activities in wounded tissue (2 mm thick) of sweetpotato (SP, left) and cassava (CV, right) incubated at 30°C and over 90% RH

infected tissue, respiratory rate, CN-insensitive respiration, and PPS were much higher than in wounded tissue. The respiratory rate was biphasic; it increased one day after incubation twofold higher than in fresh tissue, as seen in the case of wounded tissue, then increased again up to about twofold higher than in wounded tissue.

The respiratory rate in CV tissue was activated in response to wounding during one day of incubation, and increased again 3–4 days after incubation. The first and second increases may be based on wounding and PD development, respectively (Uritani and Reyes, 1984).

### Polyphenol production

In response to wounding of SP, polyphenols were produced succeeding the rise in activities of some enzymes of the phenylpropanoid pathway such as PAL and 4-hydroxylase. The compounds were CA, isoCA, pseudoCA, and caffeic acid. The production reached the maximum after 2–3 days, and decreased very slowly, and the amounts were in the range of 14–25  $\mu\text{mol g}^{-1}$  fresh wt (500–886  $\text{mg } 100 \text{ g}^{-1}$ ). These were produced much more when infected by *C. fimbriata*.

Polyphenols were not present in fresh CV tissue just after harvest, but were produced during the four days of incubation, but not so much, in parallel with the increase in PD. These were  $\pm$  catechin and (+) gallocatechin. Rickard (1985) indicated the occurrence of leucoanthocyanidins in addition to the above two. When tissue blocks of the thickness of 15 mm were incubated at 26–30°C for some days, the amounts of the compounds were 48–59  $\text{nmol g}^{-1}$  fresh wt (1.4–1.7  $\text{mg } 100 \text{ g}^{-1}$ ). No special difference in the amount of the compounds was found among A, B, and C parts in wounded tissue during four days of incubation. In the tissue adjacent to the soft rot part of MD-suffered CV, the amount was about 460  $\text{nmol g}^{-1}$  fresh wt (13.4  $\text{mg } 100 \text{ g}^{-1}$ ).

### Coumarin production

Coumarin compounds were neither present in fresh SP tissue, nor produced in wounded tissue. However, in response to penetration of some pathogenic fungi such as *C. fimbriata*, coumarins (showing bluish fluorescence) were produced in the tissue very closely adjacent to the infected region, at an earlier stage than the production of polyphenols and terpenoids (shown later). The main fluorescent compounds were identified as umbelliferone (7-hydroxycoumarin) and scopoletin (7-hydroxy-6-methoxycoumarin). Other chemicals produced were esculetin (6,7-dihydroxycoumarin) and two glucosides, namely, skimmin (umbelliferone-7- $\beta$ -glucoside) and scopolin (scopoletin-7- $\beta$ -glucoside). It is interesting that the coumarins were generally produced by some continuous injury such as fungal penetra-

tion by *C. fimbriata*, insect damage by larvae of *C. formicarius*, and treatment with toxic chemicals such as mercuric chloride.

The coumarins were produced in the SP tissue to a much lesser extent than the polyphenols. According to the time course analysis, the amounts of umbelliferone were 83 and 135  $\text{nmol g}^{-1}$  fresh wt (1.35 and 2.19  $\text{mg } 100 \text{ g}^{-1}$ ), after one and two days of incubations, respectively; and those of scopoletin were 6.6 and 20.0  $\text{nmol g}^{-1}$  fresh wt (0.13 and 0.38  $\text{mg } 100 \text{ g}^{-1}$ ), respectively.

The coumarins showing bluish fluorescence were not present in fresh CV roots, but produced in A, B, and C parts in the roots soon after harvest, in response to wounding, earlier than vascular discoloration. This was different from the case of wounded SP tissue, in which these were not produced. There was a tendency for the coumarins to be produced more in B part than in A and C parts. Even when CV was subjected to MD, the increase was not so strong as compared with the case of PD. The compounds were identified to be scopoletin, scopolin, and esculin (esculetin-6- $\beta$ -glucoside). Researchers at CIAT and TPI detected the production of scopoletin independently (Wheatley, 1982; Rickard, 1985).

Scopoletin was produced soon after wounding, reaching a maximum [17.0  $\text{nmol g}^{-1}$  fresh wt (327  $\mu\text{g } 100 \text{ g}^{-1}$ )], after about 18–24 h of incubation, then decreased, but again increased, possibly because of PD appearance. Both scopolin and esculin were produced with about 10 h lag, succeeding scopoletin production, and reaching the maximum [37.1 and 42.0  $\text{nmol g}^{-1}$  fresh wt (713 and 748  $\mu\text{g } 100 \text{ g}^{-1}$ , respectively)] after about 40–48 h incubation, then decreased. In the tissue adjacent to MD-discoloured tissue, the content of scopoletin was 1.0  $\text{mg } 100 \text{ g}^{-1}$ . The amounts of the coumarins in wounded (in relation with PD) and MD-suffered CV tissues were less than that in infected SP tissue.

### Terpenoid production

The terpenoid compounds were either absent in fresh SP, or hardly produced in wounded tissue, except for some phytosterols. However, when infected by some pathogenic fungi such as *C. fimbriata*, many kinds of sesquiterpenoids were produced in the infected region and in tissue closely adjacent to the infected region, succeeding the induction of some enzymes pertaining to the pathway such as 3-hydroxy-3-methylglutaryl CoA reductase. The main component is ipomeamarone (Ip), which was first isolated by Hiura (Uritani, 1978), and was recognized as the first example of phytoalexins as proposed by Mueller (Uritani, 1978), since it showed some inhibitory action. At the present time, about 30 sesquiterpenoids have been isolated from infected SP including the derivatives such as  $\beta$ -selinene and butenolide types, by

Uritani (1978, 1985) and Woolfe (1992). The compounds in the infected region were initially produced in small amounts, then increased rapidly, reaching the maximum whose amount was several per cent, after 3–5 days. The infection was thus prevented. The time course of the production was similar to that of polyphenols, but dissimilar to the coumarins. The compounds were produced only by continuous injury such as fungal penetration, insect (as *C. formicarius*) damage, and some toxic chemicals treatment, as for the coumarins.

When CV tissue suffered from either PD or MD (by *B. theobromae*), many terpenoids were produced in the discoloured tissue. More than 22 diterpenoids and 4 steroids were isolated from the oily material (about 7.25 g 5 kg<sup>-1</sup> fresh tissue) extracted from the discoloured region of PD-, or MD-suffering CV by methanol and ethyl acetate. However, quantitatively, the production was much lower in discoloured CV tissue than in infected SP tissue. The four steroids seemed to be produced through the oxidation of phytosterols. The identified 22 diterpenoids were composed of the *ent*-pimarane family (9 components), *ent*-beyerane family (10 components), *ent*-antisane family (2 components), and *ent*-kaurane family (1 component). Fifteen compounds among these were proven to be new structures, and one main component belonging to the *ent*-beyerane one showed inhibitory action on spore germination of *B. theobromae* (Sakai and Nakagawa, 1988; Uritani *et al.*, 1994). Although the inhibitory activity of each of the components was not tested, the diterpenoids were regarded as phytoalexins, since similar diterpenoids from diseased rice leaves were indicated to be phytoalexins. The above compounds were produced succeeding the coumarin production. The total amounts of steroids were assumed to be almost not changing during incubation. The amounts obtained for diterpenoids were 0, 14, 163, and 283 µg g<sup>-1</sup> fresh wt, in discoloured region of PD-suffering tissue blocks (15 mm thick) incubated for 0, 2, 3, and 7 days, respectively.

### Ethylene formation

In wounded SP tissue discs (1 mm thick), ethylene amounts formed for 1 h at the first and second days after incubation, were 0.020 and 0.012 nmol g<sup>-1</sup> fresh wt h<sup>-1</sup>, respectively. On the other hand, in *C. fimbriata* infected tissue blocks (10–15 mm thick), ethylene formed in the amounts of 5.2 and 68.5 nmol g<sup>-1</sup> h<sup>-1</sup> at the first and second days, respectively. Those data indicated that trivial amounts were produced in wounded tissue, but tremendous amounts in infected tissue. Ethylene was formed in the tissue part very closely adjacent to infected region through induction of the participating enzymes, but not by the fungus, which produced ethylene in a negligible amount.

For CV roots, the formation was 0.134 nmol g<sup>-1</sup> fresh wt h<sup>-1</sup> after one day of incubation (10–15 mm blocks). Sweetpotato tissue blocks incubated in the same way formed 0.008 nmol g<sup>-1</sup> fresh wt h<sup>-1</sup>. Thus, ethylene was formed more highly in wounded CV tissue than in wounded SP tissue. When CV roots were stored for three days, and cut into the blocks, some of them showed severe PD, but others showed only mild changes. Ethylene was formed (by the two kinds of blocks) in the amounts of 0.12 and 0.036 nmol g<sup>-1</sup> fresh wt h<sup>-1</sup>, respectively. Hence, ethylene production was higher in the tissue which suffered from severe PD than in the tissue showing only the mild grade of PD (Uritani and Reyes, 1984). Further, ethylene production was higher in wounded CV tissue than in wounded SP. However, infected SP tissue showed the highest production.

### Changes in proteins

In response to either wounding or infection, many kinds of metabolism were activated, as indicated in the former sections, in both SP and CV. This suggested that the patterns of xymograms of proteins could change in response to wounding and infection in both roots. From the xymogram of the soluble proteins, it was proposed that the main protein showing strong antigenicity, present in healthy SP tissue, was degraded and converted to other proteins in response to wounding and infection (Uritani and Stahmann, 1961). The protein was called component A, composed of A1 and A2. It was assumed that the protein may be a kind of storage protein even if it would have some kinds of enzymatic activity. The assumption was proven by Li and Oba (1985) and Maeshima *et al.* (1985). The protein of 25 kDa molecular weight, accounted for 60 to 80% of soluble proteins. The components may correspond to A and B, respectively. They are each composed of 199 amino acids as the whole protein. Hattori *et al.* (1985) and Nakamura *et al.* (1993) isolated the cDNA, elucidated the DNA sequences, and the mechanism of the transcription and translation, forming preprosporamin, next prosporamin, then sporamin in the vacuole.

The zymogram on the polyacrylamide gel electrophoresis using the soluble proteins in CV roots which had been denatured by heating in the presence of SDS and 2-mercaptoethanol was also investigated. This showed that the main proteins were composed of four components, whose MWs were about 23, 30, 37, and 53 KDa. It was assumed that the proteins might be the storage proteins, since the densitograms of the soluble protein samples in undamaged parts adjacent to the PD- and MD-discoloured regions were different from that of fresh CV tissue (Uritani *et al.*, 1992). Shewry *et al.* (1992) also showed the similar zymogram

of the soluble protein from healthy CV tissue, and purified some of the components. According to this study, the absence of any major components raised the question whether or not the storage proteins were present. It is emphasized that, if present, these must be insoluble in water, since the soluble proteins will be lost during traditional processing to remove cyanogenic glucosides.

### Chilling injury

Both SP and CV roots were injured by chilling. The range of temperature for storage of SP roots under adequate condition is 9–12°C. When CV roots were stored in the same range, they were injured and suffered from infection by non-pathogenic fungi. This was supported by the experimental data showing Arrhenius Plots of succinoxidase and cytochrome c oxidase activities of Mt from SP and CV roots. The transition point of the temperature for activation energy was lower in SP-Mt than in CV-Mt (Maeshima *et al.*, 1980).

### Discussion

A remarkable difference in cytology between CV and SP was that vascular streaking or vascular discoloration after harvest occurred in CV, but not in SP. Further, lignin-, then suberin-containing cells were formed in SP tissue following wounding and after prevention of fungal penetration, but such changes hardly occurred in CV tissue, except for lignin formation at PD-discoloured tissue part, B-part. The reason for such differences between SP and CV must be further investigated. When CV tissue was wounded, the changes in physiology and biochemistry were partly similar to those of wounded SP tissue, as seen in respiratory increase, enhancement of some enzymes, and polyphenol production. When CV tissue was either wounded or infected, coumarins, terpenoids, and ethylene were produced and a biphasic increase in respiration occurred, just as infected SP tissue, although the levels of the changes were less than in infected SP tissue. It is important to elucidate the mechanism of the occurrence of those changes in wounded CV tissue, since such changes are generally caused only in response to continuous injury as fungal infection in SP and other plants. Such knowledge will help illuminate the reason for the occurrence of vascular streaking in CV after harvest.

Those reactions in wounded CV tissue may be related to exposure to active oxygen at the wounded site while stored on the earth after harvest, accompanied by transmission of some signaling factors such as salicylic acid (SA) and jasmonic acid (JA), production of which might be regulated by cytokinin (CK) in the roots of CV, as suggested by Ohashi, whose group

showed regulation of levels of SA and JA in wounded tobacco plants by CK (Sano *et al.*, 1996). The above factors may transmit the signal for the metabolic activation through the apoplast and secondary phloem. Further, the loss of water mainly through the secondary xylem may contribute also to induction of the above changes. Time-course assay of such signaling factors must be investigated in wounded and infected CV tissues, under the comparison with wounded and infected SP tissues.

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# Relationship between tuberization and the appearance of a neutral invertase activity in *Pachyrhizus erosus* (L.) Urban

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Changes in soluble carbohydrates and PAGE pattern of tap root proteins were monitored in yam bean [*Pachyrhizus erosus* (L.) Urban] tap root under inductive (short days) and non-inductive (long days) environments. Up to five weeks after sowing (WAS), tap roots contained essentially sucrose. Under the short-day environment, a neutral invertase (NI) activity appeared from 6 WAS, concomitantly with glucose and fructose accumulation. Under the long-day environment, soluble carbohydrates as well as the pattern of proteins remained unchanged throughout root development. From these data it is concluded that NI controls the accumulation of hexoses in tap roots and is regulated by short days.

Keywords: Carbohydrates; Invertases; Legume; *Pachyrhizus erosus*; Proteins; Tuberization

Yam bean [*Pachyrhizus erosus* (L.) Urban] is a tropical legume which produces a tuber under photoperiodic conditions (Zinsou, 1992). The actively growing yam bean tuber utilizes large amounts of sucrose imported from source leaves for growth and accumulation as glucose, fructose, and starch (Vaillant and Desfontaines, 1995). Conversion of sucrose to hexoses often is the primary starting point for sink metabolism and may be one of the important factors in the control of the synthesis and accumulation of reserve materials in tuberous roots. The first step in the breakdown of sucrose in plant tissues is the cleavage of the glycosidic bond by either invertase (EC 3.2.1.26) or Suc synthase (EC 2.4.1.13).

In attempting to identify biochemical indicators of tuberization in yam bean, the influence of long days (LD) and short days (SD) on soluble carbohydrates and the pattern of root proteins during development were analysed.

## Materials and Methods

### Plant material and growth conditions

*Pachyrhizus erosus* seeds were sown in September 1994 (SD sowing) and in April 1997 (LD sowing). Plants were allowed to grow in a greenhouse under natural photoperiod conditions (INRA Guadeloupe) as described by Robin *et al.* (1990). The evolution of compounds during development were carried out by taking samples of the tap root at five, six, seven, eight, and

nine weeks after sowing (WAS). Tubers were stored at  $-32^{\circ}\text{C}$  in 95% (v/v) ethanol for carbohydrates compounds analysis or frozen at  $-80^{\circ}\text{C}$  for enzymatic activity studies.

### Identification of carbohydrates

The soluble sugars of tap roots were extracted as described by Vaillant and Desfontaines (1995) and separated by high performance liquid chromatography (HPLC) on a Polypore-Ca column (250 mm  $\times$  4.6 mm i.d., Brownlee Labs Inc.) equilibrated with degassed ultra-pure water heated at  $85^{\circ}\text{C}$  at a flow rate of 0.3 mL  $\text{min}^{-1}$ .

### Proteins extraction

Tuber samples were ground by a mortar and pestle in 20 mL of ice-cold buffer containing 50 mM Tris-HCL pH 8.4, 100 mM NaCl, 2 mM DTT, 0.02% Triton X-100, and 0.4 mM Pefabloc SC (protease inhibitor obtained from Merck). The homogenate was centrifuged for 20 min at 12 000 g. The supernatant was collected and desalted by centrifugation on Sephadex G-25 columns (Pharmacia). Protein concentration was estimated by the method of Bradford (1976).

### Neutral invertase assays

A quantity of 200  $\mu\text{L}$  of reaction mixtures contained 50 mM HEPES-NaOH pH 7.5, 200 mM of sucrose, and 20  $\mu\text{L}$  of desalted extract. Samples were incubated at  $30^{\circ}\text{C}$  for 10, 30, and 60 min. The reaction was stopped by adding 200  $\mu\text{L}$  of Somogyi reagent followed by

immersing the reaction mixtures in a boiling water bath for 10 min. Absorbance at 525 nm was measured and compared to a standard curve for glucose. Activities were expressed in nmol sucrose hydrolysed per minute and per milligram protein.

**Acrylamide gel electrophoresis**

Samples were separated on non-denaturing 8% polyacrylamide gels (purchased from Novex, San Diego, U.S.A.). The running buffer was 25 mM Tris base and 0.2M glycine pH 8.3. The samples were run at 125 V constant voltage, at about 4°C. Proteins were visually identified by staining with coomassie brilliant blue R-250.

**Activity staining on electrophoretic gel**

Neutral invertase (NI) activity was directly revealed on gels. The staining solution contained 50 mM Hepes pH 7.5, 100 mM sucrose, 5 mM ATP, 0.7 mM NAD, 2 mM MTT, 0.8 mM PMS, 10 mM MgCl<sub>2</sub>, 2 U mL<sup>-1</sup> hexokinase, 2 U mL<sup>-1</sup> phosphoglucose isomerase, and 2 U mL<sup>-1</sup> glucose-6-phosphate dehydrogenase. The gel was incubated in the dark at 37°C until bands of activity appeared.

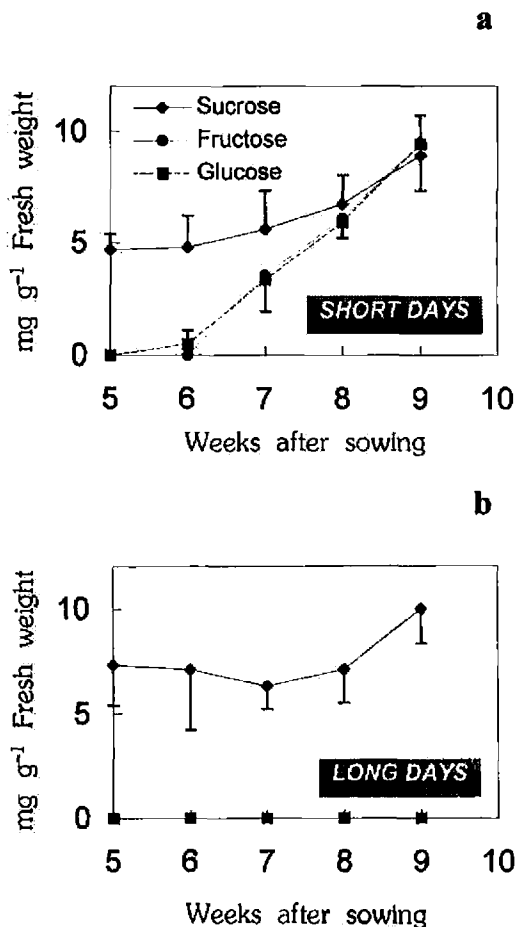
Sucrose synthase activity was detected (Morell and Copeland, 1985) by incubating gels at 37°C for 30 min in Hepes-KOH pH 8, 100 mM sucrose, and 2 mM UDP. The gels were rinsed in water and incubated in 1N NaOH containing 1% (w/v) TTC for 10 min at 37°C.

**Results**

Between 5 and 9 WAS, the fresh weight of tap root increased by 15.46 and 0.31 g, under LD and SD, respectively (Table 1). Changes in the soluble carbohydrate content of tap root are shown in Figure 1. At 5 WAS, tap root contained essentially sucrose (4.8 and 7.3 mg g<sup>-1</sup> of fresh weight, respectively, under SD and LD). Sucrose then increased constantly to reach 8.9 and 10.1 mg g<sup>-1</sup> FW at 9 WAS, for SD and LD, respectively. The levels of glucose and fructose were negligible in tap root under LD throughout development. On the other hand, glucose and fructose levels strongly increased

**Table 1** Changes in the weight of tap root during development under short days (SD) and long days (LD)

Weeks after sowing	Fresh weight of the tap root (g)	
	SD	LD
5	0.25±0.07	0.44±0.09
6	0.27±0.11	0.70±0.19
7	0.42±0.08	1.75±0.67
8	0.44±0.17	4.61±1.05
9	0.56±0.14	15.90±5.11



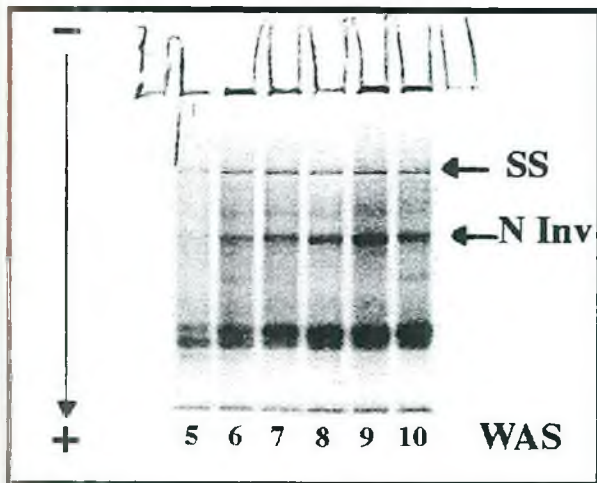
**Figure 1** Changes in soluble sugars concentration in tap root of *Pachyrhizus erosus* (L.) Urban during development under (a) short days (SD) and (b) long days (LD)

from 6 WAS under SD, to reach 9.4 mg g<sup>-1</sup> of fresh weight at 9 WAS. The ratio of glucose to fructose was close to one during development. The ratio (glucose + fructose):(sucrose) was 2.1 and 0.1 under SD and LD, respectively. Also, the amount of protein (on the basis of the dry weight) increased by 20%. Within tuber tissues, the activity of neutral invertase was higher in the flesh (about 8 times at 7 WAS) than in the peel which contained essentially sucrose (more than 90% of soluble sugars). The developmental profiles of tap root proteins presented in Figure 2, showed that the sharp increase of NI activity under SD, coincided with the appearance of a band in PAGE. This band which corresponded to a NI activity (as shown by gel staining for NI), was not present under LD.

**Discussion**

The initiation and the bulking of a tuber sink in *P. erosus* is under the dependence of SD (Zinsou, 1992; Vaillant and Desfontaines, 1995). It coincided with the accumulation of equal amounts of glucose and fructose from 6 WAS under SD, whereas those sugars were negligible under LD. In the absence of tuber bulking





**Figure 2** Per cent PAGE of tap root total proteins: 40 µg protein were loaded for each sample. Activity of sucrose synthase (SS) and neutral invertase (NI) are visualised by arrows

(long-day development), sucrose was the predominant sugar in the tap root as the main sinks for carbon were apex and secondary roots (Robin *et al.*, 1990). There was convincing evidence for a substantial increase in NI activity accompanying hexose accumulation in the tuber under SD.

There have been many reports on detection and characterization of invertases in various plants (Krishnan and Pueppke, 1990; Ranwala *et al.*, 1991; Chen and Black, 1992; Van den Ende and Van Laere, 1995). In higher plants, different invertase isoforms (EC 3.2.1.26) are located in different cellular compartments. Glycosylated forms with acidic pH optimum are found in the vacuole and (or) apoplast whereas non-glycosylated forms with a neutral pH optimum are located in the cytoplasm. Forms of each enzyme have specific functions requiring independent regulation. In sugar beets, NI appeared when the roots began to develop and increased with sucrose accumulation (Masuda *et al.*, 1987) whereas in carrot roots (Ricardo, 1974) and potato tubers (Zrenner *et al.*, 1996), it is involved in the regulation of the ratio of hexose to sucrose. Acid invertase may predominate in rapidly elongating tissues such as corn radicle tips (Hellebust and Forward, 1962), bean internodes (Morris and Arthur, 1985), snap bean pod (Sung *et al.*, 1994), and vacuoles of red beet storage roots (Milling *et al.*, 1993).

In *P. erosus*, glucose and fructose had been shown (Vaillant and Desfontaines, 1993) to have a vacuolar localisation in the tuber. The data show that the accumulation of hexoses under SD is correlated with the specific induction of a NI which may play a key role in the cleavage of sucrose before transport into storage.

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# Relationship between anthocyanin composition and paste colour in purple-fleshed sweetpotato

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**Genotype diversity in anthocyanin composition and the colour of paste from the storage roots of sweetpotato [*Ipomoea batatas* (L.)] is described. Investigation of anthocyanin extract by high performance liquid chromatography (HPLC) revealed that the proportion of six major anthocyanins varied among genotypes; these six anthocyanins were identified as acylated cyanidin and peonidin derivatives. The colour of paste made from purple-fleshed sweetpotato also varied, ranging from red purple to blue purple. Genotype diversity in paste colour was evaluated by their two reflectance values,  $L^*$  and  $b^*:a^*$  ratio. A significant positive correlation was observed between  $b^*:a^*$  ratio and the relative percentage of peonidin type of pigments. An increase in peonidin type of pigments increased the degree of redness in the paste.**

**Keywords:** *Ipomoea batatas* (L.); Anthocyanins; Cyanidin; Peonidin; Paste colour

Anthocyanins are natural pigments responsible for the red, purple, and blue flower colours of many plants. Utilization of anthocyanins in the storage roots of sweetpotato [*Ipomoea batatas* (L.)] is one of the new breeding objectives at Kyushu National Agricultural Experiment Station (KNAES) in Japan. Sweetpotato is regarded as a good source of stable anthocyanins for colorant production (Odake *et al.*, 1994). Ayamurasaki was released in 1996 as a new cultivar for colorant production, and it contains much more anthocyanins in the storage roots than an indigenous variety Yamagawamurasaki (Yamakawa *et al.*, 1997). Ayamurasaki is expected not only to be used for the anthocyanin production, but also for making purple paste and flour.

Further improvement of purple-fleshed sweetpotato is needed for processing use. Higher anthocyanin concentration, higher proportion of purple and stable pigments, and more brilliant purple paste are required by sweetpotato processors. In sweetpotato, not much information is available about genotype differences in anthocyanin composition and concentration. In this study, anthocyanin concentration and composition of the six major pigments were examined in breeding lines. In addition, the relationship between the paste colour and anthocyanin composition in the storage roots was investigated in an attempt to improve paste colour.

## Materials and Methods

### Plant materials

Purple-fleshed sweetpotato clones, including 46 breeding lines and 4 local varieties, were grown

in the field in KNAES at Miyakonojo, Japan. The storage roots were harvested about 150 days after planting (at standard harvesting) and used as the materials for anthocyanin analysis.

### Anthocyanin extraction and determination of total anthocyanins

A one-gram disc with a diameter of 10 mm, and about 2 mm thick was extracted with 20 mL of 50% acetic acid for 16 h. The extracts were diluted fourfold in a McIlvaine's buffer solution (adjusted to pH at 3.0) and measured spectrophotometrically by reading at 530 nm. Total anthocyanins (colour value) were calculated by the following formula:  $10\% E_{530} \times 4 \times 20$ , where  $E_{530}$  = spectrophotometric reading at 530 nm.

### High performance liquid chromatography (HPLC) analysis of anthocyanin extracts

The (HPLC) analysis was performed according to the method of Odake *et al.* (1992). The extract solution was filtered through a 0.45  $\mu\text{M}$  PTFE filter and used for HPLC analysis. The HPLC was run on Inertsil ODS-2 (250 mm  $\times$  4.6 i.d., Gasukuro-Kogyo) columns at 35°C with a flow rate of 1 mL  $\text{min}^{-1}$ , monitoring at 530 nm. The solvent system was a linear gradient elution for 40 min from 25 to 85% solvent B (1.5%  $\text{H}_3\text{PO}_4$ , 20% HOAc, 25% MeCN in  $\text{H}_2\text{O}$ ) in solvent A (1.5%  $\text{H}_3\text{PO}_4$  in  $\text{H}_2\text{O}$ ). In all samples, 10  $\mu\text{L}$  of the extract were injected. Six pure standards of sweetpotato anthocyanins from Yamagawamurasaki were used to determine retention times of different anthocyanins. Identification of each peak was based on the retention order of the anthocyanins.

## Measurement of paste colour

Three roots per genotype were sampled and longitudinally cut in half. Half of those cut portions was steamed. Pigments were examined for a symmetrical piece of root as described above. The steamed root pieces were packed in transparent plastic bags and pressed by hand to crush them. The surface colour of individual pastes was measured at three points with a Minolta CR-200 chromameter. Colour reflectance values  $L^*$ ,  $a^*$ , and  $b^*$  of the paste were scored.

## Results

### Anthocyanin concentration and composition in the storage roots

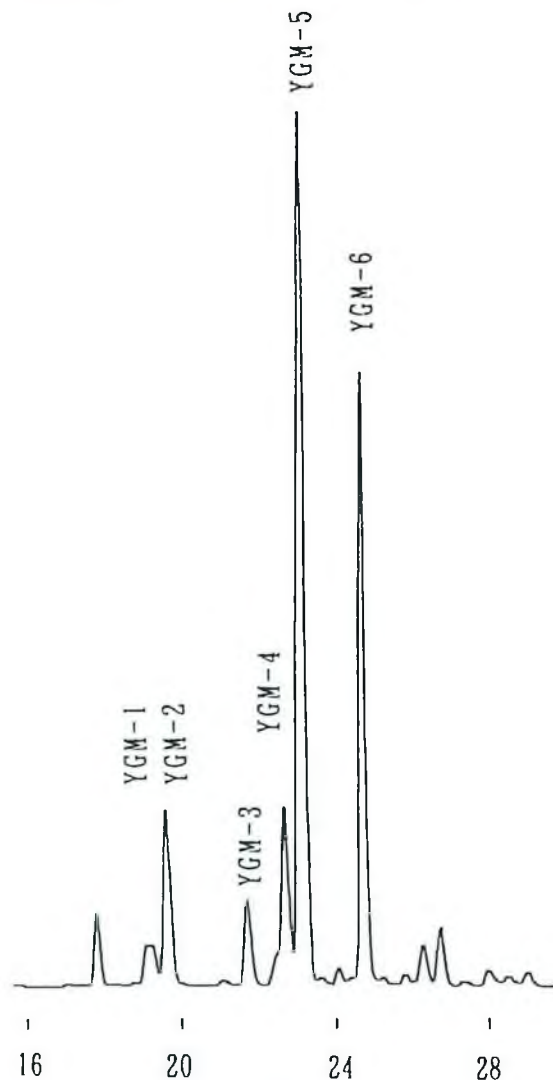
A typical elution pattern of the anthocyanins of sweetpotato cv. Ayamurasaki under the HPLC conditions used is shown in Figure 1. Six major peaks were identified (YGM-1, -2, -3, -4, -5, and -6) by comparing their retention times and elution order with the pattern in a previous report (Otake *et al.*, 1992). Scatter plots of colour value and peonidin (YGM-4, -5, -6):cyanidin (YGM-1, -2, -3) ratio showed a wide genotype difference in these two characteristics (Figure 2). No clear relationship between colour value and peonidin:cyanidin ratio was observed ( $r = 0.28$ ). The mean colour value was 5.4, with a range from 0.1 to 16.5. The colour value of Ayamurasaki was 13.6, which was approximately twice as high as that of Kyushu 109, and about four times higher than Yamagawamurasaki.

### Relationship between paste colour and composition of pigments

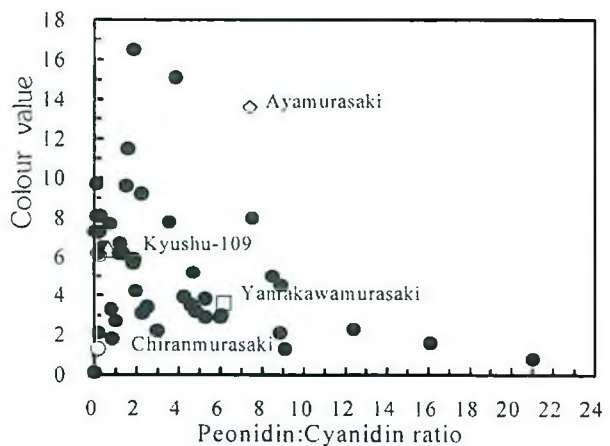
The paste made from purple-fleshed sweetpotato varied from red purple to blue purple. In scatter plots of  $L^*$  and  $b^*:a^*$  ratio, two groups were recognized: one was blue dominant at a  $b^*:a^*$  ratio of less than -1.4, while the other was red dominant with a  $b^*:a^*$  ratio of more than -1.1 (Figure 3). The  $L^*$  value was correlated negatively with colour value in the storage roots, while no correlation was found between  $b^*:a^*$  ratio and colour value (Table 1). A significant positive correlation was observed between  $b^*:a^*$  ratio and peonidin:cyanidin ratio in the storage root (Table 1). An increase in proportion of peonidin type of pigments increased the degree of redness in the paste.

## Discussion

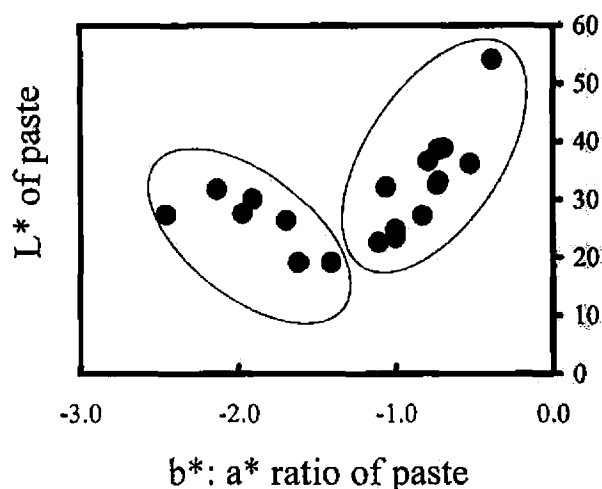
Of the six major pigments in sweetpotato, the chemical structure of two anthocyanins, YGM-3 and YGM-6, were determined as the 3-(6,6'-caffeylferulysophoroside)-5-glucosides of cyanidin



**Figure 1** High performance liquid chromatography (HPLC) chromatogram of anthocyanins extracted from purple-fleshed sweetpotato cv. Ayamurasaki. YGM-1, -2, and -3 are cyanidin derivatives; YGM-4, -5, and -6 are peonidin derivatives.



**Figure 2** Scatter plots of colour value and peonidin:cyanidin ratio in purple-fleshed sweetpotato.



**Figure 3** Scatter plots of  $L^*$  and  $b^*:a^*$  ratio of purple-fleshed sweetpotato paste

**Table 1** Correlation coefficients between two colour reflectance values ( $L^*$  and  $b^*:a^*$  ratio) of paste and two anthocyanin characteristics of the storage roots

	Colour value of the storage root	Proportion of peonidin in the storage root
$L^*$ value of paste	-0.586**	0.410*
$b^*:a^*$ ratio of paste	0.036	0.802**

\*, \*\*, significant at 5% and 1% level, respectively

and peonidin (Otake *et al.*, 1992). Additionally, two anthocyanins (YGM-2 and YGM-5) were identified as the 3-O- $\beta$ -D-glucopyranosyl-2-O- $\beta$ -D-glucopyranosyl-5-O- $\beta$ -D-glucosides of cyanidin and peonidin (Goda *et al.*, 1997). The remaining two pigments YGM-1 and YGM-4 have been identified as cyanidin and peonidin derivatives, respectively (Terahara *et al.*, unpubl.). These six major sweetpotato anthocyanins are classified into two groups based on the chemical structure of the aromatic B-ring: YGM-1, -2, and -3 are cyanidin derivatives, and YGM-4, -5, and -6 are peonidin derivatives. Methylation of

the 3'-hydroxyl group of cyanidin leads to peonidin. In this study, wide genotype differences in peonidin:cyanidin ratio and colour value were observed. To achieve higher anthocyanin concentration and more purple pigments, selection for both the peonidin type and high colour value is necessary. However, no line was found with a higher peonidin:cyanidin ratio and higher colour value than those of Ayamurasaki.

Anthocyanin composition influenced not only the quality of pigments but also paste colour. In this study, paste colour was characterized by two colour reflectance values were  $L^*$  and  $b^*:a^*$  ratio. The  $L^*$  values was correlated negatively with anthocyanin concentration in the storage roots. This suggests that the amount of anthocyanin in the roots affects the brightness of purple paste. In addition, a significant positive correlation between  $b^*:a^*$  ratio and peonidin:cyanidin ratio in the roots shows that an increase in proportion of the peonidin type of pigments increased the degree of redness of the paste. Clearly, genotype differences in paste colour is mainly due to the anthocyanin concentration and the proportion of cyanidin and peonidin types of pigments.

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# Screening sweetpotato for drought tolerance in the Philippine highlands and genetic diversity among selected genotypes

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Local germplasm of high-yielding traditional cultivars and open-pollinated (OP) progenies of sweetpotato [*Ipomoea batatas* (L.)] were screened for drought tolerance in swidden and post-rice conditions in the Philippine highlands. Several clones from OP progenies and traditional cultivars showed some drought tolerance based on the yields of marketable storage roots. Under swidden conditions, the local cultivar Tokano, had the highest yield. Likewise, the traditional cultivar Kalbo-oy, produced the best yields in post-rice paddies. To obtain genetic markers that will be used in future breeding, genetic diversity analysis was performed. Genomic deoxyribonucleic acid (DNA) from seven sweetpotato genotypes, selected from those which produced storage roots under drought conditions, was subjected to Random Amplified Polymorphic DNA (RAPD) analysis. Twenty decamer primers were tested and 16 yielded DNA-amplification products. Among the 97 scored bands, 77 were polymorphic (79.38%). Genotype-specific DNA markers were also identified. The pairwise marker difference between genotypes ranged from 0.378 to 0.936, indicating a broad range of genetic diversity. A phenogram of the genetic relationships among those genotypes is presented.

Keywords: *Ipomoea batatas* (L.); Drought tolerance; Genetic diversity; Genotypes; Post-rice; Swidden; Random Amplified Polymorphic DNA (RAPD)

Sweetpotato [*Ipomoea batatas* (L.)] is an important crop in the Philippine highlands (Tandang *et al.*, 1990). It is a main crop in swidden farms or 'kaingin' and a relay crop in rice paddies and can be grown easily with simple cultural management and little farm inputs (Gayao, 1987). Swidden is a farming practice in sloping areas which involves clearing of the natural vegetation, then burning when it is dried out. One month after, the area is prepared for planting of crops. In spite of the adaptability of the crop in the Philippine highlands, several problems exist that hinder optimum production. In a survey (Ganga *et al.*, 1994), problems identified by sweetpotato farmers were, variety related (long maturity period, yield degeneration, and susceptibility to insects and diseases), rodent damage, and drought. Drought has become a major abiotic stress in sweetpotato fields. It is, therefore, important to identify sweetpotato genotypes suitable for post-rice and swidden conditions in order to sustain sweetpotato production in the Philippine highlands.

In the past decade, the sweetpotato improvement programme of the Northern Philippines Root Crops Research and Training Center (NPRCRTC) focussed on the screening and evaluation of germplasm for high yield, disease and insect resistance, short maturity period, and high dry matter (DM) content. A number of genotypes possessing one or more of the mentioned characters were selected. Likewise, hybridizations with these genotypes in polycross nurseries were performed. Open-pollinated progenies generated from the polycross nurseries were evaluated on-station and selections were made for desirable agronomic characters (Ganga *et al.*, 1994). Because of the drought problem in the locality, selected high-yielding cultivars and clones obtained from open-pollinated progenies were screened in swidden and post-rice conditions.

The NPRCRTC's genebank maintains a wide array of germplasm which include traditional cultivars, advanced breeding lines, released varieties, and products of polycross breeding. These were characterized morphologically using descriptors by Human (1987) and possible duplicates were identified (Ganga *et al.*, 1994). However, proper identification is difficult due to variability of some morphological characters.

Morphological traits in sweetpotato are not reliable due to their paucity and are easily influenced by the environment (Prakash and

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He, 1996). A more reliable way to characterize germplasm would be the use of genetic markers.

Restriction fragment length polymorphisms (RFLPs) have been used in phylogenetic studies in sweetpotato (Kowyama *et al.*, 1992). The development of Random Amplified Polymorphic DNA (RAPD) analysis offers an important tool to applied plant breeding (Williams *et al.*, 1990). The RAPD markers were employed to study the genetic relationships and diversity in sweetpotato cultivars in the U.S.A. (Jarret and Austin, 1994; He *et al.*, 1995; Thompson *et al.*, 1997) and clonal cultivars of sweetpotato in Australia (Connolly *et al.*, 1994).

This paper presents the results of screening trials of sweetpotato genotypes for drought tolerance in the Philippine highlands and the use of RAPD markers in assessing the genetic relationships among seven selected genotypes from the drought tolerance trials, performed at the Jacob Blaustein Institute for Desert Research, Sede Boker, Israel.

## Materials and Methods

### Screening of sweetpotato genotypes in the on-station, post-rice, and swidden fields

#### Study sites

The study sites, La Trinidad and Kapangan are towns of Benguet Province situated in the mountain ranges of the Cordillera region with elevations of about 1200 m above sea level (asl). The climatic condition of the area is tropical, with an average temperature of 21°C. The dry season lasts from December until the middle of May, while the rest of the year is wet.

On-station trials were conducted in La Trinidad. Large numbers of sweetpotato germplasms were evaluated in the preliminary trials on-station which were researcher-managed; however, the usual farmer's practices of sweetpotato production were followed, i.e., no fertilizers and pesticides were applied. In this study, the field screening of open-pollinated progenies for drought tolerance from local sweetpotato cultivars was conducted on-station where the soil is classified as a clay loam.

Kapangan is a major sweetpotato producing area in Benguet province. Sweetpotato is mostly cultivated in swidden fields and rice paddies. Swidden farms have a slope of 18% and above, rain-fed, and soils are loam to clay loam. Swidden field screening of selected high-yielding sweetpotato cultivars in this study were conducted in three sites of Kapangan: Cayapes, Pudong, and Bileng-Belis. Likewise, screening of sweetpotato genotypes under post-rice conditions were conducted in Bileng-Belis where the soil is classified as loam to sandy loam.

### Data collection

Meteorological data such as temperature, relative humidity, and rainfall were obtained from the Philippine Atmospheric Geophysical and Astronomical Service Administration (PAG-ASA) weather station located in La Trinidad, Benguet.

Soil matric potential was measured using installed irrometers (Irrometer Co., Model S) in the on-station and post-rice trial sites. However, irrometers were not installed in the swidden site due to a steep slope.

Drought score was based on leaf wilting and taken when irrometer readings reached 60–70 centibars indicating a drought level condition and when plants show wilting. The rating scale (Beekman, 1985) used was: 1 = no stress or all plants were turgid; 3 = 30% of the leaves wilted or 30% of the plant population were wilted; 5 = 50% of the leaves wilted or 50% of the population wilted; 7 = 80% of the leaves wilted or 80% of the population wilted; and 9 = complete wilting.

Recovery rating was recorded after a rain spell that occurred during the experiments which resulted in rehydration of the plants to normal soil matric potential (20 centibars). The scale (Beekman, 1985) was: 1 = no recovery; 3 = 30% of the leaves recovered; 5 = 50% of the leaves recovered; 7 = 80% of the leaves recovered; and 9 = complete recovery.

Storage root yield was determined after discarding the two outer plants from each plot. All storage roots with diameter of 3 cm and above were weighed at harvest.

### Data analysis

Statistical analysis was done using ANOVA (analysis of variance) and Duncan's Multiple Range Test (DMRT) for mean comparison.

### Drought screening of sweetpotato genotypes

The succeeding sets of experiments: on-station screening of open-pollinated progenies from traditional cultivars, screening of 26 open-pollinated genotypes under post-rice conditions, and screening of selected high-yielding cultivars under swidden conditions were conducted during the dry season (January to May) of 1995 and 1996. Kalbo-oy, a traditionally grown cultivar and previously identified as tolerant to drought conditions in greenhouse or controlled environment studies (data not shown) was used as tolerant check in the trials. The usual cultural practices employed in sweetpotato fields like weeding and vine lifting were followed including conventional plowing of the land before planting.

In the on-station screening of open-pollinated progenies from traditional cultivars, a total of 1500 true sweetpotato seeds from eight local cultivars obtained through open-pollination, were germinated in December 1994 at La Trinidad,

Benguet. These were transplanted in January 1995 in the field using a randomized complete block design (RCBD) divided into four blocks. Seedlings of sweetpotato were planted in a single row distanced 60 cm between hills with a total of 32 hills per treatment. Irrigation was carried out during the first month until seedlings were established. Drought was imposed by withholding water from the fifth week until harvest. Drought scores were recorded twice at 79 and 100 days after planting (DAP) while recovery ratings were recorded at 116 and 137 DAP. Plants were harvested at 150 DAP.

Screening of 26 sweetpotato genotypes under post-rice condition was conducted in a paddy previously cropped with rice at Beleng-Belis, Kapangan, Benguet, from January to June, 1995. Apical portions of sweetpotato cuttings measuring 30 cm were planted at a distance of 30 cm between hills and rows, and laid out in RCBD with three blocks. Forty sweetpotato cuttings were planted for each genotype per replication. Irrigation was carried out for a month until establishment. After four weeks, irrigation was stopped until harvest. Drought scores were recorded at 46 and 66 DAP while recovery ratings were gathered at 106 and 133 DAP. Plants were harvested at 150 DAP.

Swidden field screening of high-yielding local sweetpotato cultivars was conducted in three sites of Kapangan, Benguet, namely, Cayapes, Pudong, and Bileng-Belis from December 1995 to July 1996. Seven cultivars were common in Cayapes and Pudong sites. In Bileng-Belis, a different set of cultivars was screened except for Kalbo-oy and Dakol OP. Apical portions of sweetpotato stems were used as the source of planting material, each measuring 30 cm long. One cutting per hill was planted in mounds in slanting position with a total of 40 plants per cultivar per replication. Planting space was 30 cm between hills and rows. Treatments were laid out using RCBD with three replications. The plants totally depended on natural rainfall throughout the growing season. Plants were harvested at 180 DAP.

#### Analysis of genetic diversity among selected genotypes

##### *Plant material*

Seven high-yielding genotypes (Kalbo-oy, Tocano, VSP6, SPS 36, SPS 35, Dakol OP, and NPSP 322) were used for RAPD analysis. Storage roots from the genotypes were made to sprout and young leaves were used for deoxyribonucleic acid (DNA) extraction.

Kalbo-oy and Tocano are traditional cultivars which produced the highest yield under post-rice and swidden conditions, respectively. VSP6 is a Philippine Seed Board approved variety bred at the Visayas State College of Agriculture (ViSCA) Baybay, Leyte, Philippines. SPS 35, SPS 36, and Dakol OP are clones from open-pollinated

progenies. NPSP 322 is an advanced line from ViSCA.

##### *DNA extraction*

Total genomic DNA was extracted from young leaves of sweetpotato as described by Jarret and Austin (1994).

##### *DNA amplification and analysis*

Twenty decamer primers obtained from Operon Technologies, Alameda, California (Kit O) were used. Taq DNA polymerase was obtained from Advanced Biotechnologies. Total PCR reaction mixture of 25  $\mu$ L contained 2.5 mM  $MgCl_2$ , 200  $\mu$ M of each dNTP's (USB); 10 pmol primer, 1 unit Taq DNA polymerase and 50 ng genomic DNA, and 1  $\times$  reaction buffer (Advanced Biotechnologies). A negative control reaction which contains all components except DNA was included every running time with a different primer. Each reaction mixture was overlaid with 30  $\mu$ L of mineral oil. The DNA amplifications were performed in a thermal cycler (Minicycler<sup>TM</sup>, MJ Research, Watertown, MA). The cycling parameters used were; 40 cycles of 94°C, 1 min; 35°C, 1 min; 72°C, 2 min; and 1 cycle, 5 min at 72°C.

Amplification products were analysed by gel electrophoresis on 1.4% agarose gel in 1  $\times$  Tris-Acetate-EDTA buffer and visualized by staining with ethidium bromide. Molecular sizes of DNA amplified products were estimated using 100 bp DNA ladder (Gibco BRL).

##### *Statistical analysis*

Bands were scored as 1 (band present) or 0 (band absent). Genetic variation among genotypes was estimated using Nei's coefficient of genetic distance (Nei and Li, 1979): Similarity =  $2N_{ab}/(N_a + N_b)$  where:

- $N_{ab}$  = number of shared amplification fragment with the same molecular weight shared between genotypes a and b;
- $N_a$  = number of scored amplification fragment in genotype a; and
- $N_b$  = number of scored amplification fragment in genotype b.

A phenogram of the genotypes was constructed by a cluster module using the SYSTAT statistical package (Wilkinson, 1990).

## Results and Discussion

### Screening of sweetpotato genotypes in the on-station, post-rice, and swidden conditions

Screening trials of sweetpotato genotypes for drought tolerance were conducted during the dry season of 1995 and 1996. Minimum and maximum temperature and relative humidity in

the on-station, post-rice, and swidden trials were monitored for 150–180 DAP. The minimum and maximum temperatures ranged from 10 to 16°C and 22 to 25°C, respectively. Relative humidity ranged from 77 to 91%. The rainfall distribution pattern throughout the growing season is shown in Figure 1A. Sweetpotato genotypes screened for drought tolerance in the on-station and post-rice in 1995 received a total of 65 mm of rainfall throughout the growing season. On the other hand, sweetpotato cultivars screened for drought tolerance in swidden farms in 1996 received a total of 110 mm. The rainfall data show that drought imposition was not totally met due to occurrence of rain during the growing season. However, the amount of rainfall was not sufficient for optimum growth and development. Furthermore, the general trend shows that rainfall was low during the early stages of growth, but high at the

later stages indicating that during critical periods of vegetative and bulking stages, water was limiting. Martin (1987) stated that although sweetpotato can tolerate drought at the end of its life cycle, it is hardly considered as a drought-tolerant crop. In the present study, although the plants did not experience severe drought stress, the results were still significant considering a transient or temporary drought conditions.

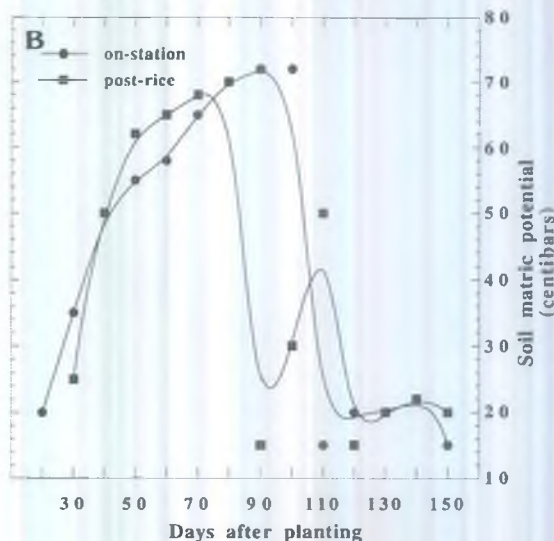
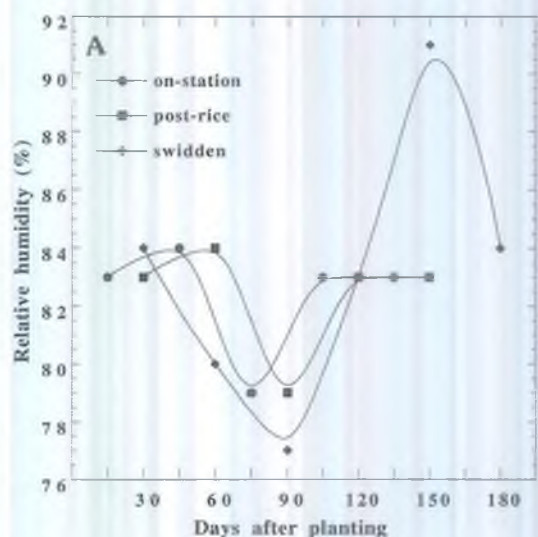
Soil matric potential (SMP) taken in the on-station and post-rice experiment is shown in Figure 1B. In the on-station screening of open-pollinated sweetpotato progenies, SMP, at 40–100 DAP, ranged from 50–72 centibars indicating that soil moisture condition had fallen to a drought level, however, SMP decreased at 100 DAP and at the later stages due to occurrence of rain. Under post-rice conditions, SMP at 40–80 DAP, ranged from 50–70 centibars and decreased at 90–100 DAP after a rain that occurred at 81 DAP. At 110 DAP, SMP reached 50 centibars, however, SMP decreased at the later stages of growth due to rainfall occurrence.

The drought score, at 79 and 100 DAP, recovery rating and storage root yield of eight open-pollinated progenies screened for drought tolerance on-station were monitored from January to May 1995. Drought imposition did not significantly affect these parameters in any of the eight cultivars (Dakol OP, NPS 48, NPS 322, NPS 350, NPS 524, NPS 712, NPS 713, and NPS 973). Although no significant differences were observed, Dakol OP produced the highest storage root yield of 13.89 t ha<sup>-1</sup>. NPS 350, NPS 524, and NPS 713 gave comparable storage root yields.

Storage root yields of the open-pollinated progenies subjected to drought conditions were higher (5.71–13.89 t ha<sup>-1</sup>) than the minimum and average yield per hectare in the Philippine highlands. Under normal conditions, the average yield per hectare in the Philippine highlands is 12.04 t ha<sup>-1</sup>, ranging from 2.49 to 49 t ha<sup>-1</sup> (MAF, 1985) with the use of local or traditional cultivars. High storage root yields of the open-pollinated progenies could be attributed to drought tolerance. Noteworthy, these progenies were produced from a polycross nursery involving elite parental selections possessing good agronomic traits such as high yield, resistance to sweetpotato weevil, high DM content, and short maturity period. It is possible that adaptive traits to the stress conditions were transmitted from parents to offsprings.

Under post-rice conditions, drought score and recovery rating did not vary among the 28 genotypes although significant differences for storage root yield were observed. All genotypes produced storage root yields that were lower than the check cultivar, Kalbo-oy (6.56 t ha<sup>-1</sup>). SPS 36 produced the highest yield (2.48 t ha<sup>-1</sup>) among the genotypes.

Storage root yields of the genotypes under



**Figure 1** A. Rainfall distribution pattern (mm) in the on-station, post-rice, and swidden conditions; B. Soil matric potential (centibars) in the on-station and post-rice conditions. (—●—), on-station; (—■—), post-rice; (—◆—), swidden



post-rice condition were very low compared to the tolerant check, Kalbo-oy. These clones were selected on-station for high yield and other good agronomic traits such as red skin, resistance to diseases and insects, and high DM content. The poor performance of these clones may be caused by the variable environment in the post-rice, and by the drought conditions where they existed. Levitt (1980) stated that plants owe their drought tolerance to different factors. The storage root yields of the sweetpotato genotypes may not only be affected by drought conditions, but also by other factors in the rice paddy, such as fertility of the soil. These emphasize the need to select for other traits other than yield that may enhance stress adaptation in specific target environments. Secondary traits of adaptive value whose genetic variability increases under drought were reported in wheat (Yadava and Bhatt, 1989); soya bean (Rose *et al.*, 1992); pearl millet (Van Oosterom *et al.*, 1995); rice (Fukai and Cooper, 1995); and maize (Bolanos and Elmeades, 1996). In sweetpotato, a number of characteristics such as leaf area index, internode length, leaf-angle of young leaves (Bacusmo and Carpena, 1986), lateral vines which produced roots (Villamayor, 1988), canopy cover, vine number, and harvest index (Anselmo *et al.*, 1992) were found to be associated with storage root yield. Although selection for these traits was not done in this study, it is worth considering these traits in evaluating existing sweetpotato germplasm in the locality.

The storage root yield of 11 sweetpotato cultivars (data not presented) were evaluated at three sites under swidden conditions. The cultivars were Dakol, Ganga, Kalbo-oy, Karumbasa, Komendal, Tokano, VSP6, NPSP 322, NPSP 350, NPSP 973, and NPSP 524. At the Cayapes site, cultivars evaluated did not significantly differ in storage root yield, but Karumbasa produced the highest yield. Cultivars at the Pudong site, likewise, did not significantly differ in their storage root yield; however, Tokano produced the highest yields compared to the other cultivars. Results in Bileng-Belis showed that Kalbo-oy and NPSP 322 were the most promising cultivars as indicated by their higher yields, although no significant differences were observed.

Under swidden condition, storage root yields of the selected cultivars were generally higher than the minimum yield reported in the Philippine highlands. These cultivars were tested on-station and in farmers' field in the locality, thus, yield is more or less stable. Luh and Moowaw (1979) concluded that sweetpotato genotypes are known to be highly variable in their interaction with the environment. The apparent interaction of the genotype and environment requires the need for local testing and selection to produce the best adapted lines. Belhassen *et al.* (1995) stressed that high yield

potential should be a criterion for selection under conditions of low environmental constraints, while in areas of high or unpredictable environmental constraints, yield stability in space and time should be given priority.

The response of sweetpotato genotypes to drought conditions in the on-station and post-rice based on drought score and recovery rating was not significantly different. This shows that these parameters were not reliable indicators for detecting drought tolerance among the sweetpotato genotypes in the present study. In past studies (Levitt, 1980; Passioura, 1996), yield was used as a measure of varietal drought resistance because it could substitute failure of other measurements. In this study, therefore, yield was used as a basis of comparison among the sweetpotato genotypes evaluated under water deficit conditions. This study suggests the need for a continued search for drought-tolerant genotypes in the Philippine highlands, until a variety with stable yield performance across locations and seasons is achieved. Yield can be a criterion for selection, but other traits must also be given importance.

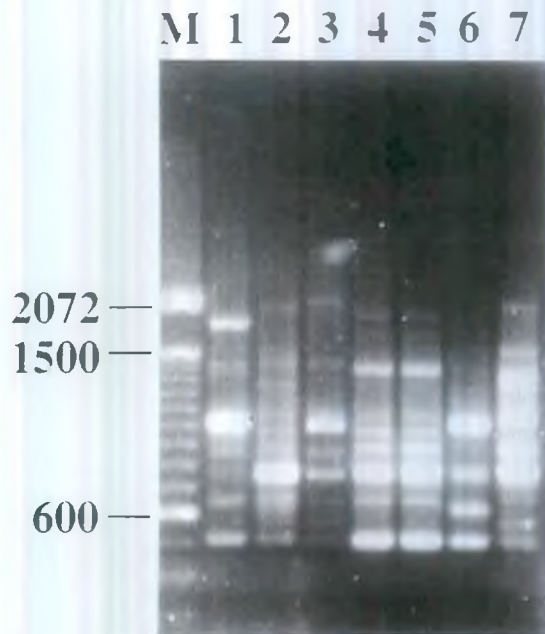
#### Analysis of genetic diversity among selected genotypes

Sweetpotato genotypes which produced high storage yields in the three experiments represent a genetically diverse population. Seven genotypes were selected and further analysed using RAPD.

To ensure genetic uniformity among the plant materials, RAPD was performed on different sprouts arising from the same storage roots. The DNA fragments generated by five primers showed no DNA polymorphism among sprouts taken from the same storage roots of three sweetpotato genotypes. Likewise, no DNA polymorphism was observed using DNAs extracted from sprouts taken singly or bulked (data not shown).

Of the 20 primers used, 16 primers yielded DNA-amplification products in all seven selected genotypes of sweetpotato. A representative RAPD pattern generated by primer OPO-11 is illustrated in Figure 2. The number of DNA bands amplified by each primer varied from 1–10 with an average of 6.07 bands per primer. The size of the amplified DNA products ranged from 300 to 2072 bp. Among the 97 scored bands, 77 (79.38%) were polymorphic. Four primers did not produce any amplification products (Table 1).

Genotype-specific bands are shown in Table 2. Ten primers generated polymorphic amplification fragments that can distinguish each genotype. Genotype NPSP 322 can be identified by OPO-11-800, OPO-15-1300, and OPO-20-700. Tokano is not represented by a unique marker but can be identified by sharing with NPSP 322 for OPO-20-500 and Kalbo-oy for OPO-20-700.



**Figure 2** A representative RAPD profile of sweetpotato genotypes obtained with primer OPO-11; lane 1, NPS 322; lane 2, Tokano; lane 3, Kalbo-oy; lane 4, SPS 36; lane 5, SPS 35; lane 6, Dakol OP; lane 7, VSP6; and lane M, molecular sizes in base pairs (bp)

Genotypes SPS 35 and SPS 36 can be identified by OPO-03-600 and OPO-08-2000, respectively. VSP6 can be identified by markers OPO-03-900 and OPO-18-700.

Nei's estimate of similarity (Nei and Li, 1979) was used to construct a similarity matrix.

Similarity values (Table 3) ranged from 0.378 to 0.936. The closest relationship was observed between SPS 35 and SPS 36 (similarity index of 0.936). SPS 35 and Tokano had the farthest relationship with a similarity index of 0.378. Cluster analysis of the RAPD markers of sweetpotato genotypes was based on 97 markers generated by 16 primers. As seen in the phenogram (Figure 3), NPS 322 is clustered separately from the others. Clustered by pairs are: SPS 35 and SPS 36; VSP6 and Dakol OP; and Tokano and Kalbo-oy.

Sweetpotato genotypes were observed to be clustered by their source except for Dakol OP and VSP6. Dakol OP is an open-pollinated selection obtained from a polycross nursery where VSP6 was one of the parents. The closeness (similarity index of 0.735) of the two genotypes implies that VSP6 is probably the paternal parent of Dakol OP, since in a polycross nursery, open-pollination occurs and only the maternal parent is known. In this case, identification of the parentage of progenies would be an immediate application of RAPD.

Genotypes SPS 35 and SPS 36 had a very close similarity index (0.936) owing to the fact that these are open-pollinated selections and their parents are local cultivars commonly grown in La Trinidad, Benguet. Kalbo-oy and Tokano are traditional cultivars planted in the highlands for many years. The clustering of these genotypes shows a narrow genetic base involving traditional cultivars in the locality. A great

**Table 1** List of primers used in the study, their sequences, number of bands obtained, their molecular size range, and number of variable bands among them

Primer	Sequence	Amplification products		
		Total bands	Size (bp)	Variable bands
OPO-02	5'-ACGTAGCGTC-3'	4	800-1500	4
OPO-03	5'-CTGTTGCTAC-3'	6	600-1700	5
OPO-04	5'-AAGTCCGCTC-3'	7	300-2000	4
OPO-05	5'-CCCAGTCACT-3'	5	1300-3000	5
OPO-06	5'-CCACGGGAAG-3'	10	500-2000	10
OPO-07	5'-CAGCACTGAC-3'	6	1000-2000	5
OPO-08	5'-CCTCCAGTGT-3'	4	1400-2072	4
OPO-09	5'-TCCCACGCAA-3'	5	500-1300	5
OPO-10	5'-TCAGAGCGCC-3'	1	1200	1
OPO-11	5'-GACAGGAGGT-3'	9	500-1800	4
OPO-13	5'-GTCAGAGTCC-3'	5	700-2000	1
OPO-15	5'-TGGCGTCCTT-3'	6	700-1500	5
OPO-16	5'-TCGGCGGTTC-3'	10	600-2000	9
OPO-18	5'-CTCGCTATCC-3'	6	700-2000	6
OPO-19	5'-GGTGCACGTT-3'	4	500-1300	4
OPO-20	5'-ACACACGCTG-3'	9	700-1500	5
OPO-01	5'-GGCACGTAAG-3'	No amplification	—	—
OPO-12	5'-CAGTGCTGTG-3'	No amplification	—	—
OPO-14	5'-AGCATGGCTC-3'	No amplification	—	—
OPO-17	5'-GGCTTATGCC-3'	No amplification	—	—

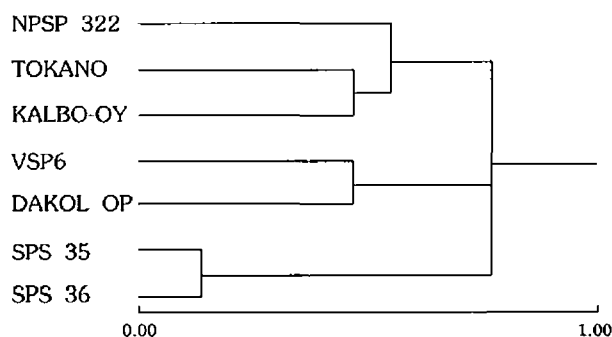
**Table 2** Sweetpotato genotypes specific Random Amplified Polymorphic DNA (RAPD) markers

Marker	NPSP 322	Tokano	Kalbo-oy	SPS 36	SPS 35	Dakol OP	VSP6
OPO-03-1500	0	0	0	0	0	+	0
OPO-03-900	0	0	0	0	0	0	+
OPO-03-600	0	0	0	0	+	0	0
OPO-04-1150	0	0	0	0	0	+	0
OPO-06-600	0	0	++	0	0	0	0
OPO-07-1900	0	0	0	0	0	++	0
OPO-08-2072	0	0	0	0	0	+	0
OPO-08-2000	0	0	0	++	0	0	0
OPO-11-1800	++	0	0	0	0	0	0
OPO-11-650	0	0	0	0	0	+	0
OPO-15-1300	+	0	0	0	0	0	0
OPO-16-900	0	0	0	0	0	+	0
OPO-18-700	0	0	0	0	0	0	+
OPO-20-500	0	+	+	0	0	0	0
OPO-20-700	+	+	0	0	0	0	0

\*, +, indicates a faint band and ++, indicates an intense band. Absence of band is indicated by 0

**Table 3** Similarity matrix generated using Nei's estimate of similarity

	NPSP 322	Tokano	Kalbo-oy	SPS 36	SPS 35	Dakol OP	VSP6
NPSP 322	1.00						
Tokano	0.577	1.00					
Kalbo-oy	0.575	0.644	1.00				
SPS 36	0.390	0.423	0.645	1.00			
SPS 35	0.395	0.378	0.610	0.936	1.00		
Dakol OP	0.432	0.447	0.568	0.634	0.640	1.00	
VSP6	0.493	0.428	0.681	0.710	0.547	0.735	1.00

**Figure 3** Phenogram of sweetpotato genotypes. The scale is 1-Pearson correlation coefficient obtained by average linkage method

number of traditional cultivars have been cultivated for over a century in the major sweetpotato-growing areas of the Philippine highlands. Establishing relationships among these cultivars was rather difficult because the only means to characterize them so far, was the use of morphological characters which were not easy to assess. The RAPD analysis is therefore

a useful tool in establishing the relationships among traditional cultivars found in the locality.

The RAPD analysis results revealed high degree of genetic polymorphism with 4.85 bands per primer. Compared with other crops, sweetpotato manifests high genetic diversity. Previous studies on sweetpotato diversity by Connolly *et al.* (1994) and He *et al.* (1995) revealed 3.7 and 16.7 bands per primer, respectively. Prakash and He (1996) found that U.S.A. cultivars despite their close relatedness, possess sufficient variation. High polymorphism among the genotypes analysed in this study may be due to the different sources and nature, i.e., traditional, introductions, and products of polycross breeding. The results indicate a relatively wide genetic base of sweetpotato germplasm in the Philippine highlands which makes the process of selection for desirable traits difficult. This justifies the potential of DNA markers as a tool in identifying core collections to be used as parents in hybridization blocks.

A large-scale RAPD analysis should be made using other sweetpotato genotypes to establish reliable genetic markers for each of the

available genotypes in the Philippine highlands. These markers can be used in future breeding programmes to identify and select drought-tolerant genotypes.

## Acknowledgements

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# Evaluation of germplasm and improved crop management practices for sweetpotato production in the U.S. Virgin Islands

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Sweetpotato [*Ipomoea batatas* (L.)] germplasm was evaluated and used to determine the contribution of cultural practices including mulching and irrigation on yield of sweetpotato and the reduction of sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers) infestation, the most economically devastating pest of sweetpotato in the United States Virgin Islands (USVI). The germplasm evaluation trials were conducted using cultivars obtained from the U.S.A., Puerto Rico (USDA-TARS), St Kitts, and the USVI. Results indicated a high per cent SPW infestation on storage roots of high-yielding cultivars, but local cultivars produced lower yields than introduced cultivars. Most cultivars from Puerto Rico were very well adapted for production in the USVI. Mulching generally increased the yield of cultivar Sunny, with the plastic mulch producing the highest yield of medium-sized roots while grass mulch reduced SPW infestation of storage roots. Irrigation significantly increased marketable yields of storage roots compared to the rain-fed treatment. Additionally, irrigation maintained at 40 kPa produced the highest yield of medium-sized storage roots which was significantly higher than the 20 kPa and rain-fed treatments. These studies indicate that for improved sweetpotato production in the USVI, growers should consider the use of locally adapted cultivars, mulching, and an optimum irrigation level.

Keywords: *Ipomoea batatas* (L.); Plant nutrition; Potassium; Mulches; Irrigation scheduling; Germplasm evaluation; Cultural practices; *Cylas formicarius*

Sweetpotato [*Ipomoea batatas* (L.)] is a crop of major economic importance in the Caribbean. Sweetpotato is a significant crop for farmers in the United States Virgin Islands (USVI) where it is the most popular root crop. The crop is grown year-round and the fresh storage roots are used for home consumption and commerce. Roots and foliage of the crop are also utilized as feed for farm animals.

One of the world's highest-yielding crops, sweetpotato has a total food production and food value per unit area exceeding that of rice, while requiring relatively low fertilizer inputs (Selleck, 1982). Sweetpotato ranks high in energy, carbohydrates, vitamin A (especially orange-fleshed cultivars), Vitamin C, calcium, and iron. The storage roots of sweetpotato are also a good source of dietary fibre (Hill *et al.*, 1984).

Sweetpotato is considered to be moderately drought-tolerant due to its extensive root system and low growth habit (Hammett *et al.*, 1982). The crop is deep-rooted and fairly good yields have been obtained under low moisture conditions. Research has clearly demonstrated that yields can be significantly increased by irrigation in areas where rainfall distribution is erratic or insufficient. If sufficient moisture is supplied to establish the plant, it will survive dry conditions

better, later in the season. Water requirements have been established at 18 mm week<sup>-1</sup> in the early season increasing to as much as 27–44 mm week<sup>-1</sup> in mid-season. Evapotranspiration has been recorded at 2.6 mm day<sup>-1</sup> for the first 45 days, 3.9 for the next 45 days, and 2.5 for the last 30 days (Jones, 1961).

In Puerto Rico, it was determined that a minimum of 900 mm of well distributed rainfall is needed during the five months growing season on an Oxisol. The highest yield and quality of sweetpotato can be produced when about 25 mm of rain or irrigation water is received every 7–10 days (Stall *et al.*, 1983). There is a need to precisely quantify irrigation amounts needed for optimum yields and quality.

Relatively little information exists on the use of mulches in sweetpotato production. Hochmuth and Howell (1983) increased total and marketable sweetpotato yields with the application of black plastic mulch. However, row covers and black polyethylene mulch increased the weight and number of large transplants but did not alter the yield of storage roots grown from the transplants (Walker and Randle, 1986).

Various insect pests and rodents attack sweetpotato in the USVI. However, the sweetpotato weevil (SPW), *Cylas formicarius elegantulus* (Summers), is the most economically

devastating. High populations of SPW are present throughout the year in the USVI (Proshold *et al.*, 1986). The SPW attacks nearly all parts of the sweetpotato plant and can develop successfully on mature stems or storage roots (Vasquez and Gapasin, 1980). Even low-level infestations can render sweetpotato roots unfit for human consumption (Proshold, 1983), because of toxic substances produced in the roots in response to SPW feeding (Uritani *et al.*, 1975). These chemicals impart a bitter taste to the storage roots.

Soil cracks are a major route for SPW to access storage roots, especially for cultivars that set roots near the soil surface (Talekar, 1991). The application of mulches can reduce SPW infestation levels by preventing the SPW from reaching the soil surface, covering cracks in the soil, and conserving soil moisture, thereby reducing the number of cracks in the soil caused by moisture stress.

Because of its limited flight activity (Sherman and Tamashiro, 1954), host specificity to the genus *Ipomoea*, and the characteristic mode of damage to the roots and crowns, the SPW may be vulnerable to suppression by cultural practices. These include crop rotation, use of SPW-free cuttings, proper field sanitation, irrigation, and mulching.

The objectives of these trials were to:

1. identify locally-adapted, consumer-acceptable cultivars which produce high marketable yields, propagate easily, have vigorous growth, compete well with weeds, and exhibit some degree of sweetpotato weevil resistance or tolerance;
2. determine the effect of irrigation rate on the production of marketable sweetpotato; and
3. determine the influence of mulches on yields and infestation levels of sweetpotato roots by SPW.

## Materials and Methods

All studies were conducted at the University of the Virgin Islands Agricultural Experiment Station on St Croix, USVI. The soil is Fredensborg loamy, fine, carbonatic, isohyperthermic, shallow, typic Calciustoll (Lugo-Lopez and Rivera, 1980). This series consists of well-drained soils (pH 7.8–8.4) formed over limestone or marl.

All plots were established with terminal vine cuttings, 0.3–0.4 m long. Plot sizes were 3 m × 3.7 m and consisted of 3 rows (banks) spaced 1 m apart. Plants were spaced 0.3 m within rows. Micro-irrigation was applied for crop establishment and to prevent moisture stress. The irrigation system consisted of 15-mm poly-hose mains and sub-mains with laterals of 15 mL Drip Strip Plus tape (Hardie Irrigation,

El Cajon, CA.) with laser-drilled orifices 0.3 m apart.

The experimental design was a randomized complete block with four replications. At harvest, 10 plants from the centre row of each plot were harvested. The foliage was cut at ground level and removed. Storage roots were then dug out of the soil, collected, and sorted, first based upon size, then SPW damage. Statistical analysis of data was performed using SAS General Linear Models procedure (SAS, 1988). All per cent data were transformed prior to analysis.

## Germplasm evaluation

The trials involved cultivars obtained from the U.S.A.: Picadito; St Kitts: SKB-2 and SKB-4; USVI: Black Rock, EDA, MC, Three Months, and CS-2; and Puerto Rico (USDA-TARS): all other cultivars.

The first three experiments consisted of 10 cultivars each. At harvest, the weight of all storage roots of marketable size (>2.5 cm diameter) was recorded as total yield. Roots infested with SPW were separated and recorded as a percentage of the total yield (% SPW damage). Uninfested roots were recorded as marketable yield.

Experiment 4 evaluated seven cultivars. In addition to total and marketable yields, data were also collected for storage root size.

Experiment 5 evaluated nine cultivars, six of which were included in previous experiments. Observations made were ease of propagation, plant vigour, and ability to smother weeds. Plant foliage was harvested by cutting the main stem at ground level. Total fresh foliage weight was recorded and sub-samples were oven-dried at 70°C to a constant weight, for dry matter (DM) determination. All storage roots were weighed and recorded as total yield. Storage roots were then separated into marketable and non-marketable categories based upon size. Marketable-sized storage roots were then examined for SPW damage. The damaged storage roots were weighed and recorded. Sub-samples of storage roots were peeled, sliced, and dried at 70°C to a constant weight, for DM determination.

## Irrigation

Sweetpotato Viola was grown in plots with controlled soil moisture levels. The treatments were rain-fed (no applied irrigation) and irrigation applied to maintain soil moisture levels at 20, 40, and 60 kPa. Tensiometers were installed in each plot to monitor soil moisture tension. The tensiometers were read daily and the irrigation system was turned on when the tensiometer readings exceeded the required soil moisture level. Water meters were installed for each treatment to record water use.

At maturity, 10 plants from the centre row of each plot were harvested. The weight and number of all storage roots were recorded.

Storage roots were divided into three size categories: small (<2.5-cm dia.), medium (marketable size), and jumbo (>12.5-cm dia. or longer than 25 cm). Each root was then inspected and rated on an SPW damage index (DI) scale of 1–6 (Jansson *et al.*, 1990): 1, no damage; 2, up to 25% of root surface area (RSA), has feeding punctures (FP) but no adult exit holes (EH); 3, 26–50% of RSA has FP but no EH; 4, >50% of RSA has FP or 1–3 EH present (or both); 5, 4–6 EH; and 6, >6 EH. The percentage of the total root biomass (all storage roots) in each damage category was recorded. The mean damage index (MDI) for each plot was calculated using the following formula, as described by Jansson *et al.* (1990):

$$MDI = \frac{\sum (s_i) \times (i) + (m_i) \times (i) + (j_i) \times (i)}{trb}$$

$$i = 1, \dots, 6$$

where *s*, *m*, and *j* are the biomass (kg) of small, medium, and jumbo-sized storage roots, respectively; *trb* is the total root biomass; and *i* is the damage category. Marketable yield were all medium-sized roots with a rating of  $\leq 2$ .

### Mulching

Two trials were conducted to evaluate mulches (grass mulch, black plastic mulch, and weed barrier) and a bare soil treatment for their effectiveness on yield and SPW infestation levels. One trial utilized the cultivar Viola, and the other, cultivar Sunny. These two cultivars were selected because they had been rated to have good culinary qualities by a consumer preference panel. The mulches were applied to the soil surface prior to planting. Holes were cut at 0.3-m spacings along the rows in the weed barrier (De Witt Pro 5) and plastic mulch (2 mil. polyethylene) to allow for the planting of the vine cuttings. The grass mulch was applied to the soil surface in a 13-cm depth. The collection of data at harvest was the same as for the irrigation trial.

## Results and Discussion

### Germplasm evaluation

The cultivars used in Experiment 1 were Miguela, Toquesita, Squish, Whity Thany, Vida, EAS-12, Tapato Fine, Tano, Limonette, and Sabino Red. Overall, best-yielding cultivars had a higher percentage of storage roots infested by SPW. Yields were relatively low. Miguela (9.4 t ha<sup>-1</sup>) and Toquesita (9.0 t ha<sup>-1</sup>) produced total yields which were significantly higher ( $P < 0.05$ ) than Tapato Fine, Tano, Limonette, and Sabino Red. The marketable yield of 4.8 t ha<sup>-1</sup> from Toquesita was higher ( $P < 0.05$ ) than the yield from Tano, Limonette, and Sabino Red, even though

Toquesita had 49% SPW damage. Miguela with a 79% SPW damage had a marketable yield of only 2.0 t ha<sup>-1</sup>. The high total yield from Miguela is consistent with traditionally high yields produced by this cultivar in Puerto Rico (Badillo-Feliciano *et al.*, 1976a).

The cultivars used in Experiment 2 were Colorette, Perla, Agata, Amatista, EAS-11, Suabor 2, Suabor, Dune, Viola, and Bonaro. Total yields ranged from 5.2 to 21.2 t ha<sup>-1</sup>, and were on average higher than from Experiment 1. Colorette produced a total yield of 21.2 t ha<sup>-1</sup> which was significantly higher ( $P < 0.05$ ) than all other cultivars except Perla, Agata, and Amatista. Colorette also produced a significantly higher quantity of marketable storage roots (9.9 t ha<sup>-1</sup>) than the other cultivars. Perla (6.8 t ha<sup>-1</sup>) had the next highest marketable yields. Agata and Amatista which had high total yields also had high SPW damage (79 and 74%, respectively) resulting in low marketable yields. In this experiment, Colorette was clearly the superior cultivar in terms of yield.

In Experiment 3, cultivars Twelve Prime, Mont Blanc, Trompo Negro, EAS-15, EAS-13, EAS-10, Black Rock, Ninety-Nine, St Georges, and Margarita were evaluated. Twelve Prime had a significantly higher ( $P < 0.05$ ) total yield than all other cultivars tested. Marketable yield from this cultivar and Trompo Negro were the highest ( $P < 0.05$ ). Mont Blanc produced a total yield of 7.4 t ha<sup>-1</sup> which was superior ( $P < 0.05$ ) to most of the other cultivars. However, Mont Blanc had only 1.7 t ha<sup>-1</sup> marketable yield due to 80% SPW damage.

Trompo Negro, despite its relatively good production of storage roots, had only 7% SPW damage resulting in a marketable yield of 5.3 t ha<sup>-1</sup>. Black Rock, a popular local cultivar for both farmers and consumers, produced low yields and had a relatively high level of SPW damage (51%).

Cultivars in Experiment 4 (Viola, Tapato, Sunny, EDA, Picadito, MC, and Three Months) had negligible SPW damage. The plot was located in an area where sweetpotato had not been established for a number of years. Total and marketable yields were generally high, due to the low incidence of SPW. Viola, Tapato, and Sunny were the best-yielding cultivars in terms of total (19.5, 16.4, and 14.3 t ha<sup>-1</sup>, respectively) and marketable (16.2, 14.2, and 13.1 t ha<sup>-1</sup>, respectively) storage roots produced. These yields were higher ( $P < 0.05$ ) than the yields of local cultivars MC and Three Months. These three cultivars (Viola, Sunny, and Tapato) were bred to produce on heavy soils in Puerto Rico (Martin, 1987). Cultivar Picadito produced the largest ( $P < 0.05$ ) marketable storage roots, weighing an average of 438 g root<sup>-1</sup>.

In Experiment 5, nine cultivars (SKB-4, CS-2, SKB-2, Sunny, Viola, Black Rock, Tapato, Trompo Negro, and Perla) were studied. Cultivars SKB-4, CS-2, and SKB-2 each pro-

duced significantly ( $P < 0.05$ ) larger quantities of fresh foliage (34.3, 28.1, and 27.2 t ha<sup>-1</sup>, respectively), than from the other cultivars. The foliage of Perla had the highest per cent DM but this cultivar produced the least foliage. This information is important because sweetpotato foliage can be consumed as a leaf vegetable or fed to animals. Foliage yield was directly related to plant vigour and the ability of the cultivars to smother weeds. Vigorous vine growth reduced the number of times the plots had to be weeded.

Trompo Negro and Black Rock produced less ( $P < 0.05$ ) fresh biomass than the other cultivars. The biomass produced by SKB-4, SKB-2, Viola, and CS-2 was over 40 t ha<sup>-1</sup> fresh, twice the amount produced by Trompo Negro and Black Rock.

In Experiment 6, the cultivars studied were Perla, Viola, Tapato, Sunny, SKB-2, CS-2, SKB-4, Trompo Negro, and Black Rock. Cultivars from USDA-TARS (Puerto Rico), had the highest total and marketable yield. This pattern was similar to the yield obtained in Experiment 4. Perla (28.6 t ha<sup>-1</sup>) and Viola (26.8 t ha<sup>-1</sup>) had a higher ( $P < 0.05$ ) total yield than the other cultivars except Tapato and Sunny. Perla, Viola, and Tapato produced higher ( $P < 0.05$ ) marketable yields (24.5, 21.9, and 21.4 t ha<sup>-1</sup>, respectively) than all other cultivars except Sunny (17.3 t ha<sup>-1</sup>). Perla, a non-sweet type cultivar, has also produced high yields of 27–42.1 t ha<sup>-1</sup> in trials in Puerto Rico (Badillo-Feliciano *et al.*, 1976b). Trompo Negro and Black Rock had the lowest total and marketable yields. The highest-yielding cultivars had a lower percentage of roots damaged by SPW. Tapato sweetpotato with an average weight of 446 g root<sup>-1</sup>, was larger ( $P < 0.05$ ) than all other cultivars tested. In Puerto Rico this cultivar produced such large roots that it was considered useful for industrial purposes (Martin, 1987).

Perla, Tapato, and Sunny produced thin vines which can make propagation, handling, and crop establishment a little more tedious than for the other cultivars. These cultivars, however, along with Viola, which produced a similar quantity of foliage, gave the highest root yields. An inverse relationship between foliage and storage root production is, therefore, apparent. Sajjapongse and Roan (1982) reported this relationship whereby excessive top growth may result in low root yield. There was a tendency for low-yielding cultivars to have a higher percentage of DM in their storage roots, compared to the high-yielding cultivars.

Overall, these trials suggest that some USDA-TARS cultivars are well adapted for sweetpotato production in the USVI. This indicates the possibility of improving the yield of sweetpotato in the USVI by utilizing introduced germplasm. The local cultivars produced low yields compared to introduced cultivars. A similar finding was reported by Huett (1976) in Australia, where cultivars from the U.S.A. yielded 3–4

times as much storage roots as the local commercial cultivar.

## Irrigation

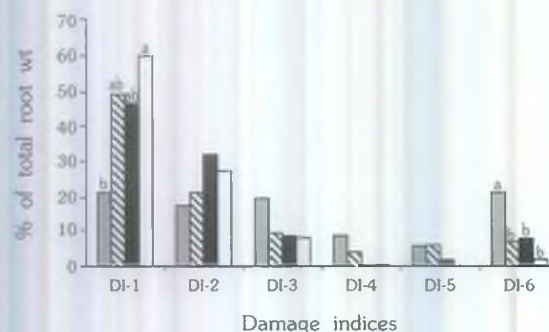
The application of irrigation did not significantly influence total root biomass (Table 1). Plants grown in the plots with soil moisture maintained at 40 kPa produced significantly more medium-sized storage roots (8.1 t ha<sup>-1</sup>) than plants grown in the 20 kPa and rain-fed plots, which produced 5.4 and 4.4 t ha<sup>-1</sup>, respectively (Table 1). All irrigated treatments produced significantly more marketable storage roots, with a lower MDI, than the rain-fed treatment. There was an inverse relationship between MDI and soil moisture levels among the irrigation treatments. A significantly higher percentage of storage roots (60.7%) from the 20 kPa treatment was rated in the Damage Index (DI)-1 (uninfested roots) category than from the rain-fed treatment (21.9%) (Figure 1). Additionally, the percentage (24.3%) of storage roots from the rain-fed treatment rated in the DI-6 (most severe) category was significantly higher than from the applied irrigation treatments with 7.6, 8.2, and 2.1%, respectively, for the 60, 40, and 20 kPa treatments. The data in Table 2 show that a significantly higher percentage (73–89%) of medium-sized storage roots from the irrigated plots were marketable compared to the rain-fed plots (33%). The rain-fed treatment produced storage roots which had a higher DM

**Table 1** Effect of irrigation on Viola sweetpotato storage roots and dry matter (DM) production (t ha<sup>-1</sup>)

Treatment	Root biomass	Medium size	Marketable	DM
Rain-fed	6.5	4.4 b*	1.5 b	0.6 b
60 kPa	9.3	7.6 ab	5.1 a	1.9 a
40 kPa	9.8	8.1 a	6.3 a	2.2 a
20 kPa	7.1 ns	5.4 b	4.8 a	1.7 a

\*Means separated by LSD<sub>0.05</sub>

Treatment means with a common letter are not significantly different



**Figure 1** Effect of irrigation on SPW damage indices 1–6 for Sunny; ■, Rain-fed; ▨, 60 kPa; ■, 40 kPa; □, 20 kPa



**Table 2** Effect of irrigation on SPW infestation of *Viola* sweetpotato

Treatment	MDI	% Medium-sized marketable	% DM
Rain-fed	3.3 a*	33.0 b†	37.7 a
60 kPa	2.2 b	73.2 a	36.4 ab
40 kPa	2.1 b	79.1 a	34.9 b
20 kPa	1.6 b	89.2 a	34.9 b

\*Means separated by LSD<sub>0.05</sub>

†Data arcsin transformed before statistical analysis was performed

DM is dry matter

MDI is mean damage index

Treatment means with a common letter are not significantly different

content than the 20 and 40 kPa irrigated treatments (Table 2). However, because of the higher yields from the irrigated plots, the total DM production was significantly higher for the irrigated plots, when compared to the rain-fed plots (Table 1). Irrigation, therefore, has potential to increase sweetpotato marketable yields while reducing SPW infestation levels, possibly by reducing the size and number of cracks in the soil.

Total irrigation water use was highest for the 20 kPa treatment but this treatment had the lowest water use efficiency (WUE) and returns to irrigation (Table 3). The water use data for the 60 kPa treatment showed the lowest water use with the highest WUE and returns to irrigation. The results of this study show that based solely on the economics of water use, sweetpotato should be grown in plots maintained at 60 kPa.

### Mulch

During the early period of crop establishment, a number of plants had to be replaced in the plastic mulch treatment, particularly for Sunny. This was due to cuttings becoming scorched when they touched the plastic mulch during the periods of high solar radiation.

### Sunny Cultivar

The application of mulches to Sunny sweetpotato did not significantly affect total root

**Table 3** Water use efficiency, water cost, and returns to irrigation of sweetpotato

Treatment	Water use (m <sup>3</sup> ha <sup>-1</sup> )	WUE <sup>a</sup> (kg m <sup>-3</sup> )	Water cost <sup>b</sup> (\$ ha <sup>-1</sup> )	Returns to irrigation water <sup>c</sup> (\$ \$ <sup>-1</sup> )
60 kPa	509.1	10.0	2153.5	2.6
40 kPa	1238.2	5.1	5237.6	1.3
20 kPa	1990.4	2.4	8419.4	0.6

<sup>a</sup>WUE, Water use efficiency in kg marketable yield m<sup>-3</sup> irrigation water<sup>b</sup>Water cost based on \$4.23 m<sup>-3</sup><sup>c</sup>Dollar return to every dollar spent for irrigation water based on a price of \$1.00 per kg sweetpotato

biomass (Table 4). Plants grown with the plastic mulch treatment had a significantly higher yield of medium-sized storage roots than plants grown with the bare soil (Table 4). Storage roots from the weed barrier plots had a higher MDI than roots from the grass mulch plots (Table 4). A higher percentage of storage roots from the grass mulch plots was rated in the DI-1 (clean roots) category than either of the synthetic mulch (plastic and weed barrier) treatments (Figure 2). A small percentage of storage roots from each treatment was rated in the categories with severe damage (DI 4–6). However, there was a trend towards higher percentages in these categories from the weed barrier treatment. The total percentages for DI 4–6 were 1.1, 4.8, 9.7, and 23.4 for the grass, plastic, bare soil, and weed barrier, respectively. Fairly high percentages (>20 %) were in the DI-3 category for the bare soil, plastic, and weed barrier treatments (Figure 2). This is an unmarketable category with the least SPW damage. Therefore, a little improvement in the level of SPW control, perhaps by the inclusion of other biological or cultural practices (e.g., irrigation or pheromone) may cause a substantial improvement in the quantity of marketable yield. These practices need to be investigated.

The grass mulch plots produced a higher percentage of the total root biomass with a DI rating of ≤2 (marketable quality) than did the weed barrier plots. Marketable yields of 18.4,

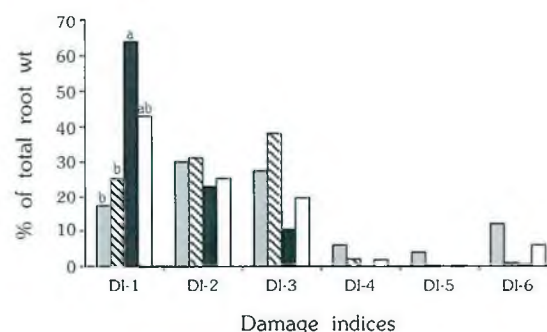
**Table 4** Yield of storage roots of Sunny sweetpotato in response to mulch application (t ha<sup>-1</sup>)

Treatment	Root biomass	Medium size	Marketable	MDI
Weed barrier	25.6	22.4 ab*	9.3	2.8 a
Plastic mulch	29.7	26.0 a	17.0	2.3 ab
Grass mulch	26.1	21.9 ab	18.4	1.5 b
Bare soil	15.4 ns	11.7 b	7.8 ns	2.1 ab

\*Means separated by LSD<sub>0.05</sub>

MDI is mean damage index

Treatment means with a common letter are not significantly different

**Figure 2** Effect of mulching on SPW damage indices 1–6 for Sunny; □, Weed barrier; ▨, Plastic mulch; ■, Grass mulch; □, Bare soil

17.0, 9.3, and 7.8 t ha<sup>-1</sup> were obtained from the grass, plastic, weed barrier, and bare soil treatments, respectively (Table 4). More than half (56%) of the medium-sized storage roots from the weed barrier treatment were rendered unmarketable due to SPW damage. In comparison, 85% of the marketable-sized storage roots from the grass mulch treatment were observed to be marketable. This indicates that the weed barrier is not an effective physical barrier to SPW, when growing Sunny sweetpotato.

### Viola Cultivar

The grass mulch treatment produced the highest yield of total root biomass and medium-sized roots. The plastic mulch treatment had the lowest yield, in contrast to the results for Sunny, where the yield from the plastic mulch was highest for both parameters. Storage roots from the weed barrier treatment had a significantly higher MDI (Table 5) and a lower percentage (22.3%) of roots in the DI-1 category, than all the other treatments. There was a trend for the weed barrier treatment to produce the highest percentage of roots in the unmarketable categories, DI-3 to DI-6. Plants grown with weed barrier produced storage roots of which 23.7% were rated in the most severe damage category (DI-6). The percentage (52.5) of roots from this treatment was lower in the categories considered marketable (DI ≤2) compared to the other treatments, which ranged from 79.8–89%. Marketable yields of 16.5, 15.5, 10.4, and 9.5 t ha<sup>-1</sup> were obtained from the grass, bare soil, plastic, and weed barrier, respectively. The percentage (50.7) of medium-sized storage roots from the weed barrier treatment which were marketable (Table 5), was significantly lower than all the other treatments (77.6–87.9%).

These trials have shown that a differential response to the various mulches exists between the two cultivars. Generally, there was an increased yield response from the Sunny cultivar to all mulches, which resulted in increased marketable yield. The MDI tended to be higher for Sunny than for Viola. Regarding the enhancement of marketable yield, the application of a grass mulch tends to be beneficial to both cultivars.

**Table 5** Effect of mulches on SPW infestation of Viola sweetpotato

Treatment	MDI <sup>1</sup>	% Medium-sized marketable
Weed barrier	3.1 a*	50.7 b <sup>†</sup>
Plastic mulch	1.4 b	87.9 a
Grass mulch	1.9 b	77.6 a
Bare soil	1.6 b	87.8 a

\*Means separated by LSD<sub>0.05</sub>

<sup>†</sup>Data arcsin transformed before statistical analysis was performed

<sup>1</sup>MDI is mean damage index

Treatment means with a common letter are not significantly different

In the weed barrier treatment, for both cultivars, SPW damage was more severe than for the bare soil treatment. When the weed barrier was removed from the soil surface during harvesting, the number of weevils present made it obvious that this treatment was not effective. It appears that the weed barrier created a micro-environment that was favourable to the SPW.

Plastic mulch increased the total yield of Sunny, but not Viola. Hochmuth and Howell (1983) have reported higher yields from black plastic mulch, similar to the results obtained for Sunny. Plastic mulch did not, however, reduce the SPW infestation levels for either of the two cultivars, compared to the bare soil treatment. Similar findings have been reported in Florida by Jansson *et al.* (1987) where no difference in SPW damage was found in four cultivars between plots with and without plastic mulch. Conversely, Talekar (1987) reported that the application of plastic film to plots reduced SPW infestations compared to plots without mulch.

The grass mulch treatment appears to have the most potential for controlling SPW, particularly for Sunny cultivar. This is in agreement with results obtained in Taiwan by Talekar (1987), where the application of rice straw mulch was found to reduce SPW infestations compared to non-mulched plots. This finding has special implication to the USVI and the other Caribbean countries, where grass is easily and inexpensively obtained.

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# Yield stability differences among sweetpotato genotypes under field and controlled environments

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Two studies were conducted in 1995 and 1996 in the field and under controlled environments to test the yield and dry matter (DM) content stability of selected sweetpotato genotypes. In the field study, initial screenings of over 300 genotypes were conducted. Six genotypes were selected and further tested under a controlled environment for their adaptability in the Tuskegee University Nutrient Film Technique system for inclusion in crop production for long-duration space missions. In both studies, plants were grown for 120 days. Data collected over the two-year period were combined and comparisons of genotype performance were done between the two growing conditions. Regression analysis on overall mean for the two environments showed that yield for the six selected genotypes were highest when plants were grown under field conditions. For all six genotypes, there were differences shown in yield performance when they were grown in the greenhouse or under the controlled environment. There was a significant interaction between environment and genotypes for root yield while DM accumulation was independent of environment. Yield stability analysis indicated that lower-yielding genotypes regress closer to unity than higher-yielding genotypes, while variance due to deviations from regression was closest to zero for genotypes whose mean yields were closest to the grand mean.

Keywords: Hydroponics; Nutrient film technique; Plant breeding; Space agriculture

Studies carried out by Collins *et al.* (1987) showed that sweetpotato yield was sensitive to environmental changes and that many other traits of interest to sweetpotato breeders were also highly influenced by the environment. Therefore, assessment of genotype  $\times$  environment interaction will allow breeders to make better germplasm selection.

Yield stability parameter estimates (Eberhart and Russel, 1966) which identify genotypes with stable yield performances across diverse environments can greatly assist the breeder in reducing cost and time spent on evaluation of less stable genotypes. Martin *et al.* (1988) showed that by using one key environment as part of their yield stability analysis, they were able to predict yield stability for genotypes over 12 environments. Ortiz and Izquierdo (1994) also found that yield stability estimates were capable of predicting stable high-yielding open-pollinated tomato hybrids in various environments. Poysa *et al.* (1986) found that tomato genotypes with low yields had greater yield stability than genotypes with high but unstable yields.

Sweetpotato has been selected by the National Aeronautics Space Administration as a crop for advanced life support (ALS) systems. This has resulted in the need to screen and select genotypes specifically for ALS environments. Current selection of sweetpotato geno-

types for ALS systems involves evaluation and screening in the field and under a controlled environment (David *et al.*, 1994; Mortley *et al.*, 1991). Initially, genotypes are evaluated in the field and then tested for adaptability to Tuskegee University, Nutrient Film Technique (NET) system under a controlled environment. Utilizing yield stability analysis will allow for greater efficiency and predictability of high-yielding, stable genotypes under controlled environments, and also result in greater utilization of limited resources, as well as reduce the time required in the identification of genotypes that need further physiological evaluations which are specific for ALS environments.

The objectives of this study were to evaluate sweetpotato genotypes for yield performance and to assess the phenotypic yield stability of the selected genotypes under field and controlled environments.

## Materials and Methods

### Field studies

Field evaluations were conducted in 1995 and 1996 on the Tuskegee University Experiment Station in Tuskegee, Alabama. Seeds of 10

accessions resulting in 300 genotypes were initially evaluated in the field. Six of these genotypes (J6/76, NC C58, J6/66, TU-82-155, J6/62, and J8/1) were selected based on yield and dry matter (DM) content and placed in replicated trials. Dry matter selection criterion was based on a DM of 25% or higher. Sweetpotato vine cuttings were planted 18 cm apart on 75-cm rows, 1 m apart. All plots received a pre-plant application of 56 kg N, 60 kg P, and 112 kg K ha<sup>-1</sup>. Four weeks after planting, ammonium nitrate was applied at 38 kg N ha<sup>-1</sup>. Six weeks later, murate of potash was applied at the rate of 112 kg K ha<sup>-1</sup>. In both years, plants were grown under rainfed conditions. Plants were harvested 120 days after planting. At harvest, roots were graded and weighed based on current USDA standards. For DM determination 50-g samples were taken from five randomly selected roots of US #1 grades and dried at 70°C for 48 h.

### Controlled environment studies

Sweetpotato vine cuttings, each 15 cm long, from each of the six genotypes used in the field trials were placed in a recirculating NFT system as described by Hill *et al.* (1984) and Morris *et al.* (1989) and planted 25 cm apart in 0.15 m × 0.15 m × 1.2 m growth channels. Each channel contained four plants of the same genotype. Growing vines were held in place in the growth channels by a flat plate assembly (Morris *et al.*, 1989) and plants were allowed to grow for 120 days.

Nutrient solution of modified half-strength Hoagland's solution (Hoagland and Arnon, 1950) with a 1:2.4 N:K ratio was supplied from a reservoir volume of 30.4 L. The solution was pumped to the opposite end of each growth channel by a submersible pump (Teel Model 1p680 A horse power; Dayton Electric, Chicago) and spread across each channel (1% slope) in a thin film as it flowed back into the container. Nutrient solution flow rate was set at 1 L min<sup>-1</sup> using a bypass line to each container and was adjusted with a control valve. Nutrient solution protocol consisted of daily water replenishment to maintain the reservoir volume. Nutrients were replenished as needed when the electrical conductivity of the nutrient solution fell below 1200 µS cm<sup>-1</sup>. The solution pH was adjusted to 6.0 by adding (1N) HCl or NaOH at the time of planting and allowed to fluctuate between 6.0 and 4.0. Plants were also grown in an environmental growth chamber for 120 days at ambient CO<sub>2</sub> levels, a diurnal temperature cycle of 28/22°C, 70% relative humidity, and irradiance between 400–500 µmol m<sup>-2</sup> s<sup>-1</sup>. At harvest, plants were weighed for storage roots yield and DM was determined.

The design used for both studies was a complete randomized design with two replications in time. Each genotype's phenotypic stability was determined by regression analysis (Eberhart and

Russel, 1966). A genotype was considered stable if  $b_1$  (coefficient of regression) = 1 and  $s^2_d$  (variance due to deviations from regression) = 0. To determine the consistency of the stability characteristic  $b_1$ , an F statistic was used to test if it differed significantly from unity. Analysis of variance was also conducted and standard error of means used to evaluate yield performance in both environments.

## Results and Discussion

There were significant differences among genotypes for yield and DM accumulation (Tables 1 and 2). Storage root yield ranged from 488 ± 271 to 1380 ± 589.7 g plant<sup>-1</sup> under field environment and 251.5 ± 14.9 to 982 ± 25.5 g plant<sup>-1</sup> under the controlled environment. Generally, field root yields were higher for all genotypes than under the controlled environment. This reduction in yield under the controlled environment may be as a result of the high moisture conditions under the flat plate assembly. In addition, oxygen levels may be lower in the root environment of the genotypes grown under the controlled environment than that of the field. However, genotypes which were high producers under the field condition maintained this trend under the controlled environment except J8/1 which showed a large decrease in yield under the controlled environment.

**Table 1** Mean root yield of six sweetpotato clones under field and controlled environment

Clone	Yield (g plant <sup>-1</sup> )	
	Field	Controlled environment
J6/76	593.5±522.6 <sup>a</sup>	826.0±21.2
NC C58	1351.0±0.7	744.5±84.2
J6/66	583.0±337.9	982.0±25.5
TU-82-155	614.0±16.9	612.0±46.7
J6/62	488.0±271.5	252.5±12.0
J8/1	1380.0±589.7	251.5±14.9

<sup>a</sup>Standard error of means

**Table 2** Mean dry matter content of six sweetpotato clones under field and controlled environment

Clone	Dry matter (%)		
	Field	Controlled environment	Combined
J6/76	21.8±4.1 <sup>a</sup>	19.3±3.5	20.55±3.4
NC C58	17.0±3.2	14.6±3.1	15.80±2.2
J6/66	28.8±2.3	18.0±4.0	23.40±6.7
TU-82-155	16.7±0.14	15.0±1.7	15.85±1.3
J6/62	29.2±1.2	22.5±0.07	25.85±3.9
J8/1	26.9±2.8	25.6±1.7	26.25±2.0

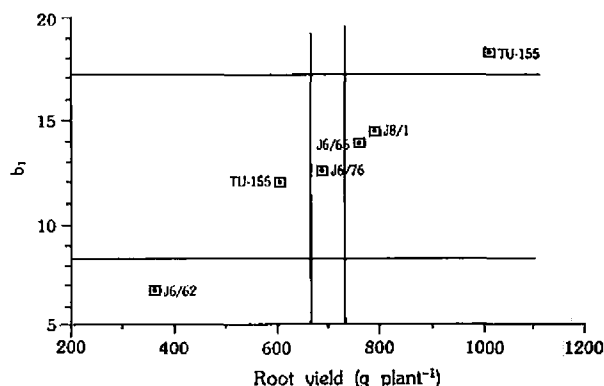
<sup>a</sup>Standard error of means

Dry matter accumulation followed similar trends to root yield in the field condition versus the controlled environment. However, yield performance was inversely related to DM accumulation, with high yielding genotypes showing lower DM accumulation. Combined mean DM yields for both environments showed that J8/1, J6/62, and J6/66 consistently produced greater yields than the grand mean of both environments (Table 2). Although J8/1 was the lowest producer under the controlled environment for root yield, its combined mean DM accumulation was significantly higher than the grand mean.

Analysis of variance showed a significant genotypes  $\times$  environment interaction for root yield and a nonsignificant interaction for DM accumulation (Table 3) which indicates that the genotypes tested showed different root yield responses in the two environments. As a result, yield stability analysis was performed only on root yield to identify high yielding but stable genotypes. The genotypes J6/66, J8/1, and NC C58 were considered high yielding since their overall means were significantly greater than that of the grand mean of the environments (Figure 1) and showed a consistent stability characteristic of  $b_1$ , as variance due to deviations from regression ( $s^2_d$ ) was not significantly greater than 1. However, conclusions

**Table 3** Combined analysis of variance for root yield and dry matter content for six sweetpotato clones

Source of variation	Mean squares	
	Yield (g plant <sup>-1</sup> )	Dry matter (%)
Environment (E)	203450.0*	106.3*
Genotypes (G)	299713.5*	87.9*
G $\times$ E	322072.5**	14.0
Coefficient of variability (%)	36.14	11.72



**Figure 1** Regressive coefficient  $b_1$  of the cultivar means over environmental index plotted against cultivar means over field and controlled environments

**Table 4** Means and two yield stability parameters for root yield of six sweetpotato clones

Clone	Yield (g plant <sup>-1</sup> )		
	$\bar{x}$	$\beta$	$s^2_d$
J6/76	709.80 $\pm$ 330	12.70	0.48
NC C58	1047.80 $\pm$ 353	18.70	0.81
J6/66	782.50 $\pm$ 302 ab	14.00	0.60
TU-82-155	613.00 $\pm$ 28	11.98	0.63
J6/62	370.30 $\pm$ 207	6.63	0.80
J8/1	815.80 $\pm$ 735	14.61	2.98

\*Means within columns are not significantly different at  $P < 0.05$   
 $\beta$  = coefficient of regression  
 $s^2_d$  = variance due to deviations from regression

could not be drawn as to their stability as their means over both environments did not regress very close to unity.

For all genotypes, regression coefficient estimates were not significantly different from unity (Table 4) even though genotypes did not regress very close to unity as was expected. In contrast, estimates were very close to zero. These results are opposite to those of Eberhart and Russel (1966) who showed that estimates can only be obtained from testing several environments and that a good estimate of regression coefficient can be achieved with fewer environments. Since one of the testing environments in the study used environmental parameters that were controlled, this may explain the low values obtained for stability estimates. Therefore, utilizing only parameter estimates, J6/76 would be considered the most stable genotype and would be expected to perform well under favourable as well as adverse conditions. The remaining genotypes may be considered as possessing average yield stability except J8/1 which was considered unacceptable for ALS environment.

The inconsistency shown in the present results from stability analysis could have been due to the small sample size used in the study, as well as, the fact that only two environments were investigated. Further, the wide variation that existed between the two environments may also have contributed to these results. Grafius (1969) emphasized the importance of the variation in environmental stress in measuring the G  $\times$  E interaction. He stated that if stresses are excessive, yields might be reduced to unacceptable low levels, and that an environment with intermediate levels of stress factors, might permit selection of lines with stress tolerances that would convey yield superiority in the widest range of environments. Therefore, the ideal environment for yield testing may not be one that gives maximum yields (Martin *et al.*, 1987).

Although results were somewhat inconsistent, yield stability analysis can be used as a tool for identifying stable high yielding cultivars for ALS systems. A larger germplasm pool needs to be assessed in the future.

## Acknowledgements

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# The potential for extending the shelf-life of sweetpotato in East Africa through cultivar selection

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The potential of sweetpotato is limited by the perishable nature of the storage tubers. An extension of storage-life could be brought about, both by improving the storage environment and by breeding for extended shelf-life. The latter has the advantage in that it would result in an increased storage-life, both under conditions designed for long-term storage and under normal marketing conditions without incurring additional costs for producers and traders. To determine the feasibility of selecting cultivars for extended shelf-life, it is important to determine the genetic range in perishability among existing germplasm and the effect of the growth environment. In addition, a practical methodology for selection should be established. If possible, physiological characteristics associated with perishability should be identified to allow the development of indirect selection techniques. The progress of collaborative work conducted in Tanzania to address these issues is presented. Twenty-nine sweetpotato varieties, representative of the wide genetic variability of germplasm available in Tanzania, were assessed for their perishability. Under simulated Tanzanian marketing conditions, the major forms of deterioration observed were weight loss (primarily due to water loss) and rotting. Varieties varied considerably in both their rates of weight loss and of rotting, and these two characteristics were significantly correlated. Market observations have indicated that roots are subjected to considerable mechanical damage during normal transport and marketing. Thus, a range of varieties were tested for their rate of deterioration following simulated damage. In initial trials the ranking of varieties was not affected by the damage treatment, indicating that simulated damage need not be used during the selection procedure. These results suggest that indirect selection on the basis of physiological parameters may form part of the breeding programme in Tanzania.

Keywords: Sweetpotato; Shelf-life; Germplasm; Deterioration; East Africa

The short shelf-life of the sweetpotato storage tuber after harvest is a serious constraint to the use of the crop for food security in tropical regions. Sweetpotato can be kept for several months if it is maintained at 13–15°C (Picha, 1986; Woolfe, 1992). Even at tropical temperatures it is possible to maintain tubers in good condition for up to 4–5 months (Devereau, 1995), if the tubers are undamaged and stored in pits. However, in the tropics, marketing may involve transport over long distances and it is often not possible to maintain tubers consistently under ideal conditions, and they deteriorate rapidly. In Tanzania, it has been observed that tubers rarely keep for longer than two to three weeks (NRTCP, 1997; Bancroft, R. pers. commun.). An extension of shelf-life through better handling techniques and the use of cultivars with better keeping quality would improve the potential for transporting and trading the commodity. With the increasing urbanization of the East African population, the provision of food to urban centres is of growing importance.

A study of Tanzanian markets (NRTCP, 1997) has shown that the main forms of deterioration are rotting and weight loss, both of which were assessed in these trials. It has also shown that the level of tuber damage in the market can be very high, with a high proportion of tubers showing cuts, breaks, bruises, insect infestation, and rodent damage. Damage increases the rate of deterioration with breaks and cuts having the greatest effect. In this study the effect of damage of tubers by cutting, on the subsequent rate of deterioration, was tested for a range of cultivars to determine if cultivars differ in their susceptibility.

This would allow the examination of the potential for breeding for less perishable cultivars with the following specific objectives:

- (i) to determine whether a sufficient range of storability exists within sweetpotato germplasm available in East Africa for breeding to be successful;
- (ii) to establish a suitable method for screening germplasm for storability; and
- (iii) to identify physiological characteristics associated with storability and thereby facilitate cultivar selection.

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## Materials and Methods

During 1996–97, trials were conducted at A.R.T.I. Ukiriguru (Lake Zone, Tanzania) on a wide range of sweetpotato germplasm, including local cultivars, recent crosses, and recently introduced cultivars. Storage tubers were tested for their rate of deterioration when stored under conditions simulating those to which tubers are normally subjected during marketing.

Storage tubers were obtained from two field trials. Nine cultivars were included in Trial 1 and 22 cultivars in Trial 2. Two cultivars (SPN/0 and Mwanamonde) were common to both trials. Cultivars were selected from local and introduced germplasm to provide a wide range of known characteristics, but included only those cultivars known to give reasonable yields.

Both trials were planted on 28 December 1996, with planting of extra cuttings on 17 January 1997 due to poor establishment as a result of drought. Both trials were planted as RCB designs. Trial 1 had four replicates with plots of 6 m × 6 m (3 plants m<sup>-1</sup>), while Trial 2 had two replicates with plots of 6 m × 2 m. Trials were harvested on 23 June 1997.

Tubers of marketable size (greater than 2.5 cm diameter) and good quality (i.e., low damage) were selected for post-harvest evaluation. For each cultivar these were divided into replicates (6 for Trial 1 and 3 for Trial 2) with 25 roots per replicate wherever possible.

To simulate normal marketing conditions, roots were stored in woven polythene sacks, which were closed by tying for two days to allow curing, then opened and rolled down to half height for the remainder of the storage period.

Two treatments were carried out for each cultivar for Trial 1. Tubers were either stored undamaged, or with simulated damage, consisting of two latitudinal cuts on each tuber. The cuts were made one third of the way along the tuber from each end, to a depth to reach the centre of the tuber.

At the start of the trial, tubers of each cultivar were assessed for a range of physiological and morphological characteristics including cortex thickness, latex production, hardness (measured by a penetrometer), concentration of soluble solids in root sap [measured by refractive index (R.I.)], and dry matter (DM) content. The methods used for these assessments are summarized in Rwiza *et al.* (1996).

For measurement of weight loss, six roots were selected at random from each replicate and were numbered using a marker pen. The weight of each of these roots was recorded at the start of the trial and at weekly intervals.

The extent of external rotting for each replicate was assessed at the start of the trial and at weekly intervals by sorting the tubers into six categories of visible per cent surface rotting (0; 1–10; 11–25; 26–50; 51–75; and 76–100 designated 1, 2a, 2b, 3, 4, and 5, respectively). An overall rotting score for each cultivar was

calculated for each replicate as  $(n_1 + 2.n_{2a} + 2.5.n_{2b} + 3.n_3 + 4.n_4 + 5.n_5)/(n_1 + n_{2a} + n_{2b} + n_3 + n_4 + n_5)$  where  $n_1$  is the number of tubers with a score of 0;  $n_{2a}$  is the number with a score between 1 and 10% and so on.

At the start of the trial and at weekly intervals two tubers were selected randomly from each sack for destructive assessment of internal rotting, and soluble solid content (R.I.) of tuber sap. For internal rotting, tubers were cut in half and scored in a similar way to that used for external rotting.

## Results

Tubers for Trial 1 were stored in an undamaged or damaged state to determine the effect of damage on the rate of deterioration of tubers of different sweetpotato cultivars. The effect of damage on the rate of weight loss and the rate of rotting at one, two, and three weeks of storage is shown in Table 1. Although the difference between treatments was significant in some cases, no interaction between treatment and cultivar was observed and the two treatments were, therefore, combined for all subsequent analyses and results are present by variety only.

The extent of externally observable rotting could be assessed using all the tubers of each

**Table 1** Trial 1 — The effect of damage treatment on the rate of deterioration of tubers

(a) Weight loss			
Treatment	% Wt loss (at one week)	% Wt loss (at two weeks)	% Wt loss (at three weeks)
Undamaged	12.0	23.6	35.7
Damaged	12.1	25.9	38.6
Treatment effect	ns	*	+
C.V. %	14.8	15.2	16.2
(b) Rotting <sup>1</sup>			
Treatment	Overall rotting (at one week)	Overall rotting (at two weeks)	Overall rotting (at three weeks)
Undamaged	1.51	2.45	3.64
Damaged	1.38	2.57	3.60
Treatment effect	*	+	ns
C.V. %	10.80	7.20	8.80

ns, Not significant; +, \*, significant at 10% and 5% level of probability, respectively

<sup>1</sup>Average score when tubers were sorted into the following categories: (1, 0% surface showing visible rotting; 2a, 1–10%; 2b, 11–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100%)

C.V. is Coefficient of Variation

sample, whereas internal rotting was assessed each week by destructive sampling of two randomly selected tubers of each sample. For this reason the coefficient of variation for internal rotting was high (23.4%). Nevertheless, in all cases the cultivar effects were very significant, and there was a wide range of perishability observed for the cultivars used in these trials. For example, in Trial 2, the weight loss over a two-week period ranged from 8.4 to 30.6%, while the overall rotting score ranged from 1.44 to 2.82 (data not shown). The ranking of cultivars was consistent over the whole period of storage (data not shown).

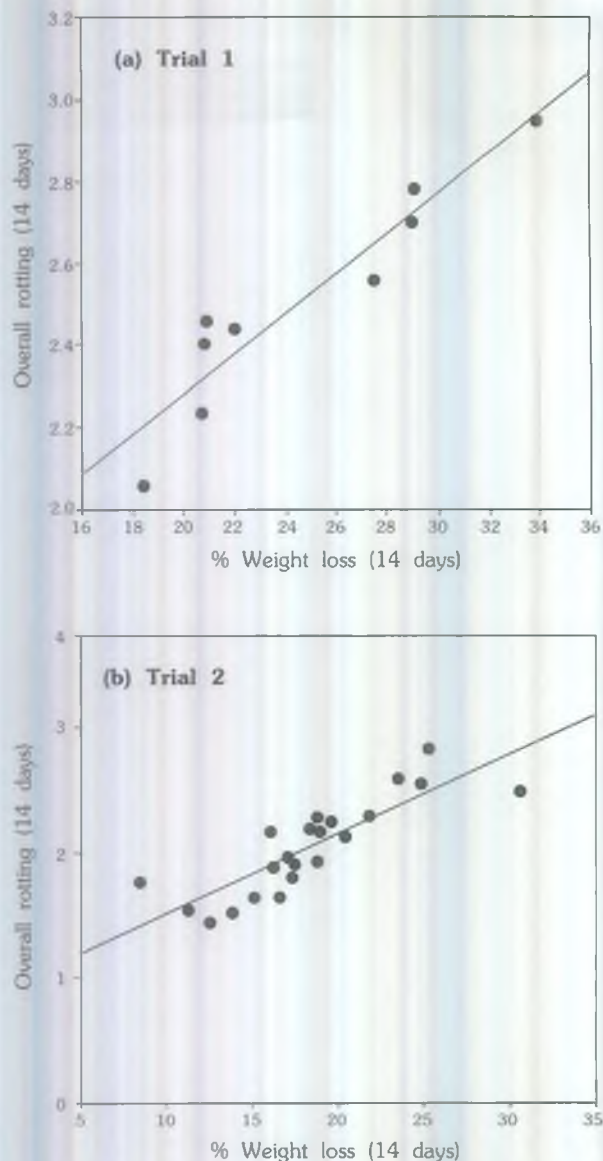
The yields for Trial 2 (14.43 t fresh wt ha<sup>-1</sup>) were considerably higher than for Trial 1 (6.19 t fresh wt ha<sup>-1</sup>), probably as a result of better drainage and lower pest pressure.

An important observation was that the amount of weight loss and the amount of rotting were highly correlated for both trials. This is illustrated in Figure 1 (a, b) which shows the relationship between rotting and weight loss at two weeks. Of particular note are the high correlations between weight loss after one week and subsequent rotting. This suggests a causative link between weight loss and rotting and the possibility that initial rate of weight loss could be used to predict susceptibility to rotting.

A number of physiological parameters that might be associated with rates of deterioration were measured to see if they could be used for indirect assessment of perishability. Latex contains a high level of phenolics, and is thought to be associated with resistance to pathogens. The extent of latex release when roots were broken was therefore assessed subjectively using a 1–5 score. During farmer assessment of cultivars in Tanzania, farmers have indicated that they perceive that cultivars with a thick cortex are less susceptible to damage and keep better. Cortex thickness was therefore recorded. Tuber hardness, sugar content (indicated by refractive index of the root sap), and DM content were also assessed. The only variable that showed a consistent relationship with rates of deterioration (indicated by weight loss and rotting after two weeks), was DM content at the start of storage. Contrary to the normal perception that tubers with low DM deteriorate more rapidly, a significant positive correlation was found between DM content and both weight loss and rotting for both trials (Figure 2a, b).

## Discussion

The results presented show a wide range in storability within both local and introduced sweetpotato germplasm, which indicates that there is great potential for breeding for extended shelf-life. Although the results of only a single trial are presented here, these are consistent with the results of less extensive trials carried out at the same location during the pre-

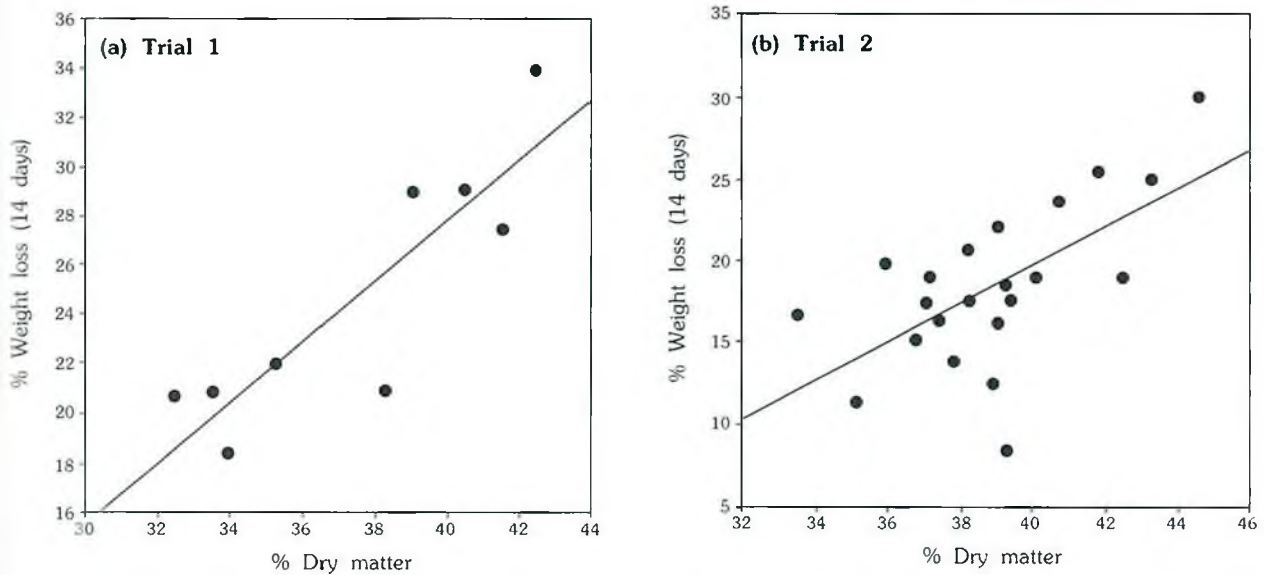


**Figure 1** The relationship between overall rotting and per cent fresh weight loss after two weeks of storage of roots of (a) 9 sweetpotato cultivars in Trial 1 and (b) 22 sweetpotato cultivars in Trial 2; Correlation coefficient  $r = 0.943^{***}$  in Trial 1 and  $0.846^{***}$  in Trial 2

vious two years. This finding agrees with that of studies that have previously been conducted in other regions of the world (Campbell and Collins, 1987; Woolfe, 1992).

Although further work needs to be done to investigate the basis of differences in storability, the strong relationship between rates of weight loss and rotting suggests either that there is a causative link between the two (e.g., weight loss may promote rotting) or that there is a common factor providing resistance against both. An investigation of periderm structure in a range of cultivars and its role in perishability would be very valuable.

The positive relationship between initial DM content and rate of deterioration needs further investigation. At this stage it is not known



**Figure 2** The relationship between initial per cent dry matter content and per cent fresh weight loss after two weeks of storage for roots of (a) 9 sweetpotato cultivars in Trial 1 and (b) 22 sweetpotato cultivars in Trial 2; Correlation coefficient  $r = 0.871^{**}$  in Trial 1 and  $r = 0.63^{**}$  in Trial 2

whether this is a causative relationship or not. In East Africa, high DM is very important for consumer acceptability, so it would be counter-productive to breed for low DM. For this reason, cultivars that keep well, and yet have high DM (falling below the line in Figure 2a, b) will be particularly useful within breeding programmes. A notable example is Bilagala in Trial 2.

For a breeding programme, in the absence of indirect selection techniques, any method for assessing rates of deterioration needs to be as simple as possible. Measurement of weight loss is simpler and more objective than assessment of rotting. The results presented here suggest that weight loss during the first week of storage is a good indicator of subsequent rates of deterioration. If further studies confirm this, then it may be possible to select on the basis of weight loss alone.

In this study the effect of damage (cuts) on rates of deterioration did not vary with cultivar, indicating that effective cultivar selection could be carried out without the use of a damage treatment. In previous years other methods of damaging have been tried, which involved battering the tubers to scuff and bruise the surface. This methodology did not affect the ranking of cultivars. Despite these results, given the extent of damage which has been observed in Tanzanian markets, it is still believed that other forms of damage (e.g., breaking tubers) should be tested.

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# Genetic variation in physical properties of flour from selected Asian yams (*Dioscorea* spp.)

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Flours from a total of 15 genotypes of *Dioscorea alata*, *D. esculenta*, *D. hispida*, *D. pentaphylla*, and *D. rotundata* were evaluated to identify variation in starch-related functional properties affecting their quality for use in food processing. Significant differences were obtained among and within species for these properties. Very low amylose flours (3.8–7.4%) such as *D. esculenta* showed high swelling volume (SV), lower gelatinization temperatures (GT), low paste viscosities and high breakdown, and soft, sticky, and cohesive gel textures. *Dioscorea rotundata* flours gave slightly higher apparent amylose contents (AAC) and GT compared to *D. esculenta*, but moderately high and variable paste viscosities and firm, non-sticky gel textures. *Dioscorea hispida* flour had low AAC and GT but extremely high paste viscosity and low paste stability. Flours from wild and cultivated genotypes of *D. pentaphylla* and *D. alata* showed a range of properties with potential for use in noodle-making and for industrial uses requiring high viscosity. Genotypes BSUP 115 and BSUP 126 (*D. pentaphylla*) showed strong resistance to mechanical disintegration (shear-thinning). In *D. alata*, LA 077 purple flour gave the highest paste viscosity values and setback ratios, followed by the processing type BSUA 093, BSUA 102, and wild staple substitute BSUA 101; the Puerto Rican IA 227 and Chinese IA 401 flours gave extremely low paste viscosities, and somewhat firm, non-sticky gel textures.

Keywords: *Dioscorea*; Yam; Swelling volume; Gelatinization temperature; Paste viscosity; Gel texture; Starch processing; Apparent amylose

Tropical root crops have long served as staple foods for hundreds of millions of people (Purseglove, 1972; Onwueme, 1978). However, the major constraints to their utilization in processed foods are lack of improved varieties, storage losses, and limited processing technologies (Obimpeh, 1991). Crop quality depends on physicochemical properties which have direct or indirect effects on the final food product. Genotype and environment affect root and tuber composition and properties.

The two important cultivated species of *Dioscorea* are *D. alata* and *D. esculenta*. There are several well-known varieties of these but only the purple types of *D. alata* are used in the food industry. The indigenous *D. hispida*, *D. pentaphylla*, and *D. rotundata* species are occasionally gathered from the forest as buffer foods in times of food shortage. Each of the wild yams has its own unique eating characteristics that are exploited by villagers for specific uses (Salda, 1994). The tubers of *D. hispida*, when detoxified, dried, and milled into flour make a good wheat flour substitute in cookies and cakes using 100% and 25% substitution, respectively (Salda, 1994).

Bartolini and Gundaya (1995) stated that domestic and export demand for yam and its processed products is growing continuously. Exported products cater to Filipino communities in other countries such as the United States and

in Europe (Giron, 1995). So far, yams are utilized mainly as ice cream flavouring, powdered yam, preserves, and candies. New processes, products, and equipment are needed to improve the commercial potential of yam and upgrade it from its present status as a minor crop. Analysis of genetic resources for quality-related traits is an essential step in selecting and breeding improved processing varieties of higher value. The objectives of this paper were to describe the genetic variation in functional properties of flour in 15 local and introduced yam varieties, and to identify genotypes having unique properties potentially useful in product development.

## Materials and Methods

### Yam samples and flour preparation

Accessions of *D. alata*, *D. esculenta*, *D. hispida*, *D. pentaphylla*, and *D. rotundata* were obtained from the germplasm collections of the two root crop centres in the Philippines, viz. the Northern Philippines Root Crops Research and Training Center (NPRCRTC) at Benguet State University, and the Philippine Root Crops Research and Training Center (PRCRTC) at Visayas State College of Agriculture. The others were collected for this study in

the Philippines, while the China entry was a market sample from Hong Kong (Table 1). The accessions were multiplied under uniform field conditions over an 8–9 month growing season in 1996 at the Kadoorie Agricultural Research Center, Hong Kong. Philippine-grown samples of *D. pentaphylla* and *D. hispida* were used in the analysis due to low yield obtained in Hong Kong.

The flour samples were prepared by washing, peeling, and slicing using a locally-made wooden chipper with blade adjusted to 1–2 mm thickness, and oven-drying at 65–70°C for 48 h. The dried chips were crushed by hand and finely ground using a Tecator cyclone mill. The flour was packed in Ziploc plastic bags and kept in air-tight containers until use. The highly toxic *D. hispida* (containing dioscorine alkaloids) was detoxified by village processors in the Philippines.

#### Apparent amylose content (AAC) of flour

Apparent amylose content was determined following the method described by Morrison and Laignelet (1983), slightly modified by using 10 mg yam flour instead of the normal 50–100 g sample. Samples were replicated twice. Defatting was not done as yams, like other root crops, contain insignificant amounts of fat that would interfere in the iodine blue complex (Madamba *et al.*, 1992).

**Table 1** Yam genotypes studied

Genotype	Local name	Country of origin
<i>Dioscorea alata</i>		
LA 077	Purple yam	Philippines
BSUA 093	Sampero	Philippines
BSUA 101	Balolong	Philippines
BSUA 102	Padikot	Philippines
IA 401	Yu Tau	China
IA 227	Gimelos	Puerto Rico
<i>Dioscorea esculenta</i>		
IE 001	Doli	Puerto Rico
LE 007	Apali	Philippines
LE 033	Tugui	Philippines
BSUE 109	Native Tugue	Philippines
<i>Dioscorea hispida</i>		
BSUH 111	Karut	Philippines
<i>Dioscorea pentaphylla</i>		
BSUP 115	Kapungaw	Philippines
BSUP 126	Gassey	Philippines
<i>Dioscorea rotundata</i>		
IR 001	Iwo	Nigeria
HR 043	—	Nigeria

#### Swelling volume (SV)

Swelling volume was determined following the method described by Crosbie *et al.* (1991) using flour samples of 0.4 g. The SV (mL g<sup>-1</sup>) of the sedimented gel was calculated from its height in the constant bore tube used.

#### Gelatinization temperature

The gelatinization temperatures of yam flour were measured in duplicate using a Mettler DSC20 differential scanning calorimeter (Mettler, Naenikon-Uster, Switzerland). Flour samples (3 mg flour adjusted to 14% moisture content) were weighed into a 40 µL pan and distilled water was added using a microsyringe. The pan was hermetically sealed and left for 1 h to allow samples to equilibrate. Samples were heated from 40°C to 110°C at increments of 10°C min<sup>-1</sup>. An empty pan was used as reference. The onset (T<sub>o</sub>), peak (T<sub>p</sub>), and completion (T<sub>c</sub>) temperatures, and the enthalpy of gelatinization (ΔH, J g<sup>-1</sup>) were recorded.

#### Viscoamylography

A Rapid Visco-Analyzer (RVA) model 3D (Newport Scientific Pty. Ltd., Warriewood, Australia) was used to determine the pasting properties of yam flours. Samples (4 g, adjusted to 14% moisture content; or reduced sample size as specified) were weighed directly in the aluminum RVA canister. Distilled water was added to a total sample weight of 28 g. A 22 min programme profile was activated under constant shear, held at 50°C for 1.0 min, heated to 95°C in 7.5 min, held at 95°C for 5 min, cooled to 50°C in 7.5 min, and held at 50°C for 1 min. Apparent viscosity was recorded in RVU (1 RVU ≡ 10 cp). Data taken were peak viscosity (PV), holding or hot paste viscosity (HPV), and final or cooled paste viscosity (CPV). Stability ratio was calculated as HPV/PV. All tests were replicated twice.

#### Gel texture

Samples from the RVA test (the paddle removed immediately after the test) were covered with parafilm and kept overnight at room temperature. Texture profile analysis was performed using a QTS-25 texture analyser (Stevens Advanced Weighing Systems, Leonard Farnell and Co., Ltd., England) fitted with a cylindrical 7-mm flat-ended probe. A two-cycle compression programme with test speed of 30 mm min<sup>-1</sup> and 2.0 g trigger point was used. Two tests were done per sample per replicate. Parameters recorded were hardness (maximum load on cycle 1), cohesiveness (total positive work done on cycle 2 divided by total positive work done on cycle 1), adhesiveness (total negative area in cycle 1), and springiness (distance beam travels compressing the sample in cycle 2).

## Results and Discussion

### Apparent amylose content of flour

The AAC of flour ranged from 3.9 to 21.4%, with the lowest values generally found in *D. esculenta* and fairly uniformly higher values in the other species (Table 2). The AAC in *D. alata*, *D. pentaphylla*, and *D. rotundata* is comparable to the 21% reported by Rasper and Coursey (1967) from *D. alata* and other African varieties. The highest individual AACs were obtained from genotypes IR 001 and HR 048 (Nigerian origin) of *D. rotundata*; BSUP 115 (highland wild yam) of *D. pentaphylla*; IA 227 (Puerto Rico), IA 401 (China), and LA 077 (ViSCA entry) of *D. alata*. The range is within the 12 to 15% reported by Nkala *et al.* (1994) for African wild yam (*D. dumetorum*) starch but is much lower than the 34% reported by Cruz-Cay and Gonzalez (1973) in Caribbean Florida yam (*D. alata*) starch. The low amylose yams could be utilized in frozen sauces, desserts, or sweets because of the low staling rate, and for alcohol production. Interme-

diante and higher amylose yams would be more suitable for extruded products and flat noodles. Further study in this area is recommended to determine the overall acceptability of varieties in food applications.

### Swelling volume

Genotypes having high AAC gave low SV, (and vice versa) up to 145 mL g<sup>-1</sup> (Table 2). The swelling was so high that the supernatant layer was indistinct. This property was exhibited by all genotypes of *D. esculenta* and *D. hispida*. Tester and Morrison (1990) similarly showed that swelling is related to amylopectin content in starch. In restricted swelling starches, amylose can act as a diluent and as an inhibitor of swelling by forming insoluble complexes with some of the amylopectin. This could explain some of the varietal differences found in plant species such as wheat (Crosbie *et al.*, 1991). Leach *et al.* (1959) explained that the variation in swelling and solubility patterns of starches (present in the flour), when heated in water, is due to the different character and strength of the micellar network within the

**Table 2** Genetic variation of yam in apparent amylose content (AAC), swelling volume (SV, mL g<sup>-1</sup>), peak viscosity (PV), holding viscosity (HPV), final viscosity (CPV), stability ratio (SR)<sup>1</sup>, hardness (Hard), cohesiveness (Cohes), adhesiveness (Adhes), and springiness (Spring) of yam paste or gel

Genotypes	AAC	SV	PV	HPV	CPV	SR	Hard	Cohes	Adhes	Spring
<i>Dioscorea alata</i>										
LA 077	21.4	75	508	480	704	0.95	72	0.6	124	2.12
BSUA 093	15.6	79	403	304	383	0.75	127	0.6	117	10.2
BSUA 101	14.8	82	345	346	430	1.00	216	0.8	35	22.1
BSUA 102	14.9	51	398	328	401	0.82	66	0.6	110	15.4
IA 401	18.5	87	156	128	163	0.55	157	0.6	85	21.6
IA 227	20.1	95	77	83	54	1.09	45	0.6	55	16.4
<i>Dioscorea esculenta</i>										
IE 001	5.8	96	67	32	50	0.47	218	1.0	0.1	0.1
LE 007	3.8	143	108	63	90	0.58	241	0.8	0.1	0.1
LE 033	7.1	145	22	12	16	0.55	137	0.7	53	9.2
BSUE 109	7.4	145	18	9	11	0.50	32	0.7	96	20.1
<i>Dioscorea hispida</i>										
BSUH 111	11.2	131	745	456	658	0.61	113	0.8	80	20.1
<i>Dioscorea pentaphylla</i>										
BSUP 115	20.4	54	413	342	477	0.83	21	1.0	0.6	0.0
BSUP 126	19.2	48	86	139	188	1.63	20	1.0	0.1	0.0
<i>Dioscorea rotundata</i>										
IR 001	19.2	100 d	410	197	516	0.48	77	0.6	106	21.3
HR 048	17.2	104 c	482	198	198	0.41	52	0.6	109	21.6
LSD <sub>0.05</sub>	0.6	3	74	35	60	na	49	0.3	64	8.1

LSD<sub>0.05</sub> indicates least significant difference between genotype means within a column; <sup>1</sup>Stability ratio, SR = HPV/PV

granules. The extent and strength of molecular association is in turn influenced by factors such as molecular weight and molecular weight distribution, conformation, and length of outer branches of amylopectin.

High SV is a property preferred for the production of adhesives, fillers, binders (in paper making), sweeteners, or frozen sauces (Whistler *et al.*, 1984). Starches with high AAC and restricted swelling are preferred for the production of starch noodles (Kim and Seib, 1993). Restricted swelling and textural differences are known to be associated with the non-starch lipids present in wheat flour, but this has not been confirmed in yam.

### Pasting properties

The RVA viscograph results (Table 2) show the time and temperature dependent changes in viscosity during simulated processing conditions of cooking in excess water under continuous shear. *Dioscorea esculenta* gave very low pasting viscosities (Table 2), with PV ranging only from 18 to 108 RVU, and with fairly low stability ratios. This indicates rapid and unrestricted swelling at relatively low temperature and fragmentation and solubilization of the swollen starch granules that are mechanically ruptured due to starch paste agitation (Cruz-Cay and Gonzalez, 1973). These low-pasting properties were associated with low AAC and high SV values.

The pasting properties of *D. alata* varied significantly among the six genotypes (Table 2). They had high stability ratios due to relative absence of shear-thinning (high HPV). Their pasting properties were within the range reported by Cruz-Cay and Gonzalez (1973) and Soni *et al.* (1985). The Puerto Rican genotype IA 227 was similar to the pasting pattern of *D. esculenta* genotypes except that time to peak viscosity was delayed. Genotype LA 077, a dark purple fleshed Philippine yam showed the highest PV, HPV, and CPV of the *D. alata* accessions, and had high AAC.

The viscosity of genotypes of *D. rotundata*, *D. hispida*, and *D. pentaphylla* increased with time and produced irregular pasting curves at the standard 4-g flour in 28-g total sample concentration for the RVA (data not shown). Such profiles were also observed in potato starch by Haase *et al.* (1995) who postulated that the starch paste adheres to the canister wall during shearing. Reduction of yam flour concentration to 3.5 g from 4 g in 28 g total sample weight gave a smooth RVA profile (data not shown). The *D. hispida* showed a very high peak viscosity and a marked breakdown. The two genotypes of *D. pentaphylla*, with low and indistinct peaks and little breakdown, had similar pasting patterns to yam variety Kuan-Shu treated with sodium chloride (Lii and Chang, 1978). This behaviour reflects the stability of the swollen granule against mechanical disinte-

gration (Cruz-Cay and Gonzalez, 1973), and indicates strong bonding force within the granule (Lii and Chang, 1978). Accordingly, these may be good materials as food stabilizers or gelling agents, and for industrial uses in which high thickening is required.

### Texture quality

Genotypes IE 001 (Puerto Rico), and LE 007 and LE 033 (Philippines) gave hard gels, with high cohesiveness and low adhesiveness and springiness (Table 2). Flour gel texture of *D. pentaphylla* had high cohesiveness but was low in the other parameters, while *D. rotundata* exhibited high adhesiveness and springiness.

The relative contents of amylopectin and amylose play a key role in food product texture (Whistler and Daniel, 1985). Low amylose starches have lower GTs and higher enthalpies but weak bonding forces, thus producing softer gels. These characteristics could explain the observations of highland farmers (Salda, 1994) that cooked tubers from *D. esculenta* and BSUA 101 (farmer's type) are somewhat softer in texture with good chewing characteristics even when kept as left-over foods. Philippine highland farmers prefer tasty and soft textured yams as these may be served as early breakfast or snack foods especially during heavy field work. The mealy and processing types (those that easily crumble when cooked, e.g., BSUA 093) contain higher levels of amylose. These varieties, when cooked, were claimed to have grainy, floury, and dryer cooking textures especially when they are freshly harvested. This could be one reason why ice cream and haleya processors add about 5–10% of the moist- or soft-type varieties to obtain the desired consistency of the product (Chan, L. pers. commun.). Another practice is the storage of yam tubers for some weeks to give more acceptable consistency. The altered cooking characteristics of the stored tubers could be due to the partial conversion of starch to sugar.

These varied texture characteristics of yam flour, with similarities to other commercial flours or starches, may be useful in product development of noodles, snacks, and baby food products. It will also be of interest to determine the functional properties of wheat:yam composite flours and yam starch doughs and sheets. Such uses would provide sustained demand for yam, and reduce storage losses and marketing and transportation costs.

### Conclusion

The amylose content of starch in yam flours determines the cooking characteristics and contributes substantially to the variation in SV, pasting properties, GT, and gel texture. Depending on genotype, properties were found which could be applicable both in food and

non-food industries. Among these properties, are intermediate to high amylose content with restricted swelling, high paste viscosities and GT, and film-forming characteristics that could be used in noodle making, and for industrial products which require high thickening power. Genotypes of *D. alata* and *D. rotundata* seem to be most suitable for these uses.

Low amylose content with high SV, low GT, low paste viscosities, and soft, elastic, and sticky gel textures would be applicable in making frozen sauces, desserts, and sweeteners. Such properties were shown by the four genotypes of *D. esculenta*. The shear-thinning resistant pasting profile of *D. pentaphylla* may indicate its possible use as a food stabilizer, gelling agent, and in pasta products. This species is becoming rare and action should be taken to conserve its genetic variation. The results of this study indicate a diversity of starch-related flour properties in Philippine yam genetic resources.

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# Application of embryo culture in germinating African cassava mosaic disease resistant gene mapping population

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In a study to map out the genes conferring resistance to African cassava mosaic disease (ACMD), a cost-effective protocol for zygotic embryo culture developed at the International Institute of Tropical Agriculture (IITA) using 1/2 strength Murashige and Skoog's basal media, was employed to germinate the F<sub>1</sub> populations. The F<sub>1</sub> crosses were generated from cross combinations between ACMD resistant and susceptible and local cassava germplasm. These crosses were TMS 30572 × TME 117, TMS 30572 × TME 4, and TMS 30555 × TME 3, giving three mapping populations. Four weeks after explanting embryo axes, progenies of the F<sub>1</sub> cross of TMS 30555 × TME 3 performed best with 88% of the total explanted embryos producing two or more nodes, followed by those of the F<sub>1</sub> cross of TMS 30572 × TME 4 with 79%, and TMS 30572 × TME 117 with 77%. A small percentage of each of the mapping population germplings had 4 to 5 nodes, and 3, 5, and 9%, in F<sub>1</sub> crosses of TMS 30572 × TME 117, TMS 30572 × TME 4, and TMS 30555 × TME 3, respectively.

Keywords: African cassava mosaic disease; Zygotic embryo; Embryo axes; Mapping population

Cassava (*Manihot esculenta* Crantz) is a major calorie-producing crop for over 200 million people in Africa. However, the yield in Africa of 7.7 M t ha<sup>-1</sup> compares unfavourably with yields of 13.0 M t ha<sup>-1</sup> in Asia, 11.2 M t ha<sup>-1</sup> in Oceania, and 12.4 M t ha<sup>-1</sup> in South America (CIAT, 1994). Prominent among the problems associated with this low yield in Africa, are the impact of the endemic diseases and arthropods on the continent that have overcome the defensive strategies of cassava through adaptation and mutation. African cassava mosaic disease (ACMD) caused by either of two geminiviruses [African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV)], is widespread and economically damaging (Swanson and Harrison, 1994; Thresh *et al.*, 1994).

Crop improvement by breeding and selection for more productive cultivars and multiple pest resistance can overcome the problem. Cultivars with stable resistance offer an advantageous and practical long-term solution for controlling cassava pests because the control strategy is economical, easy to use, and compatible with other control measures (Mahungu *et al.*, 1994). In a collaborative project between the International Institute of Tropical Agriculture (IITA) and the International Centre for Tropical Agriculture (CIAT), research has been initiated to map the genes conferring resistance to ACMD and develop molecular markers for this trait in African cassava germplasm. This will facilitate the de-

ployment of ACMD resistance into cassava germplasm in the region and especially in areas with high disease pressure. Mapping populations were generated between ACMD resistant and susceptible germplasm through controlled hybridization.

Germination of cassava seeds have been reported to be poor and erratic (Martin, 1976; Biggs *et al.*, 1986). Embryo culture is an efficient tool to increase the germination of embryos of many crop species (Raghavan, 1985), including cassava (Biggs *et al.*, 1986). To ensure successful germination of these hybrid seeds and to facilitate the production of planting materials for evaluation as well as safe germplasm movement across national boundaries, embryo culture technique developed at IITA was tested for the germination of ACMD resistant gene mapping populations.

## Materials and Methods

Parental lines selected for the production of mapping populations were improved genotypes, TMS 30572 (resistant to ACMD) and TMS 30555 (susceptible to ACMD), and local germplasm, TME 3 and TME 4 (both resistant to ACMD) and TME 117 (susceptible to ACMD). The cross combinations were TMS 30572 × TME 117, TMS 30572 × TME 4, and TMS 30555 × TME 3 (giving three map-

ping populations).

The  $F_1$  seeds of these mapping populations were pre-treated in dry hot air (hot oven) at 60°C for 14 days. This was to eliminate cassava bacterial blight (CBB) infective particles that may reside in the seed endosperrn. Five hundred seeds from each mapping population were used. Procedures for treating the seeds and embryo culture were the modifications described by Ng (1992).

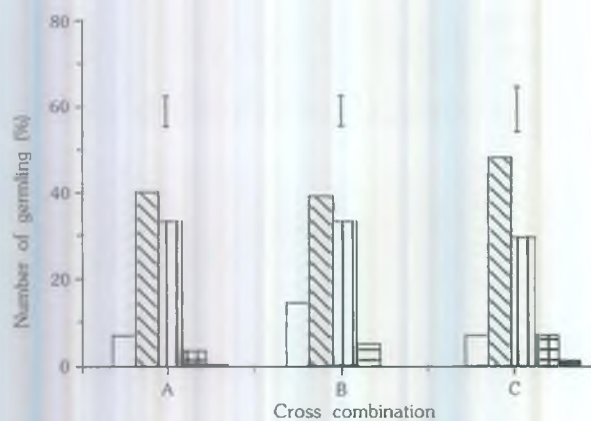
Seeds were soaked in concentrated tetra-oxo-sulphate (vi) acid ( $H_2SO_4$ ) for 2 h, with occasional agitation to soften the hard seed testa. They were afterwards thoroughly rinsed in running tap water. The seeds were surface-sterilized with 70% ethanol for 5 min, followed by 10% sodium hypochlorite NaOCl solution with few drops of Tween 20 for 20 min with one rinsing in sterile distilled water. The seeds were soaked for a second time in 5% NaOCl solution for 10 min and rinsed with two changes of sterile distilled water. Seeds were then soaked overnight in sterile distilled water before embryo axes were excised with a scalpel (with blade No. 11) and forceps. The embryo axes were cultured in 1/2 strength Murashige and Skoog's basal medium (Murashige and Skoog, 1962) supplemented with 50 mg  $L^{-1}$  inositol, 3% sugar, and 0.7% agar. The pH of the medium was adjusted to 5.7 prior to sterilization. The medium was melted and 5 mL dispensed to each test tube (125 mm  $\times$  15 mm) and sterilized at 12°C and 1.1 kg  $cm^{-2}$  for 15 min.

Cultures were incubated at 25°C (night) to 30°C (day), with 12 h light and 12 h dark. Illumination was provided by daylight fluorescent bulbs providing 31  $\mu mol m^{-2} s^{-1}$ . Observations on germination of the cultures were carried out daily during the first week and thereafter at two and four weeks after culturing. Data on germling vigour were analysed by analysis of variance (ANOVA) using SAS (SAS, 1989).

## Results and Discussion

Elongation of root and shoot axes were observed from explanted embryos in all crosses in less than one week after culturing.

At four weeks after explanting embryo axes, progenies of the  $F_1$  cross of TMS 30572  $\times$  TME 117 had 8% of the total explanted embryos producing one node, 41% two nodes, 33% three nodes, 3% four nodes, and less than 1% five nodes (Figure 1). For the  $F_1$  cross of TMS 30572  $\times$  TME 4, 14% of the total explanted embryos produced one node, 40% two nodes, 34% three nodes, 5% four nodes, and less than 1% five nodes. The  $F_1$  cross of TMS 30555  $\times$  TME 3 had 7% of the total explanted embryos producing one node, 49% two nodes, 30% three nodes, 8% four nodes, and 1% five nodes. At four weeks after culturing, the majority of plantlets had reached two- or three-nodes stages. There were 74%



**Figure 1** Performance of cassava germlings from three mapping populations; A =  $F_1$  cross of TMS 30572  $\times$  TME 117, B =  $F_1$  cross of TMS 30572  $\times$  TME 4, and C =  $F_1$  cross of TMS 30555  $\times$  TME 3; Vertical bars =  $LSD_{0.05}$   
 □, 1 node; ▨, 2 nodes; ▩, 3 nodes; ▤, 4 nodes; and ■, 5 nodes

for mapping population of crosses TMS 30572  $\times$  TME 117 and TMS 30572  $\times$  TME 4, and 79% for TMS 30555  $\times$  TME 3.

A total of 85% of the embryos cultured produced plantlets with one node and greater in cross TMS 30572  $\times$  TME 117, 93% in cross TMS 30572  $\times$  TME 4, and 95% in cross TMS 30555  $\times$  TME 3. Among these three mapping populations, cross TMS 30555  $\times$  TME 3 had the best growth performance; 88% of the cultured embryos formed plantlets with two nodes and greater, followed by TMS 30572  $\times$  TME 4 (79%). TMS 30572  $\times$  TME 117 had the lowest (77%). However, 3, 5, and 9% of the plantlets of crosses TMS 30572  $\times$  TME 117, TMS 30572  $\times$  TME 4, and TMS 30555  $\times$  TME 3, respectively, had four nodes and above (Figure 1). At this stage, plantlets are ready for micro-propagation. This shows the proficiency of the protocol for embryo germination of cassava seeds earlier described by Ng (1992) used at IITA. This procedure was also used successfully to culture isolated embryos of several wild *Manihot* species (Ng and Ng, 1996).

Variability was observed in the germling vigour. This was illustrated by the consistent manner of nodal proliferations among progenies of the populations within each of the crosses. This phenomenon could be attributed to the differences in seed quality, seed handling and treatment, as well as embryo excision. Whether the variability has genetic basis is not certain. No studies have been reported on any genetic basis for seedling vigour in *Manihot esculenta*.

## Conclusions

The protocol used at IITA for embryo culture of cassava is very efficient. It will serve as a means of breaking seed dormancy and increasing the recovery of plants from cassava hybrid

seeds. This technique will also allow preparation of aseptic seedling material which can be rapidly multiplied *in vitro* prior to field trials and evaluation among different national programmes.

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# Advancement of sweetpotato breeding for high starch content in Japan

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The starch content of Japanese sweetpotato [*Ipomoea batatas* (L.)] cultivars is rated among the highest in the world and has been accomplished via a national breeding programme conducted for more than 50 years. The first step of the programme was to accumulate genes for high starch content in local cultivars whose starch content ranged from 14 to 20%. A starch content of 15–20% with higher storage root yield was obtained from the offsprings. Genes from foreign cultivars were then transferred to the local cultivars. Cultivar 'Koganesengan' was developed from this cross producing a starch content of 22–26% and considered to be a high storage yield cultivar. Wild relatives were also used to increase genetic variation, and 'KI23' (19% starch content but no storage root) a hexaploid wild plant was crossed with cultivated sweetpotato. The resulting hybrids were used to backcross (BC) with improved cultivars. From the resulting BC<sub>2</sub> progenies, 'Minaniyutaka' was selected and released, which had a lower starch content (19–22%) but produced more storage root than 'Koganesengan'. Recently, 'Hi-starch' and 'Satsuma-starch' were released. Both had the highest level of starch content (28–30%) among the cultivars released in Japan.

Keywords: Sweetpotato; Breeding; Autohexaploid; Starch content; Japan

Sweetpotato [*Ipomoea batatas* (L.)] is one of Japan's most important upland crops, especially in the south-western area, because of its superior ability to convert solar energy to carbohydrates and its tolerance to environmental extremes, such as drought and typhoon, which cause significant losses of upland crop production.

Sweetpotato was first introduced into Japan in the 1600s. By the middle of the 19th century it had spread throughout Japan except Hokkaido, the northern-most area. The production area had increased to 400 000 ha in 1940–50, when Japan had suffered from both direct and after effects of World War II. During this period, sweetpotato contributed to human food supply and fuel production. As food supply improved, the crop was mainly used for starch production which accounted for 30–40% of total sweetpotato consumption. Its starch was used for glucose production which required cultivars with high starch content. Therefore, a national breeding programme for developing sweetpotato cultivars with high starch content and high storage root yield was initiated by the collaboration of national institutes and universities. However, sweetpotato production in Japan has been declining significantly due to the change in the Japanese diet and the higher price of domestic sweetpotato starch relative imported corn starch.

Domestic sweetpotato starch had been protected for the last 20 years against importation of corn starch but recent World Trade Organization (WTO) trade policy would not permit the continuation of this protection.

A much higher tuber yield and starch con-

tent from new cultivars are required to reduce the cost of producing sweetpotato starch and to make it more competitive. The theory, strategy, and history of high starch content breeding in Japan are reported here.

## Theoretical Basis

Since sweetpotato is an autohexaploid and mainly self-incompatible species, and progeny segregation is extremely complicated, the mode of inheritance in quantitative characters has not yet been completely elucidated. The concentration of genes controlling starch content in a cultivar is essential for the development of cultivars with high starch content. The easiest and fastest method is inbreeding by selfing and sib-cross. Inbreeding raises the frequency of desirable genes and improves the selection efficiency. However, while inbreeding depression occurs in total storage root yield, and moderately in total storage root number and total vine length, it does not occur in dry matter (DM) content (Table 1; Sakai, 1964; Tamiya *et al.*, 1992). On the other hand, a higher degree of heterosis is observed through top crosses of inbred lines in the storage root yield rather than in DM content (Sakai, 1964). Differences in the inbreeding depression and heterosis of agronomic characters in the progenies of self-compatible genotypes suggested that each character was controlled by different genes. Sakai (1964) reported that DM content was controlled by additive gene effects, and total storage yield by dominant gene effects. He also concluded that the best method to develop new cultivars

**Table 1** Degree of inbreeding depression<sup>1</sup> after self-fertilization in several sweetpotato cultivars<sup>2</sup>

Variety	Total vine length	Total root weight	Total root number	Dry matter content
Norin 1	52	31	—	88
Norin 7	68	46	—	90
Nakamurasaki	38	41	—	86
Shichifuku	82	34	—	98
Kyushu 19	52	34	—	83
Shirosatsuma	—	53	59	101
Minamiyutaka	—	63	67	97
CS69136-2	—	78	102	111
CS69136-33	—	87	85	90
Chikei 7130-2	—	74	100	103
Kankei 25	—	42	67	87
Means	58	53	80	94

<sup>1</sup>Means of S<sub>1</sub> progeny/means of parent (%)<sup>2</sup>After Sakai (1964) and Tamiya et al. (1992)

with high DM content and high storage root yield was the development of high DM inbred lines accompanied by crossing among them or with leading cultivars.

### Strategy of Breeding for High Starch Content

Based on the analysis described above, enhancement of general and specific combining abilities is required to develop cultivars not only for high yield, but also high starch content and cultivar development. General combining ability influences characters controlled by additive gene effects such as starch content, and specific combining ability affects characters controlled by dominant gene effects such as storage root yield. The first step in developing high starch content and high storage root yield cultivars is developing inbred lines which are derived from different parents.

The second step is the evaluation of specific combining ability of each inbred line. Cross combinations and inbred lines selected in these steps are used in the breeding programme. Inbred line development is continuously conducted through the evaluation of general and specific combining ability to contribute to further cultivar improvement.

### History of Breeding for High Starch Content

Local cultivars were used at the first stage of the Japanese breeding programme, and introductions and wild species were used subsequently for development of new cultivars and for inbred lines.

### Use of local cultivars

Before 1940, five major cultivars, 'Genji', 'Beniaka', 'Shichifuku', 'Oiran', and 'Taihaku' were planted on about 70% of sweetpotato fields in Japan and their starch content was relatively low, 14–20% (Table 2). When a systematic sweetpotato breeding programme was started in 1937, less than 10 parents including these cultivars were used as parents in the programme. This programme produced several excellent cultivars, such as 'Okinawa 100', 'Gokokuimo', 'Norin 1', 'Norin 2', and 'Tamayutaka'. These cultivars had as much starch content as, and higher storage root yield than, local cultivars.

### Use of introductions

In the middle 1950s, Japanese sweetpotato breeders were of the view that no further improvement could be achieved unless the genetic base was increased. The inbreeding coefficients of breeding lines had increased to 0.1 (Sakai, 1964). A number of cultivars and breeding lines were subsequently introduced from many countries, such as the U.S.A., Mexico, Brazil, Indonesia, Taiwan, and Uganda. Among these, 'L-4-5 (Pelican Processor)' developed at the Louisiana State University, U.S.A., showed the highest starch content (23%). Cultivar 'L-4-5' was crossed with a number of breeding lines developed in Japan because of its high starch content and because its cross-incompatibility group was D which was not present in Japanese local cultivars. A prominent line was selected from a cross between 'L-4-5' and a breeding line related to another introduction from Indonesia. This line was registered by the Ministry of Agriculture and named

**Table 2** Starch content of sweetpotato cultivars in Japan

Local or released	Cultivar name	Starch content (%)
Local	Shichifuku	19.9
	Taihaku	14.2
	Yoshida	16.1
	Choshu	20.2
Released before 1960	Gokokuimo	21.7
	Okinawa 100	15.7
	Norin 1	20.8
	Norin 2	20.5
	Tamayutaka	19.6
Released after 1961	Koganesengan	24.5
	Minamiyutaka	19.2
	Shiroyutaka	23.7
	Shirosatsuma	23.6
	Hi-starch	30.3
	Satsumastarch	31.2

Investigated in 1991 and 1992 in NARC, Japan

'Koganesengan' in 1966 because of its high performance in starch production (Sakai *et al.*, 1967).

### Use of wild relatives

The efforts to increase genetic variation and to develop high starch content inbred line continued more actively after the development of 'Koganesengan'. Since 'Koganesengan' had both a high starch content and high combining ability, it was used as one of important breeding materials in this programme. In 1956, an accession (K123) a wild species closely related to sweetpotato was introduced from Mexico by Nishiyama (Nishiyama, 1959) and found to be able to cross with sweetpotato. Cultivar K123 was reported as a hexaploid type of *Ipomoea trifida* by Nishiyama *et al.* (1961) who intensively investigated its crossing ability with sweetpotato and characterized it. Although the taxonomic identity of K123 has been questioned (Jones, 1967), it was utilized in breeding programmes in an attempt to transfer its nematode resistance to sweetpotato. Hybrids between sweetpotato and K123 were used to backcross (BC) to improved sweetpotato cultivars. From BC<sub>2</sub> progenies, 'Minamiyutaka' was selected and released (Ono *et al.*, 1977). 'Minamiyutaka' was highly resistant to root lesion nematodes similar to K123 and, in addition, showed very high-yielding ability derived from some kind of heterosis. Its starch content was moderately high (19–22%).

### Enhancement of combining ability

Special crossing activities to evaluate and enhance the combining ability was conducted for cultivar development until 1990, and a number of breeding lines were developed based on their origin through inbreeding. Since hybrid seed production was restricted since 1991, more cross combinations with less number of seeds for cultivar development are carried out. Superior clones are selected and the combining ability of each cross and breeding line are evaluated simultaneously. Enhancement of combining ability for various traits is one of most important steps in sweetpotato breeding since it raises the efficiency of cultivar development.

'Shiroyutaka' (Sakamoto *et al.*, 1987), 'Shirosatsuma', 'Hi-starch', and 'Satsuma-starch' were developed from the breeding materials. Starch contents of 'Shiroyutaka' and 'Shirosatsuma' are the same as that of 'Koganesengan', but they produce more storage roots than 'Koganesengan'.

Three breeding lines, 'CS69136-2', 'CS69136-33', and 'CS7279-19G', played very important roles in the development of high starch content cultivars. Their pedigrees are shown in Figures 1 and 2. Starch content of 'CS69136-2', 'CS69136-33', and 'CS7279-19G' are approximately 24, 23, and 23%, respec-

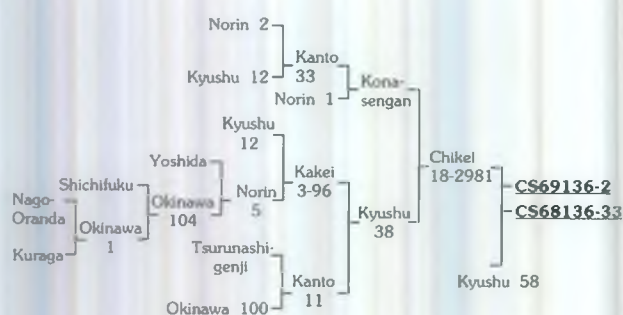


Figure 1 Pedigree chart of 'CS69136-2' and 'CS69136-33'

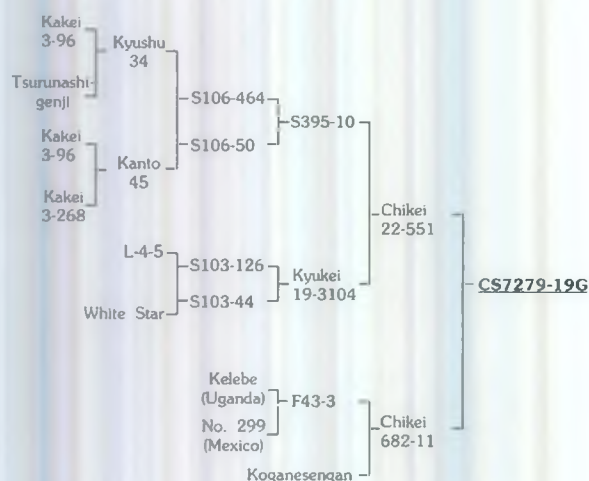


Figure 2 Pedigree chart of 'CS7279-19G'

tively. All of them are resistant to root knot nematode (*Meloidogyne incognita*) and moderately resistant to black rot (*Ceratocystis fimbriata*). Their combining ability were evaluated through test crosses with different cultivars and breeding lines. Cultivar 'CS69136-2' showed high specific combining ability to 'Tamayutaka', and produced 'Shirosatsuma' (Sakamoto *et al.*, 1989). Both 'CS69136-33' and 'CS7279-19G' had higher combining ability with respect to starch content. Their progeny is a hybrid, 'Hi-starch' with a starch content of 28–30%, which is much higher than those of both parents (Tarumoto *et al.*, 1989). The reason for such high starch content in 'Hi-starch' is not clear but it would appear that genes related to high starch content have accumulated in the cultivar. Unfortunately, in spite of its high starch content, 'Hi-starch' has not been cultivated commercially because of lower storage root productivity than that of 'Koganesengan' and susceptibility to nematodes and diseases.

Cultivar 'Satsumastarch' was developed to improve the productivity of 'Hi-starch'

(Tarumoto *et al.*, 1996), and was derived by crossing 'Koganesengan' and 'Hi-starch'. Its storage root yield is as much as, or higher than, that of 'Koganesengan' and its starch content is almost the same as 'Hi-starch'. However, disease and insect resistance has not yet been improved because both 'Koganesengan' and 'Hi-starch' are susceptible to diseases and nematodes.

### Acknowledgement

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# Analysis of genetic diversity in Guinea yams (*Dioscorea* spp.) using AFLP fingerprinting

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Genetic diversity and resulting heterotic groupings in Guinea yams (*Dioscorea rotundata* and *D. cayenensis*) and their wild relative *D. praehensilis* were assessed using Amplified Fragment Length Polymorphism (AFLP) genetic markers. Amplified Fragment Length Polymorphism generated DNA fingerprinting patterns of 19 varietal groups of cultivated yams and 1 wild species, *D. praehensilis*. Two primer combinations generated a total of 87 polymorphic loci across the 20 varietal groups analysed. Five major groups were recognized in the germplasm based on the neighbour-joining method of cluster analysis and the Statistical Analysis System multiple correspondence analysis procedure. Among these, four varietal groups which corresponded to *D. praehensilis* (cv. Grovota), *D. cayenensis* (cvs Yaobadou and Kpokpokpokpo), and *D. mangelotiana* (cv. Kappe-bolanda) were genetically distant from a core of 16 varietal groups of *D. rotundata*. Among these, two varietal groups (cvs Dobnawo and Koukou) from Cameroon clustered separately from the West African genotypes. The results of the present study showed that AFLP analysis is a sensitive and robust DNA fingerprinting technique for genomic analysis in yams.

Keywords: Amplified Fragment Length Polymorphism; *Dioscorea* spp.; DNA fingerprinting; Genetic diversity; Yams

Yams (*Dioscorea* spp.) are an economically important starch staple in tropical and sub-tropical regions of the world particularly West Africa, Asia, Far East, The Pacific, and Caribbean regions. Among the 600 described yam species, only 7 are widely cultivated for food crops. *Dioscorea rotundata* and *D. cayenensis* also referred to as Guinea yams or African yams are the most important cultivated yams in West and Central Africa where they are indigenous. In West and Central Africa where Guinea yams have been domesticated for about 7000 years (Coursey, 1976), farmers have selected genotypes that best fit their needs and, thus, have generated a large number of traditional varieties. In addition, different ethnic groups in the region have contributed to this selection, thus leading to numerous vernacular names given to the same varieties according to ethnic groups. This nomenclature has led to confusion in the exact numbers of yams varieties under cultivation in the region, hence the need to characterize the germplasm.

Yam germplasm characterization has been carried out using morphological descriptors (Martin and Rhodes, 1978; Akoroda and Chheda, 1983; Onyilagha and Lowe, 1985; Hamon *et al.*, 1986) and a combination of morphological characters and isozyme markers (Hamon and Touré, 1990a, b; Dumont *et al.*, 1994). These markers, although valuable for yam varietal group identification, show limited levels of inter- and intra-varietal polymorphism. In order to assess the extent of genetic variability in the yam germplasm, more polymorphic markers are needed.

Recently, various molecular markers have been applied to plant genetic resource management and plant breeding (Bretting and Widrechner, 1995). Among these, molecular genetic markers such as the Restriction Fragment Length Polymorphisms (RFLPs) and the Polymerase Chain Reaction (PCR)-based markers, such as Random Amplified Polymorphic DNAs (RAPDs) and microsatellites, have increased the possibilities to understand the ge-



netic relationships in various plant species and to develop linkage maps for marker-assisted selection (Mohan *et al.*, 1997). The RAPDs markers have been used to study the genetic diversity of plants including vegetatively-propagated species such as cassava (Tonukari *et al.*, 1997) and yams (Muzac-Tucker and Ahmad, 1995; Asemota *et al.*, 1996; Ramser *et al.*, 1996). Although RAPDs and the recently developed Random-Amplified Microsatellite Polymorphism (RAMPO) (Ramser *et al.*, 1997) are useful molecular markers that effectively discriminate yam germplasm, the number of polymorphic markers per PCR assay is still low and the reliability and repeatability of RAPDs markers are still being questioned. There is, therefore, the need to use a more robust, polymorphic, and reliable molecular marker technique to study the genetic diversity in yams.

Recently, Amplified Fragment Length Polymorphism (AFLP), a new generation of molecular marker technique has been developed that combines the uses of restriction enzymes and the amplification of a set of restricted fragments through a PCR technique and the subsequent separation of amplified fragments through a high resolution sequencing gel system (Vos *et al.*, 1995). This deoxyribonucleic acid (DNA) fingerprinting technique has already proven to be robust and fast. It also requires a minute amount of genomic DNA and usually generates more polymorphic loci compared to other PCR-based markers. Since AFLP markers are inher-

ited in Mendelian fashion, they have been used in genetic diversity studies in plant species such as wild bean, *Phaseolus vulgaris* (Tohme *et al.*, 1996), pea, *Pisum* spp. (Lu *et al.*, 1996), lens (Sharma *et al.*, 1996), and to generate genetic linkage maps in polyploid genomes such as sugar beet, *Beta vulgaris* (Schondelmaier *et al.*, 1996). In this study, the AFLP technique has been applied to determine the genetic diversity among the most widely cultivated Guinea yams.

## Materials and Methods

### Plants materials

Field-grown plants from 19 yam landraces and 1 wild yam species (*D. praehensilis*) were selected for characterization. Morphological characters of the shoot at both juvenile and adult stages and the tubers were scored using the yam descriptors published by the International Board of Plant Genetic Resources (IPBGR) (Hamon *et al.*, 1986). The varietal groups into which the selected germplasm have been classified are presented in Table 1. Among these, eight are newly described varietal groups (Mignouna *et al.*, unpubl.). The most typical morphotype of each varietal group was selected for genetic analysis. These landraces are part of yam germplasm collected from various countries of West and Central Africa and planted in the

**Table 1** List of Guinea yam varieties used for Amplified Fragment Length Polymorphism (AFLP) analysis

Species no.	<i>Dioscorea</i> species	IITA collection no.	Local name	Place of origin	Country of origin	Varietal group*
1	<i>rotundata</i>	CI-014	Kangba	Sandengne	Cote d'Ivoire	Baniakpa
2	<i>rotundata</i>	CI-033	Tanda pranka	Affuanome	Cote d'Ivoire	Lokpa
3	<i>rotundata</i>	CI-039	Koffikan	Yakasse	Cote d'Ivoire	Zrezrou
4	<i>rotundata</i>	CI-021	Sensi	Grebeu	Cote d'Ivoire	Krengle
5	<i>rotundata</i>	CI-048	Kponan	Bassawa	Cote d'Ivoire	Kponan
6	<i>rotundata</i>	CI-078	Cocoassie	Ondefiodou	Cote d'Ivoire	Cocoassie
7	<i>cayenensis</i>	CI-081	Kpokpokpokpo	Anekro	Cote d'Ivoire	Kpokpokpokpo
8	<i>rotundata</i>	CI-082	Warakou	Anekro	Cote d'Ivoire	Waraga
9	<i>rotundata</i>	CI-050	Sammancou	Trienko	Cote d'Ivoire	Sammancou
10	<i>rotundata</i>	NG-001	Agatu	Yola	Nigeria	Norforwu
11	<i>rotundata</i>	CAM-017	Koukou	Ngv	Cameroon	Koukou
12	<i>rotundata</i>	CAM-029	Dobnawo	Ngv	Cameroon	Dobnawo
13	<i>praehensilis</i>	GN-020	Grovota	Boola	Guinee	Grovota
14	<i>rotundata</i>	GN-026	Terkokonou	Boola	Guinee	Terkokonou
15	<i>rotundata</i>	GN-019	Kappe bolonda	Dounkin	Guinee	Kappe bolonda
16	<i>rotundata</i>	GN-027	Djambi Tenkerena	Kalankalan	Guinee	Soussou
17	<i>cayenensis</i>	CI-070	Kounougbe	Ondefiodou	Cote d'Ivoire	Yaobadou
18	<i>rotundata</i>	TG -032	Abalo	Sotouboua-Tabinde	Togo	Morokorou
19	<i>rotundata</i>	TG-033	Gnidou	Haho-Ahassoue	Togo	Gnidou
20	<i>rotundata</i>	CAM-034	Djalingo-2	Mbang	Cameroon	Bakokae

\*According to (Hamon *et al.*, 1986; Mignouna *et al.*, unpubl.)

experimental field of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, for maintenance and characterization.

### DNA isolation

About 5 g of young leaves were collected from field-grown plants for DNA isolation. The DNA was isolated according to the method of Rogers and Bendich (1985) but with the following modification: a leaf sample of each variety was ground in liquid nitrogen with a mortar and pestle. The fine powder was quickly transferred to 50 mL Falcon tubes and 15 mL of 2 × CTAB extraction buffer [2% CTAB (w/v), 100 mM Tris (pH 8), 20 mM EDTA, 1.4M NaCl, 1% PVP] was added, mixed, and incubated at 65°C for 1 h. One extraction with a mixture of phenol-chloroform was performed and DNA precipitated by the addition of cold ethanol. The DNA was hooked with a sterile Pasteur pipette, dissolved in Tris-EDTA (TE) buffer [10 mM Tris (pH 8); 1 mM EDTA] and treated with 50 ng of RNase at 37°C for 30 min. A second phenol-chloroform treatment was performed and DNA precipitated as above, was washed in cold 70% ethanol, and dried and dissolved in TE. Purified DNA was checked on agarose gel and the concentration determined by a DNA fluorometer (Model TKO-100).

### Double digestion and ligation of adapters

The AFLPs were generated using AFLP analysis system I/AFLP Starter Primer Kit (GIBCO-BRL Life Technologies, Inc.) following the manufacturer's instructions and with some modifications as described by Lu *et al.* (1996). The DNA (0.5 µg) of each sample was double-digested in RL (restriction and ligation) buffer containing 10 mM Tris, pH 7.5; 10 mM mg acetate; 50 mM K acetate; 5 mM DTT for 2 h at 37°C using 10 U of *Eco* RI (Boehringer) and 5 U of *Mse* I (NEN B) restriction enzymes in a total volume of 50 µL. The resulting fragments were ligated

to 5 pmol of *Eco* RI adapter and 50 pmol of *Mse* I adapter (Table 2) in the same RL buffer supplemented with 1.2 mM ATP using 1U of T4 DNA ligase (Gibco BRL) and incubated at 37°C overnight.

### Selective amplification of DNA fragments

An initial preselective amplification of fragments was carried out using digested and ligated fragments in 0.1 × TE diluted 10 times. This preselective amplification was performed using primers with one selective nucleotide *Eco* RI + A (E1) and *Mse* I + C (M1) (Table 2). The PCR amplification profiles were as follows: 20 cycles at 96°C for 30 s, 56°C for 1 min, and 72°C for 1 min.

The preselective amplified mixture was diluted 10 times and subsequent selective amplification carried out using P33-labelled *Eco* RI + (3) and *Mse* I + (3) selective nucleotide primers (Table 2). The PCR conditions used were 13 cycles at 94°C for 30 s, 65°C for 30 s, and 72°C for 1 min with a step-down annealing temperature of 0.7°C. Each cycle was followed by 22 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min. All PCR reactions were carried out in a PTC-100 thermocycler (MJ Research, Inc.).

An equal volume of gel-loading-buffer was added to PCR products and samples were denatured by heating at 98°C for 3 min. An aliquot of 1.5 µL of each sample was loaded on 4.5% polyacrylamide DNA sequencing gel containing 7.5% urea. After electrophoresis was carried out for 1.30 h at constant power of 30 W, the gel was dried on a Whatman filter paper and exposed to X-Omat autoradiography film for two days at room temperature with an intensifying screen. The film was further developed and the fragments scored visually.

### Data analysis

The presence (1) or absence (0) of each amplified fragment from the autoradiogramme was scored for each genotype. From this data, a matrix of simple matching coefficients (Sokal and Michener, 1958) was generated and the neighbour-joining (NJ) clustering (Saitou and Nei, 1987) carried out with PHYLIP 3.5 c computer software (Felsenstein, 1993). For ordination analysis, principal component analysis was carried out using Statistical Analysis Software (SAS, 1989) computer package and the genotypes were plotted in three-dimensional space.

## Results and Discussion

A preliminary survey of two six-base cutter-restriction enzymes *Eco* RI and *Pst* I in combination with a four-base cutter-restriction enzyme *Mse* I was conducted to select the most informative combination of enzymes for AFLP analysis of yam genome. The combination *Eco* RI/*Mse* I revealed 64 fragments among which

**Table 2** Oligonucleotide adapters and primers used for amplified fragment length polymorphism (AFLP) analysis of Guinea yam varietal groups

Eco RI adapter:		5'-CTCGTAGACTGCGTACC
		3'-CATCTGACGCATGGTTAA-5'
Eco RI primers:	E1	5'-GACTGCGTACCAATTCA-3'
	E2	5'-GACTGCGTACCAATTC-3'
Mse I adapter:		5'-GACGATGAGTCCTGAG
		3'-TACTCAGGACTCAT-5'
Mse I primers:	M1	5'-GATGAGTCCTGAGTAAC-3'
	M2	5'-GATGAGTCCTGAGTAACAT-3'
	M3	5'-GATGAGTCCTGAGTAACAG-3'

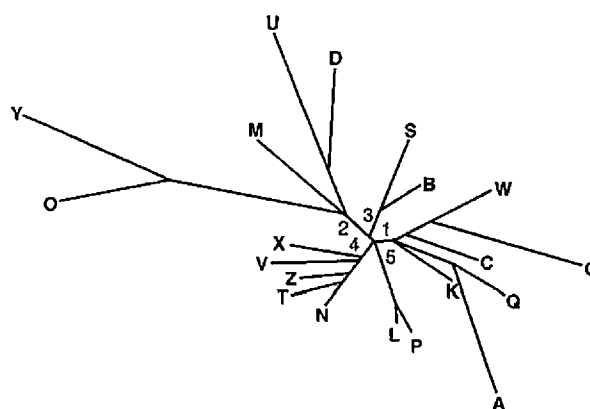
32 fragments (50%) were polymorphic compared to that of *Pst* I/*Mse* I which generated a total of 47 fragments among which only 12 (25.5%) were polymorphic. Therefore, the former combination of *Eco* RI and *Mse* I restriction enzymes was selected for generating the fragments for amplification.

Two AFLP primer combinations of *Eco* RI ACT (E2) with *Mse* I CAT (M2) and *Eco* RI ACT (E2) with *Mse* I CAG (M3) were used in the study and generated 47 and 40 polymorphic fragments, respectively, among the 20 analysed genotypes. A part of the autoradiogram from the informative primer combination is shown in Figure 1. The 87 polymorphic fragments were analysed using both phenetic and ordination methods.

The genetic diversity tree produced by neighbour joining cluster of yam varietal groups is shown in Figure 2. All the yam genotypes analysed were distinguished by their AFLP finger-



**Figure 1** Amplified Fragment Length Polymorphism-deoxyribonucleic acid (AFLP-DNA) fingerprinting of Guinea yam varietal groups for the first primer combination of *Eco* RI ACT (E2)/*Mse* I CAT (M2) Numbering according to Table 1



**Figure 2** Genetic diversity tree based on Amplified Fragment Length Polymorphism (AFLP) data generated by neighbour-joining method; Genetic distance is according to simple matching method:

- |                   |                |               |
|-------------------|----------------|---------------|
| A = Kappe-bolonda | B = Baniakpa   | C = Cocoassie |
| D = Dobnawo       | G = Grovota    | K = Krengle   |
| L = Lokpo         | M = Morokourou | N = Norforwu  |
| O = Kpokpokpokpo  | P = Kponan     | Q = Soussou   |
| S = Samancou      | T = Terkokonou | U = Koukou    |
| V = Bakokae       | W = Waraga     | X = Gnidou    |
| Y = Yaobadou      | Z = Zrezrou    |               |

prints, each genotype displaying a unique DNA profile. Five groups of cultivars could be identified as indicated on the tree. The first group consisted of five cultivars (Waraga, Krengle, Cocoassie, Soussou, and Kappe-bolonda) which corresponded to the cultivated species *D. rotundata* and the wild yam relative *D. praehensilis* (Grovota). Cocoassie and Kappe-bolonda, are peculiar in their morphological characteristics. Cocoassie, a forest-grown yam cultivar, is similar to the wild species *D. praehensilis* on the basis of several morphological traits (Hamon, 1987). The close morphological resemblance is also reflected in their genome similarity (80.2%). The most distant genotype among this group was Kappe-bolonda which showed the highest (17) number of unique fragments among all the analysed cultivars. Its morphological characteristics were similar to the forest-growing wild species *D. mangenotiana* which may suggest that Kappe-bolonda was domesticated from *D. mangenotiana*. That might explain the position (high genetic distance) of this cultivar on the genetic diversity tree compared to the other yam varieties that are classified into *D. rotundata*.

Group two was genetically distant from group one and was formed by five varietal groups (Morokorou, Dobnawo, Koukou, Yaobadou, and Kpokpokpokpo). Two varietal groups, Yaobadou and Kpokpokpokpo were the most remotely associated varieties on that branch. These varieties were characterized by eight and three specific AFLP fragments, respectively, and shared 64% of the genome information. Yaobadou is

a typical cultivar which can be easily recognised among cultivated yams and has been classified into the species *D. cayenensis*. It has some of the morphological characteristics of wild species *D. burkilliana* and might have been domesticated from *D. burkilliana* (Hahn, 1995). The highest genetic distance revealed by AFLP markers between Yaobadou and the other varietal groups analysed in the present study supports the classification of this varietal group into a distinct species *D. cayenensis* (Akoroda and Chheda, 1983; Onyilagha and Lowe, 1985). This varietal group was clearly separated from the other yam cultivars by both morphological and isozyme markers (Hamon and Touré, 1991) and, therefore, should be recognised as a separate taxon. The closest varietal group to Yaobadou was Kpokpokpokpo from Côte d'Ivoire. Kpokpokpokpo was classified as an intermediate between *D. rotundata* and *D. cayenensis* genomes (Hamon and Touré, 1990b). In the present study, it shared only 64% of its genome with Yaobadou based on AFLP information. The variety Kpokpokpokpo is cultivated only by the Akan ethnic group of eastern Côte d'Ivoire and has a particular tuber morphology that resembles a cluster of eggs. The close genetical relationships between Kpokpokpokpo and Yaobadou and their clear separation from the other yam varieties indicates that Kpokpokpokpo is genetically closer to *D. cayenensis* than to *D. rotundata*. On the same branch, two varietal groups from Cameroon, Koukou and Dobnawo, were distinct from Morokorou, a variety from Benin Republic. These two varieties were morphologically distinct from the other yam varietal groups described from West Africa. For instance, the variety Dobnawo showed a high density of spines on the stem, with heterogenous leaf characteristics made of a mix of ovate ('*cayenensis*') and orbiculate ('*rotundata*') leaf morphotypes. In contrast, the varietal group Koukou showed a spineless stem with big leaves that had long lobes and red spot on the petiole.

Dobnawo shows intermediate morphological characteristics which suggests that it may be of hybrid origin between *D. rotundata* and *D. cayenensis* or, alternatively, may have been domesticated from wild species that are particular to the Cameroonian agro-ecologies.

The third cluster consisted of only two varietal groups represented by Sammancou and Baniakpa. The variety Sammancou from Côte d'Ivoire which was used in the present study, was morphologically different both in aerial and underground organs from the one described by Hamon et al. (1986). Therefore, it is presumed that a labelling error might have occurred within the collection regarding this variety. The fourth and fifth clusters constituted five (Gnidou,

Bakokae, Zrezrou, Terkokonou, and Norforwu) and two (Lokpa and Kponan) varietal groups which were genetically close and constitute the bulk of the most cultivated and widely-spread yam varieties in West and Central Africa particularly in the savanna agro-ecologies.

To better visualize the relationships between the Guinea yam genotypes analysed by AFLP markers, a principal component analysis of the 87 polymorphic fragments was carried out. The major group of clusters as revealed by cluster analysis described above was confirmed by the three-dimensional plot (Figure 3). The first dimension discriminated the varietal groups, Yaobadou and Kpokpokpokpo, while the second dimension discriminated the Kappe-bolanda variety. The third dimension in a similar manner, discriminated the Cameroonian varieties Dobnawo and Koukou and also the wild yam *D. praehensilis* represented by the variety Grovota. The three-dimensional representation of the varieties permits a better estimation of the relationships among the remaining varieties that were dispersed around the centre of the Figure.

This study has shown that in addition to morphological and isozyme markers, AFLP markers are extremely useful to distinguish various yam varieties and to define heterotic groups that are useful for yam germplasm management and genetic improvement through breeding and selection. The present study has also shown that AFLP markers are polymorphic in yam and thus, constitute valuable genetic markers for mapping yam genome in view of tagging

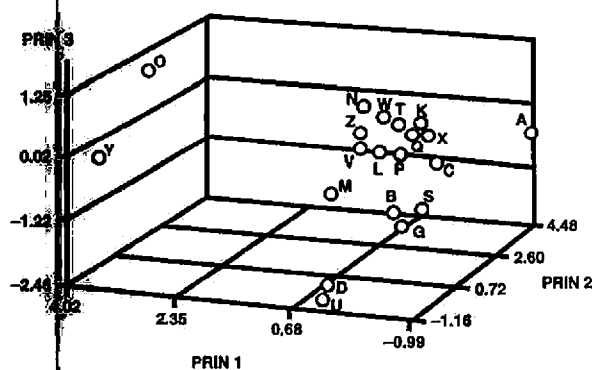


Figure 3 Three-dimensional plot based on Amplified Fragment Length Polymorphism (AFLP) data derived from the principal component analysis of Guinea yam varietal groups:

- |                   |                |               |
|-------------------|----------------|---------------|
| A = Kappe-bolanda | B = Baniakpa   | C = Cocoassie |
| D = Dobnawo       | G = Grovota    | K = Krengle   |
| L = Lokpa         | M = Morokourou | N = Norforwu  |
| O = Kpokpokpokpo  | P = Kponan     | Q = Soussou   |
| S = Sammancou     | T = Terkokonou | U = Koukou    |
| V = Bakokae       | W = Waraga     | X = Gnidou    |
| Y = Yaobadou      | Z = Zrezrou    |               |

important traits such as resistance to viruses and nematodes.

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# Network impact and scientific advances in cassava biotechnology

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The goal of the Cassava Biotechnology network (CBN) was to contribute to enhancing the value of cassava for food security and economic development. The first objectives of the CBN were to enlist advanced laboratories for cassava biotechnology research around a common strategic agenda, in order to use existing research investment cost-effectively and to stimulate relevant research in cassava-growing countries. The CBN has created linkages between national cassava research and biotechnology laboratories, both within their countries and internationally. There are presently about 125 leading cassava biotechnology projects in the world. Some of the technical progress in cassava biotechnology include the use of molecular markers such as Random Amplified Polymorphic DNAs (RAPDs), Restriction Fragment Length Polymorphisms (RFLPs), and Amplified Fragment Length Polymorphisms (AFLPs), and also cassava micro-propagation.

Keywords: Network; Cassava biotechnology; CIAT; IITA; Impact; Activities

The Cassava Biotechnology Network (CBN) was founded in 1988 mainly by the Centro Internacional de Agricultura Tropical (CIAT) in Colombia and the International Institute of Tropical Agriculture (IITA) in Nigeria. Its goal is to contribute to enhancing cassava's value for food security and economic development. The CBN's first objectives were to enlist advanced laboratories for cassava biotechnology research around a common strategic agenda, in order to use existing research investment cost-effectively, and to stimulate relevant research in cassava-growing countries.

In 1992, CBN incorporated farmers opinions directly into its research planning process. This led to development of a new vision within CBN. While CBN member laboratories work to develop advanced research tools for cassava, CBN as a network can enhance the impact of this work. It can help to create the conditions necessary for cassava biotechnology to reach its ultimate intended impact in rural areas.

## The CBN's Impact

The CBN has sought to have impact in four inter-related areas: incorporation of farmers' perspectives in cassava biotechnology research priorities; stimulation of priority research; fostering of information exchange; and the search for resources for cassava biotechnology research.

The single most important impact of the

CBN Coordination Office has been the creation of linkages between national cassava research and biotechnology laboratories, both within their own countries and internationally. In addition, CBN is actively helping to link applied research and (or) biotechnology partnerships to farmer groups for participatory research. As biotechnology tools are developed, they are being integrated into cassava research. Participatory research with cassava farmers and processors, is expected to assist the design of biotechnology-assisted research (such as new varieties) and also to increase technology transfer to the farmers. As a result, cassava research is no longer isolated from the potential of biotechnology.

## Network Growth and Recent Activities

By 1992, the network had grown from a few scientists in about four projects, to about 200 members in 25 projects. Today, active research members are more than 800; two-thirds of these work in developing countries. There are about 125 leading cassava biotechnology projects in the world.

The CBN Small Grants programme has fostered research and collaboration among members by awarding more than 40 grants since 1992. The most impact has been in fostering priority research, but iterative and interactive assessment of priorities and information exchange,

have also received a substantial proportion of the total contributions. About half of CBN grants are for research conducted in cassava-growing countries, and most involve national programme collaboration. More recent grants involve farmer collaborators as well.

The CBN international scientific meetings reflect the growth and character of the Network. The first CBN meeting in 1992, was attended by 125 researchers, mostly from advanced laboratories and international centres. The third meeting, four years later, was attended by 185 persons, more than half from national cassava research programmes, while the number attending from advanced laboratories and international centres has been constant. Funding support for CBN meetings has also grown significantly, mostly for participation of scientists in developing countries. An increasingly important group of CBN members are donors and farmer representatives. These groups began to be significant participants in the third CBN meeting and are expected to play an important role in the upcoming fourth meeting in 1998.

Participation in CBN meetings by researchers from all regions (Africa, Asia, and Latin America) permits the formation of contacts for South-South collaboration. Because of the high cost of South-South travel, funding for national programme researchers is often restricted to meetings within their own region or in the 'North'.

### Outlook: CBN Regionalization

Regionalizing CBN's structure has been discussed for several years. A first step was the allocation of 1996 CBN small grant awards according to regional priorities and recommendations. Regionalization is timely for a number of reasons.

The regional character of cassava agriculture (for example, the predominance of local food security in Africa versus income generation in Asia), and the different stages of regional technological development, can be better served in a regional structure. The CBN's partners in cassava applied research networks are organized by region. Farmer interaction with researchers is developing naturally at the regional and local levels.

An appropriate structure is needed in each region to permit a decentralized network to function effectively. A global function is needed as a continual source of animation and vision, in order to encourage farmer linkages, link regions to relevant international information and experience, promote South-South interactions, and communicate results to the international community.

As regionalization of CBN advances, it will emphasize stakeholder participation in order to generate broad-based regional ownership in CBN. This is expected to lead to enhanced sustainability, but most importantly, to creative

new structures and activities that will benefit CBN's members and objectives in ways unattainable without full participation.

### Technical Progress in Cassava Biotechnology

#### Genetic transformation of cassava

#### *Breakthrough and rapid subsequent progress*

Standard protocols for selection of transformed cells inhibit the regeneration of transformed cassava cells. Moreover, cassava has not been regenerated from callus tissue except via somatic embryogenesis. Somatic embryos are often generated from groups of cells rather than from single cells, resulting in plantlets that are only partially-transformed (Taylor *et al.*, 1996). Consequently, transformation methods developed for other crops were not readily transferable to cassava. Initial progress was difficult and slow.

*Agrobacterium* systems are considered most desirable for cassava improvement purposes, because the transformation technology does not require expensive equipment (Gonzales *et al.*, 1997). However, *Agrobacterium*-mediated transformation of cassava is genotype-dependent. Microprojectile systems are costly but have the advantage of being genotype-independent. A new regeneration system for cassava embryogenic suspension cultures (Taylor *et al.*, 1996) offers a better probability of success in targeting, isolating, and regenerating a single cell to produce a uniform transformant.

Recently genetic transformation of cassava has been achieved using both *Agrobacterium* and microprojectile transformation systems; and both embryogenic suspension cultures and somatic embryos have now been successfully used as target regeneration systems. Methods used successfully to produce confirmed transgenic cassava plants include microprojectile bombardment of suspension cultures (Raemakers *et al.*, 1996; Schöpke *et al.*, 1996) and *Agrobacterium*-mediated transformation of somatic embryos has been used in two other laboratories (Sarria *et al.*, 1995; Schöpke *et al.*, 1996; Sarria *et al.*, 1997). Additional laboratories are at advanced stages of obtaining (Vazquez *et al.*, 1997) or verifying (Arias-Garzon *et al.*, 1997) transgenic plants by this method. Regeneration via organogenesis has also recently been achieved (Li *et al.*, 1996), a method that, if broadly applicable, could shorten the time from initial explant to transformed plant. Cassava protoplasts have been transformed using microbombardment and the early stages of plant regeneration have been achieved (McDonnell and Gray, 1997; Sofiari *et al.*, 1997).

Further development of genetic transformation as a methodology for cassava breeding will re-

quire appropriate gene promoters. A constitutive promoter has been isolated from cassava vein mosaic virus (Verdaguer *et al.*, 1997) that has been shown to be active in cassava. A promoter isolated from the cyanogenesis pathway of cassava itself appears to be root-specific, which would permit targeting novel gene expression to the economic part of the plant.

Within the next three to five years, transgenic cassava carrying genes of interest is expected to be available for field trials. Biosafety protocols could be a bottleneck since only a few cassava-growing countries have a national policy or legislation in place (Krattinger, 1997). However, a number of countries are in advanced stages of developing a biosafety policy. The CBN members are working closely with their national biosafety planning groups in Brazil, Colombia, and Nigeria, to ensure that CBN's work is in compliance. At present, it appears likely that transgenic cassava research will reach the stage of the first field trials at about the same time, or shortly after, national policies are enacted (Roca, W., CIAT, and Sampaio, M.J. CENARGEN, pers. commun).

#### *Use of the transgenic approach in cassava improvement*

The next major effort will be directed at introducing useful genes to help solve problems and create opportunities relevant to concerns of cassava farmers. Among farmers' concerns, two general areas are usually top priority: total dry matter yield and production stability (including availability of desired varieties), and markets and prices (Henry and Howeler, 1995; Thro *et al.*, 1994, 1997).

*Yield level and stability*—Disease and insect resistance will be the first production-related targets. For example, every tool known, from intercropping through classical breeding to transgenic approaches, is being employed to combat a virulent new virus that has eradicated traditional cassava varieties in Uganda and spread into neighboring countries. A defective viral replicase that confers resistance to ACMV in a model system has been transferred into cassava. Transgenic plants with viral-derived (coat protein) resistance to common cassava mosaic virus, will be ready for testing in affected areas of South America, as soon as all biosafety considerations have been met (Schöpke *et al.*, 1993; Gonzales *et al.*, 1997; Taylor *et al.*, 1997). Research on transgenic approaches to resistance to bacterial blight, globally the most widespread cassava disease, and to cassava stem borer, a destroyer of planting material throughout northern South America, is in the exploratory initial stages of research (Taylor, N., Puonti-Kaerlas, J. and Roca, W., pers. commun.)

*Market and price factors*—Varietal traits relevant to markets and prices include crop quality for market advantage and specialty uses and storage life. Transgenic approaches can increase

the value of cassava by enhancing starch content and (or) starch quality (Visser and Jacobsen, 1993). Transgenic techniques are being used to study the biochemistry of the rapid physiological post-harvest deterioration in cassava. Information obtained will assist both transgenic and classical approaches to retarding deterioration for reduced crop losses and better market access. Genetic transformation will create a range of types of cyanogen metabolism for cassava, for optimizing crop management and market access. In some areas, farmers chose acyanogenic varieties for 100% consumer confidence; in other situations, when production or plant protection advantages are paramount, cyanogenic (toxic) varieties will be more appropriate. Genes for starch alteration (Salehuzzaman *et al.*, 1992) and modification of cyanogen metabolism in cassava are available (Hughes *et al.*, 1994; White and Sayre, 1997). In the long term, transformation may permit the harnessing of cassava's uniquely rapid photosynthetic mechanism (Black *et al.*, 1993) for synthesis of new products such as polymers for biodegradable plastics (Poirer *et al.*, 1992).

#### *Cassava molecular marker research*

The genetics of cassava is less well understood than the genetics of the other four major staple crops (rice, maize, wheat, and potatoes), due to the relatively small amount of basic research on cassava. Development of molecular markers for cassava is intended to address fundamental unanswered questions about the crop's origin, genome structure, organization of genetic diversity among *Manihot* germplasm resources, and genes controlling traits of agronomic interest. These studies will enhance breeders' ability to access and select desired genetic variation.

#### *Molecular marker characterization of cassava germplasm resources*

The large number of polymorphisms at the deoxyribonucleic acid (DNA) level in even closely related organisms, added to their phenotypic and environmental neutrality, makes DNA markers the most powerful available tool for the assessment of genetic diversity. Several types of DNA markers have been employed in cassava. The choice of marker is based on research objective, degree of relatedness between samples being studied, state of knowledge about target genotypes and, most important, cost and relative ease of assay.

The first DNA markers applied to cassava were Restriction Fragment Length Polymorphisms (RFLPs) (a labour intensive and costly, but highly repeatable method) and Random Amplified Polymorphic DNAs (RAPDs) (a more rapid, lower-cost method, but sensitive to experimental conditions). The RFLPs and RAPDs were used to estimate genetic distances between related and unrelated cassava clones and *Manihot* spp. accessions (Beeching *et al.*, 1993; Haysom *et al.*, 1994; Marmey *et al.*,



1994; Mignouna and Dixon, 1997) and to distinguish between clones which were suspected of being duplicates, based on isozyme data (Ocampo *et al.*, 1995). The RFLP markers based on chloroplast and ribosomal DNA clones have provided evidence that cassava has its origins in domestication of close wild relatives, including *M. tristis* and *M. esculenta* subsp. *flabellifolia* (Bertram, 1993; Fregene *et al.*, 1994). Studies with both RFLPs and RAPDs showed that agricultural cassava clones had relatively low levels of DNA polymorphism, even when their origins were agro-ecologically diverse (Angel *et al.*, 1993; Bonierbale *et al.*, 1993). While the germplasm adapted to certain edaphoclimatic zones showed a broader genetic base than germplasm adapted to other zones, accessions could not be assigned to a particular zone based on molecular patterns, due to considerable overlap of allele frequencies.

More recently, microsatellite markers have been used to study cassava. Microsatellites are nuclear DNA that occur as very short repeats of base pair units. They are inexpensive in use and have a high level of polymorphism, making them very attractive for analysis of genetic variation or mapping (Tautz, 1989), although their development can be expensive. When microsatellites were used to study genetic relationships between cassava and its wild relatives (Chavariagga, in preparation; Bonierbale, unpubl. data), results revealed that the great morphological variability in cassava is not reflected at the molecular level, in comparison to higher levels of molecular genetic diversity in closely related species.

Amplified Fragment Length Polymorphism (AFLP), a multilocus DNA fingerprint technique, has also been applied to assess genetic relationships in cassava and wild relatives. A study of wild relatives (Roa *et al.*, 1997) demonstrates that certain Brazilian and Colombian *Manihot* species (*M. esculenta* subsp. *flabellifolia* and *peruviana*, *M. cartheginensis*, and *M. brachyloba*) are more similar to cassava than is a Mexican relative, *M. aesculifolia*, a species earlier thought to be closest to cassava based on morphology (Rogers and Appan, 1973). These data support the conclusion from cpDNA- and rDNA-based RFLP studies and microsatellites, that cassava might have its origin in close relatives such as *M. esculenta* subsp. *flabellifolia*, and *M. tristis*. Results from this study and two others (Fregene *et al.*, 1997; Second, 1997) using microsatellite markers also supports the findings that cassava is genetically less diverse than its close relatives, despite its prodigious morphological variation and the wide differences in the agro-ecological origins of cultivars. A study comparing African and Latin American germplasm revealed a unique AFLP fragment, shown to be the intergenic spacer region (IGS) of rDNA, present only in some African accessions (Fregene *et al.*, 1997). This region may

be useful for genetic distance analysis and for understanding the movement of cassava from its centre of origin.

### A molecular genetic map of cassava

Because of the paucity of simply inherited morphological markers in cassava (only nine have been described to date) (Graner, 1942; Jos and Hrishii, 1976; Hershey and Ocampo, 1989), a classical genetic map does not exist for cassava. A cassava molecular map has been constructed, using as the mapping population, an intraspecific cross between TMS 30572 (female parent), an elite cassava cultivar developed at IITA, Nigeria, and CM 2177-2 (male parent), a successful cassava cultivar resulting from breeding at CIAT in Colombia, with 150 progeny. The mapping population was designed to segregate for traits of interest, including resistance to the African cassava mosaic disease (ACMD), high photosynthetic rates, good cooking quality, and tolerance to the cassava mealy bug. A sub-set of 90 plants was employed to construct the first map (Fregene *et al.*, 1997).

One hundred and fifty RFLPs, 30 RAPDs, 5 microsatellites, and 3 isoenzyme markers, segregating as single dose restriction fragments from the gametes of the female parent of the mapping population were used in constructing the molecular genetic map (Fregene *et al.*, 1997). The map consists of 20 linkage groups spanning 940cM and it is estimated to cover about 70% of the cassava genome. Average marker density is one per 7.9 cM. Since the mapping population is an F<sub>1</sub> cross between heterozygous parents, with unique alleles segregating from either parent, a second map was constructed from the segregation of 107 RFLPs, 50 RAPDs, 3 microsatellites, and 1 isoenzyme marker from the male parent organized into 24 linkage groups of total length 1200 cM. Comparison of intervals in the male- and female-derived maps, bounded by markers heterozygous in both parents or allelic bridges (Ritter *et al.*, 1991), revealed significantly less meiotic recombination in the gametes of the female compared to the male parent. A total of 30 allelic bridges were detected and employed to reconcile all but 6 of the male- and female-derived analogous linkage groups.

### Molecular marker tagging of genes controlling simple and complex agronomic traits

Marker genes may have little or no direct effect on the traits they mark, but can be used to estimate number of genes, gene action, and magnitude of genes controlling agronomic traits of interest. Molecular genetic maps provide a set of neutral genetic markers for the complete genome, and consequently a high probability of detecting linkages with any gene or genes of interest to genetics or breeding. Desirable characters that are difficult to screen and (or) breed

for by traditional methods (e.g., pests subject to quarantine exclusion, traits that are expressed only at the end of the crop's long growing cycle, and traits for which assays are complicated by environment, maturity, or pest variability) are important candidates for gene tagging. Prerequisites for mapping are (1) importance of a character; (2) difficulty of screening by direct methods (i.e., need for marker); (3) an adequate (large, variable) population, preferably with a simple pedigree; (4) a source of (mapped) markers; and (5) a reliable (even if difficult) phenotypic screening method.

The population used for genetic mapping of cassava at CIAT was designed to express traits regarded as priority for gene tagging which could benefit from the development of correlated molecular screens. These include resistance to cassava bacterial blight (CBB) and ACMD, root quality characters such as cyanogenesis, post-harvest deterioration, culinary quality, early bulking, and starch content. The RFLP mapping identified a region of the cassava genome that accounts for 80% of the variance for resistance to a strain of CBB. This ought to be a useful marker for introgressing CBB resistance into susceptible genotypes, but for the difficulty of backcrossing in cassava. Cassava suffers from strong inbreeding depression when made homozygous. Efforts are continuing to saturate this interval with more markers as the starting point for cloning the resistance gene. Similar work has begun with ACMD resistance in collaboration with scientists in Africa where the disease is found and where segregating populations can be phenotyped for disease resistance. A backcross population, derived from crossing members of the mapping population to the ACMD resistance parent, TMS 30772, has been developed to this effect and is expected to show significant variability to the disease. Resistance to ACMD is thought to be recessive (Byrne, 1984). The advent of map-based cloning of resistance genes coupled with genetic transformation protocols for cassava make genetic transformation a faster and more efficient way of combating disease epidemics.

The QTL analysis of root quality traits has revealed regions of the genome that control 8 and 10% of phenotypic variation for post-harvest deterioration (PHD) and 13% of phenotypic variance for dry matter content (CIAT, unpubl. data). These data indicate that molecular tags can be developed. An interdisciplinary approach to identifying markers for these traits will be essential.

#### *Comparative mapping and the universality of molecular markers*

Because they reflect variation at the DNA level, genetic maps based on RFLPs or other measures of homology provide tools for comparing sexually incompatible but closely related species on a whole genome level. Along with cassava, is *Hevea* rubber, for which a genetic map is

being constructed (Low *et al.*, 1995). Preliminary comparisons of genomic probes have demonstrated homology in reciprocal hybridization between *Manihot* and *Hevea* spp. (*M. sequin* and *M. bonierbale*, unpubl. results). A comparison of linkage order among mapped probes in the two genomes is planned. This process could contribute to definition of the genome structure of the two species, as has been difficult to date for cassava alone.

#### *Cassava micro-propagation: A new look at a 'mature' technology*

In 1995, at the time of the last International Society of Tropical Root Crops (ISTRIC) international meeting, it might have been thought that cassava micro-propagation was an accomplished technology. Although regeneration after genetic transformation procedures remained a serious bottleneck, meristem culture needs only location-specific adjustments for application by applied cassava research programmes for germplasm conservation and exchange (Roca, 1984; Roca *et al.*, 1991). The cost of tissue culture, compared to the value of the crop, seemed to limit micro-propagation to these uses for cassava.

Responding to a series of crises in Africa has changed that view. Tissue culture is proving indispensable for variety multiplication to overcome cassava's extremely slow rate of propagation. Without rapid availability of urgently-needed planting material in areas where cassava has been devastated by new diseases and by war, hunger and poverty will be prolonged or even intensified. These experiences have led researchers to ask if costs cannot be reduced by further research, combining knowledge of the basic biology of micro-propagation in relation to local conditions, supplies, and materials. The applicability of low-cost, robust tissue culture methods, even if less than factory-efficient, would extend far beyond Africa. For example, such a technology could support micro-enterprises in rural towns.

'Low-tech biotech' for cassava variety multiplication is still a fantasy, but it is one which CBN members hope to pursue in Uganda, Colombia, and other countries. A first step is already in progress which is a Master's thesis project at the University of Zimbabwe, also involving groups in Mozambique, which will investigate the development of robust methods for post-flask hardening of cassava plantlets. Post-flask losses are often very high; better methods for this step alone would be a great assistance to programs using micro-propagation in cassava-growing areas.

#### **Conclusion**

Genetic transformation of cassava has been completed past the pioneering phase, protocols are improving rapidly, and applications will be available in the short- to medium-term. The

DNA molecular markers are also past the initial exploratory stages and the first applications are being developed. These scientific advances are linked with interdisciplinary applied research to move cassava biotechnology from the laboratory to the field within the next two to five years. Also during that time, regional leadership and initiative in the CBN will prioritize the needs and opportunities of cassava-dependent rural communities and their countries and become increasingly active in developing biotechnology applications for cassava.

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# Promotion and adoption of yam minisett technology in Ghana

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The minisett technique has been proposed as a method that can solve the problem of lack of planting material in Ghana. Despite its apparent advantages over traditional seed yam production methods, the technique has not been widely practised by yam farmers although it was introduced several years ago. From an analysis of reports from District and Regional Agricultural Offices and interviews with selected Agricultural Extension Officers in Ashanti, Brong Ahafo, and Northern regions, the following results were obtained: the choice of emphasis on farmers in the traditional yam zones in Ashanti and Brong Ahafo regions or high yam production areas of the Northern Region produced no adoption during the promotion of the technique (1989-92). The non-traditional yam zones, even without much emphasis had more positive farmer responses. The reasons for these results are discussed.

Keywords: Yam minisett technique; Seed yam; Farmer responses; Target clientele; Traditional and non-traditional yam zones; Ghana

In Ghana, farmers use two conventional methods to get yam-planting material. One involves cutting the head of the tuber which results in one or two pieces depending on its size. The second involves a process described as milking, topping, or pricking in which the growing vine is severed from the main tubers towards the end of the growing season resulting in the development of a cluster of small tubers or seed yams. Seed yams are the most popular planting materials used by farmers in West Africa (Otoo, 1980) for the many cultivars of *Dioscorea rotundata* Poir (white yam). The size of seed yam usually planted by farmers varies from 0.5-1 kg (Otoo, 1980; IITA, 1987).

In Ghana, ordinarily, farmers have to set aside about a quarter of their yam harvests to be used as planting material (Otoo *et al.*, 1987). Therefore, the minisett technique solves the major problem of planting material encountered especially by prospective yam farmers in Ghana.

Another major problem facing commercial yam farmers in Ghana, is the post-harvest dilemma of storage and marketing of tubers milked at the peak of the rainy season (August to September). During this period, farmers have a poor bargaining position in terms of pricing. Yams harvested at the peak of the rainy season, if not quickly sold, stand the risk of easily becoming rotten due to inaccessibility and remoteness of major production areas, and the prevailing warm and moist conditions at the time of milking these early-maturing cultivars.

Since one of the main reasons behind milking is to produce seed yam for the next planting season, an alternative source of seed yam (i.e., through the minisett technique) could allow farmers to delay harvesting for as long as they wish, and harvest only on demand, thus increasing their bargaining power and earnings. In the short-run, the technique could enable farmers to produce enough planting material, avoid waste in yam production, and make seed yam available at reasonable cost.

Apart from the knowledge and skill, an established yam farmer only needs to purchase a small amount of fungicide in order to fully put the technique into practice. A prospective yam farmer on the other hand, in addition, needs to buy ware-yams from which the minisetts are made. However, it is still cheaper to produce and maintain pure clones of seed yam through the minisett technique than to buy seed yam from the market. The advantages in the use of and ease of application of the technique should ensure a high rate of its adoption by, and diffusion among, farmers. However, even though introduced in Ghana in 1989, the technique has remained at the demonstration stage with only a few trials by curious innovators.

The Department of Agricultural Extension Services (DAES) is the sole agency mandated to carry out extension education among farmers in a Unified Extension Service in Ghana. The failure of farmers to adopt the minisett technique, especially in the traditional yam-producing zones in Ghana despite the advantages of the technique needs to be assessed. The main objectives of this research were to (i) examine the role played by the DAES in the poor adoption and diffusion of this technique among farmers

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in three important yam-producing regions in Ghana, and (ii) explain the observed pattern of adoption of the yam miniset technique, and suggest an approach that could enhance its successful promotion in Ghana.

## Methodology

The main data collection and analysis involved analysis of routine monthly reports from relevant District and Regional Agricultural offices and subject files on yam miniset technique in both the Crops and Extension Service Departments of the Ministry of Food and Agriculture (MOFA) in Ashanti, Brong Ahafo, and Northern regions. These are the three major yam-producing regions in Ghana. The data were supplemented and cross-checked with discussions with agricultural officers and intensive interviews with some yam farmers.

In each region, the Regional officers in charge of Extension, Extension Training, Crops Services, and five District Agricultural Extension officers (DAEOs) who reported extension activity on the technique were randomly selected and interviewed. Thirty of the 50 farmer interviews were limited mostly to (established and prospective) migrant yam farmers in the Brong Ahafo region because they easily constitute most of the large-scale yam producers and the largest production area in Ghana. The 20 remaining farmer interviews were divided equally between farmers from the Ashanti and the Northern regions. The documentary data collection, discussions with agricultural officers, and intensive farmer interviews were conducted simultaneously between July and December 1992.

The authenticity of reports from which the documentary analysis was made is considered to be sound. They do not under-report any extension activities or adopted techniques which are being promoted. Much emphasis is placed on the writing of monthly reports by superior officers in the DAES. The general tendency is, rather, the exaggeration of activities and successes by front-line staff (whose individual reports are compiled into the District report by District officers) so as to give the impression of being hard-working.

## Results and Discussion

### Technical preparation prior to promotion of the technique

Evidence from records, informal discussions with Agricultural Officers, and some of the farmers who cooperated in some of the on-farm demonstrations suggests strongly that the initial preparation by the departments charged with the responsibility of promoting the technique

was either non-existent or woefully inadequate. The following reasons account for this impression.

### *Lack of adaptive trials on the technique prior to promotion*

Work by Kalu (1989) suggests that the original recommended miniset size of 25 g by the National Root Crops Research Institute, Umudike, which developed the technique in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan, may not be appropriate for all yam varieties in all ecological zones. There is no record of any adaptive trials by departments in any of the regions under consideration or in any part of the country. This explains why smaller sizes of minisets were used in the on-farm demonstrations (especially for cultivars of the popular white yam variety), which resulted in the production of sizes of seed yams normally roasted for on-farm feeding instead of being used as planting material by experienced, traditional yam farmers.

### *Inadequate training for change agents*

Analysis of the records indicate that only single, one-day demonstration training sessions were, at best, given to District Agricultural Officers of both departments who subsequently gave similar training to front-line staff responsible for finally imparting the skill to farmers. This training given to both categories of change agents (most of whom may never have grown yam on their own) is inadequate. The result of this inadequate training was the production of seed yams which were normally rejected by traditional farmers in most of the on-farm demonstrations. The primary aim in using on-farm demonstrations, is to impress farmers so as to convince them to adopt what is being promoted.

### Extension activities undertaken

The following extension activities were undertaken during active promotion of the technique in the three selected regions during the period 1989-92.

#### *Ashanti region*

The technique was launched in May 1989 in two districts (Sekyere West and Ejura-Sekodumasi) both of which are traditional yam-producing areas, and 15 and 150 farmers, respectively, participated. In May 1990, a demonstration plot was established in each of the five sub-districts of the Sekyere-West district involving a total of 122 male and 50 female farmers.

A priority year for the technique was declared in 1991 with plans to establish 0.4 ha-demonstration plots in all the 18 districts and additional 1.6 ha-plots in each of four other districts (Ejura-Sekodumasi, Sekyere East, Sekyere West, and Offinso) considered as high potential areas. In connection with this priority

plan, all DAEOs and one technical officer from each district were given a one-day training on yam minisett technique.

Only one district (Adansi West) reported that 7 out of 92 farmers exposed to the technique had tried it on a total area of 0.16 ha. In addition, 24 farmers, 1 secondary school, and 1 Crop Association from 2 other districts (Adansi-East and Bosomtwe-Atwima-Kwawoma), had tried to produce seed yam using the technique. All three districts from which these responses were obtained in 1991 fell outside the traditional yam zone or officially designated high potential area. By July 1992, only one district (Ejura-Sekodumasi) out of the 18 districts in the region had on-going minisett demonstration plots with nurseries in all sub-districts.

### *Brong Ahafo region*

Extension work on the technique involving demonstration plots began only in one district (Wenchi) in 1989. In 1990, 25 contact farmers were involved in another district (Techiman). Both districts are traditional yam-producing areas. A third district (Berekum) from the non-traditional yam zone area had only three demonstration plots in 1990. In 1991, on-farm demonstrations were established in all 13 districts of the region. Four out of five districts (Asutifi, Berekum, Jaman, and Tano) with over 20 cooperating farmers were from the non-traditional yam zone. In the Jaman District, a crop association which adopted the technique with the sole aim of producing yam for export was officially inaugurated in November 1991.

By July 1992, there were 71 seed yam producers in the Jaman District while a target of 40 on-farm demonstrations had been exceeded by 2 in Asutifi District. A third district from the non-traditional yam zone (Tano) had an increase from 18 in 1991 to 22 on-farm demonstrations in 1992. Two other districts had moderate target achievements (Wenchi 12 out of 19 and Berekum 26 out of 54) in on-farm demonstrations, while two others recorded very poor target achievements (Attebubu 3 out of 40, and Techiman 1 out of 55). In all, seven districts did not record any activity whatsoever, while four others (Attebubu, Berekum, Techiman, and Wenchi) recorded less work on the technique in 1992.

### *Northern region*

A monthly workshop was used at the Regional level to train 43 participants (mainly DAEO) by resource persons from the Crop Services Department in April 1989. Thirty-six other staff from a pilot district (Bole) were subsequently given a one-day training in May 1989 by resource personnel from the DAES. Two hundred and 206 farmers from Savelugu and Gushegu, respectively, were similarly given pre-season training during which the technique was one of six topics discussed.

In 1990, two districts (Yendi and Saboba)

had staff training on the technique while farmers' meetings were held in another two districts (Tolon and Saboba) presumably to demonstrate the technique. Only one district (Savelugu) had two on-farm demonstrations.

In 1991, a large number of meetings with farmers were held in which the technique was discussed in two districts (Nanumba, 30 times with 826 farmers, and Zabugu, 22 times with 454 farmers); while Farm and Home visits featured the topic in two districts (Nanumba and Yendi). Through the DCS/International Fund for Agricultural Development (IFAD) collaboration, 60 on-farm demonstrations were established by July 1991, but by August 1992, only two (Gushegu-Karaga and Nanumba) out of the total of 13 districts had on-going on-farm demonstrations on yam minisett in the Northern region, and no reported adoptions or even trials by farmers.

### *Implications of extension activities during the promotion*

The following observations stand out from the manner in which the DAES promoted the yam minisett technique during the period under consideration.

#### *The extension approach used*

Informal discussions with selected Agricultural Officers confirmed that the minisett promotion programme was established suddenly by top management. Inadequate technical preparation (discussed earlier) of front line staff (FLS) and officers involved in promoting the technique further worsened this top-down transfer of technology approach adopted by the DAES. To be successful, transfer of technology approach demands technical competence in the conduct of on-farm demonstrations, for example, in order to bring out clearly to farmers, the superiority of the technique over their traditional methods. Under the circumstances, participatory technology development approach, by involving farmers who are the more experienced in yam production in any case, in on-farm trials using a combination of different minisett sizes for different cultivars, for example, would have been more appropriate. The suggested approach could have given farmers the opportunity not only to reveal their cultural biases, but also adapt the technique to suit their needs.

#### *Duration of the active promotion*

After being introduced between 1989 and 1991 in every district and sub-district, only one district in Ashanti, two in the Northern, and six in Brong Ahafo region had on-going extension work on yam minisett technique by July 1992. Only 1 out of 15 district officers with whom discussions were held anticipated funding for yam minisett promotion in his area. The general view held by district officers was that the programme was too short-lived to have any



meaningful impact, especially on established yam farmers in the traditional yam-producing areas. During the promotion, funding for materials for demonstrations as confirmed by DAEO, was erratic to the extent that interested farmers (especially in Brong Ahafo) often had to provide all the materials required for on-farm demonstration.

The adoption and diffusion of a new technique such as yam minisett by farmers require continuous exposure to it. Research studies have clearly demonstrated that extensive delays often occur between the time farmers first hear about favourable innovations, and the time they adopt them, if they find them appropriate to their situations. For example, it took four years on average for the majority of Mid-western United States of America farmers to adopt recommended practices (van den Ban and Hawkins, 1988). The downward trend in extension activity and farmer responses after the initial launching in 1989 is attributable not only to the approach adopted in promoting it, but also to the lack of sustained policy to consistently include yam minisett promotion on its agenda and budget for a reasonable number of years. Unless there is consistency in extension programme planning, budgeting, and financing, adoption and diffusion of recommended practices by farmers may fail in most areas simply due to the *ad hoc* nature of the promotion.

An analysis of the records generally indicate a lack of enthusiasm by farmers from the traditional yam-producing areas to adopt the yam minisett technique.

One implicit assumption of agricultural extension lies in the incorrect belief that the adoption of innovations is always desirable to all social and economic strata in the society. There is the need to give circumstances and goals of farmers strong consideration first (van den Ban and Hawkins, 1988), in order to provide for the real needs to the right target clientele, at the right time.

Although a positive response to the technique was shown in 7 out of the 44 districts, analysis of the regional data shows a downward trend in 40 districts in the study area in respect of extension activities and farmer participation in the minisett technique. A positive response by farmers, as used here, refers to one or any combination of the following situations: (i) increased farmer interest reflected in higher reported numbers cooperating in on-farm demonstrations; (ii) reports of any trials by farmers; and (iii) reports of any adoption by farmers.

There were only four districts in which progress, no matter how little, was recorded in positive farmer responses over preceding years during the period of active promotion of the technique. These districts were Asutifi, Jaman, Tano, and Berekum, all non-traditional yam zones in the Brong Ahafo region. It is not coincidental that there was not a single district

within the traditional yam zone in which any positive farmer responses were recorded.

### Reasons for poor farmer responses in traditional yam zones

The following reasons deduced from intensive interviews with farmers exposed to the technique during the period of active promotion of the technique, explain the zonal disparity in farmer responses between traditional and non-traditional yam zones.

- (i) Culturally among traditional yam farmers, the bigger the sizes of yams produced, the higher one's social standing as a yam farmer. Experienced farmers know that the bigger the size of seed yam planted, the bigger the yield. It is, therefore, not surprising that responses from farmers in the traditional yam zones to the mostly under-sized seed yams produced during most of the on-farm demonstrations in these zones was very poor. Normally, the average size of seed yams used by farmers as planting material is  $\geq 500$  g (Otoo, 1980; IITA, 1987).
- (ii) Commercial seed-yam production is not yet an accepted practice in these traditional yam-zones. Traditionally, seed yam is considered as very important capital that should never be sold, especially prized cultivars. It requires very good relationship, often bordering on servant-master situations or strong family bonds between donors and recipients or sometimes buyers and sellers (in cash or kind) to obtain good cultivars of seed-yam. This usually establishes some influence of the donor/seller over the recipient/buyer which the yam minisett technique could eliminate if adopted in these areas.
- (iii) Prospective yam farmers in the traditional yam zones who are in dire need of seed yams in order to be able to establish themselves, and would, therefore, have benefited from the yam minisett technique promotion, were not identified as the target clientele, and consequently, could not have been reached by extension agents.
- (iv) Finally, established yam farmers, as rational economic beings, have not discovered the advantages that the minisett technique may have over their main traditional method of milking since this realisation and decision-making process in adoption requires cultural changes which take time and extension effort to accomplish (Jones, 1967; van den Ban and Hawkins, 1988). None of these requirements was, or could have been met, through the extension approach adopted, and within the short span of active promotion of the technique.

### Reasons for positive farmer responses in the non-traditional yam zones

Informal discussions and intensive interviews with farmers in the non-traditional yam zones indi-

cate that there is a preponderance here of prospective yam farmers, to whom planting material constitutes the greatest obstacle. Secondly, prospective yam farmers in this zone are more likely not only to have less experience in, but also little cultural behaviour attached to, yam production. Moreover, no known institutionalised interests in yam production were threatened by the adoption of the yam minisett technique in this non-traditional yam zone. Consequently, with no cultural barriers to surmount, and no interests threatened, better farmer responses to the technique were shown only in the non-traditional yam zones in this study: Adansi East and West and Bosomtwe-Atwima-Kwawoma in the Ashanti region; and Jaman, Asutifi Berekum, and Tano in the Brong Ahafo region.

#### Choice of target clientele

Although the yam minisett technique was promoted throughout the districts and sub-districts within the study area, documentary evidence from the departments charged with that responsibility indicates that established yam farmers were the chosen target clientele. Emphasis in the promotion was placed on the traditional yam zones and high yam production areas. For example, in the Ashanti region, emphasis in extension effort was placed on the four districts constituting the traditional yam zone (Ejura-Sekodumasi, Sekyere East and West, and Offinso) which were designated as high potential areas. No positive farmer responses were, however, recorded in any of these districts, even though active extension activity still continued in Ejura-Sekodumasi at the time of this study. The only positive farmer responses within the region were recorded from non-traditional yam zones (Adansi East and West and Bosomtwe-Atwima-Kwawoma).

Similarly, in Brong Ahafo region, extension activity on yam minisett was concentrated in traditional yam zones (Wenchi and Techiman) in the first two years with no progress. However, mass adoptions were recorded from non-tradi-

tional yam zones within the region immediately after the technique was introduced. Considerable progress in terms of farmer participation in on-farm demonstrations and adoptions were recorded in Brong Ahafo compared to similar areas in Ashanti simply because in the latter, the idea of concentrating on the traditional yam zones was followed strictly while ignoring other areas, even in the face of very poor farmer responses in the high potential areas.

The Northern region, with high and low production areas, can be safely described as within the traditional yam zone. No extension activity was recorded for any of the districts in the low yam production areas, and no positive farmer responses whatsoever were recorded for the entire region; not even from the high yam production areas which benefited from the promotion.

The non-traditional yam zones, even without such emphasis, reported more positive farmer responses. The choice of, and emphasis on, the promotion of the technique in the traditional yam zones were mis-directed.

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# Influence of carbon source on *in vitro* tuberization and growth of white yam (*Dioscorea rotundata* Poir.)

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The response of white yam (*Dioscorea rotundata* Poir.) nodal cuttings to eight different carbon sources (sucrose, glucose, mannose, maltose, galactose, fructose, lactose, and sorbitol) at two concentrations (3 and 5%) in modified Murashige and Skoog medium was investigated. The number of nodes per plant (NNP), plantlet fresh weight, number of leaves per plant (NLP), percentage tuberization, number of micro-tubers per plant (NTP), and micro-tuber weight per plant (TWP) were significantly different among the carbon sources ( $P < 0.01$ ). There were no significant differences in terms of micro-tuber weight per tuber and plant dry matter content. In terms of NNP and NLP, fructose, glucose, mannose, and sucrose at 3 and 5%, and sorbitol at 5% were comparable. However, nodal cuttings were unable to produce tubers in media containing sorbitol. All the explants cultured in 5% sucrose, 5% fructose, and 3 and 5% mannose produced micro-tubers. The NTP ranged from 0.9 (3% mannose) to 1.8 (5% sucrose), while 5% glucose gave the highest TWP, followed by 5% sucrose. The best carbon sources for *in vitro* tuberization were sucrose and fructose.

Keywords: White yam; Carbon source; Nodal cuttings; Micro-tuberization; Micro-tubers

White yam (*Dioscorea rotundata* Poir.) is an important food crop for millions of people in the tropics. It is the most widely cultivated yam species in West Africa, particularly in Nigeria. Yam tubers are a source of carbohydrate and also contain vitamins such as carotene, thiamine, riboflavin, niacin, and ascorbic acid. They are also high in essential amino acids and the crude protein content in the tuber which is consumed, is 11.21% (Eka, 1985). Sugars most commonly found in *D. rotundata* tubers are sucrose, fructose, glucose, and maltose in decreasing order (Ketiku and Oyenuga, 1973). The sugar content in tuber decreases as the tuber age increases.

Viral diseases and nematode attacks reduce yield drastically, and are transmitted through the seed tubers or setts to the next generation of plants. This has significant implications in germplasm exchange across the national boundaries. The production of virus-tested plants is a prerequisite for the international exchange of clonal germplasm to avoid all risks of introducing diseases and pests to non-affected areas. Plantlets have been regenerated from meristem-tip culture of white yam (Ng, 1984) and virus-tested plants were obtained through a combination of heat treatment and meristem culture, or meristem culture alone followed by rigorous virus indexing (Ng, 1988a). Micro-propagation

using nodal cultures has been reported and used for the production of plantlets for distribution (Mantell *et al.*, 1979; Ng, 1992).

Ng (1994) described three systems for the multiplication and production of virus-tested white yam propagules for international distribution. These systems described are for the production of *in vitro* plantlets, micro-tubers *in vitro*, and mini-tubers in the greenhouse in sterile soil. Micro-tubers had been proposed as the most convenient means for the international exchange of yam germplasm because they are less bulky and can be kept for several months, due to the dormancy of the tubers (Ng, 1988b).

*In vitro* tuberization has been reported in several yam species, *D. abyssinica* (Jean and Cappadocia, 1991), *D. alata* (Ammirato, 1976; Alhassan and Mantell, 1994), *D. bulbifera* (Uduebo, 1971; Mantell, 1987), *D. cayenensis* (Ng and Mantell, 1996), *D. opposita* (Mantell and Hugo, 1986), and *D. rotundata* (Mantell, 1987; Ng and Mantell, 1996). Sucrose has always been used as the carbon source in these reports. This paper describes results obtained from studies on the response of white yam nodal cuttings to eight different carbon sources with the aim of identifying the best carbon source that could maximize shoot multiplication ratio and *in vitro* tuberization of white yam.

## Materials and Methods

White yam (*D. rotundata*) genotype TDr 131, collected from Nigeria (local name 'Abi') was used in this study. Single-node cuttings from *in vitro* plants previously grown on yam multiplication medium (YMM) were used. The YMM consisted of Murashige and Skoog medium (Murashige and Skoog, 1962), supplemented with 3% table sugar, 20 mg L<sup>-1</sup> L-cysteine, 0.5 mg L<sup>-1</sup> kinetin, and 0.7% agar. These plantlets were derived from meristem culture and virus-tested as described by Ng and Ng (1991, 1997).

The culture medium used for this study was similar to the YMM except that table sugar was replaced by eight different carbon sources: glucose, sucrose, maltose, galactose, lactose, fructose, mannose, and sorbitol. Two concentrations of each carbon source, 3 and 5% were tested. The pH of the medium was adjusted to 5.7 prior to sterilization. The medium was heated to melt the agar. Twenty millilitres of the medium were dispensed to each baby food jar (100 mL capacity). The culture medium were sterilized at 121°C for 15 min.

Two single nodes were placed in each jar. Each medium treatment had five replications (jars). Jars were sealed with parafilm. Cultures were incubated under 12-h daylength and irradiation supply was 31  $\mu\text{mol s}^{-1} \text{m}^{-2}$ . Temperature was maintained at 24°C (night) to 28°C (day). The experiment was repeated twice. Data on survival, formation of primary nodal complex (swollen base at the base of the nodal cutting), micro-tuber, and roots were recorded at 4, 8, 12, and 17 weeks after culturing (WAC). At 22 WAC, plantlets were harvested. At harvest, the number of nodes per plant, number of leaves per plant, plant fresh weight, and plant dry matter (DM) content, percentage tuber formation, number of micro-tubers per plant, and tuber weight per plant were measured. Dry matter content of all components was obtained by drying the fresh samples, in an oven at 80°C, for three days. All data were analysed by analysis of variance using SAS (SAS Institute, 1989). Correlation analysis (Pearson correlation coefficients) was performed between percentage tuber formation both at 12 and 17 weeks and the percentage shoot formation, percentage root formation, and percentage swollen base at eight WAC.

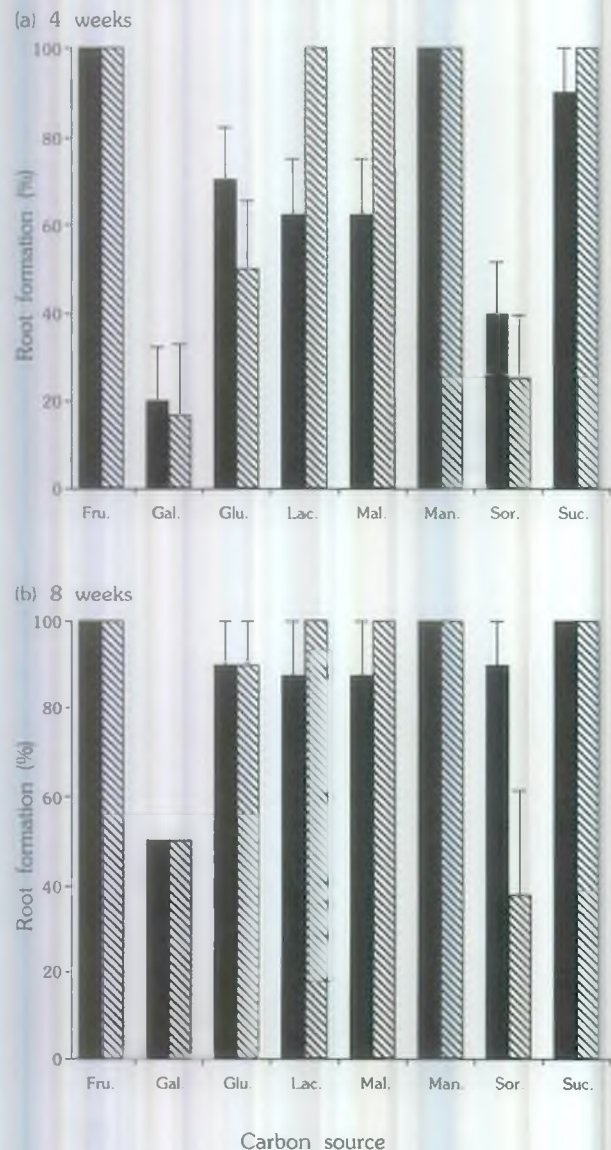
## Results and Discussion

### Growth and development

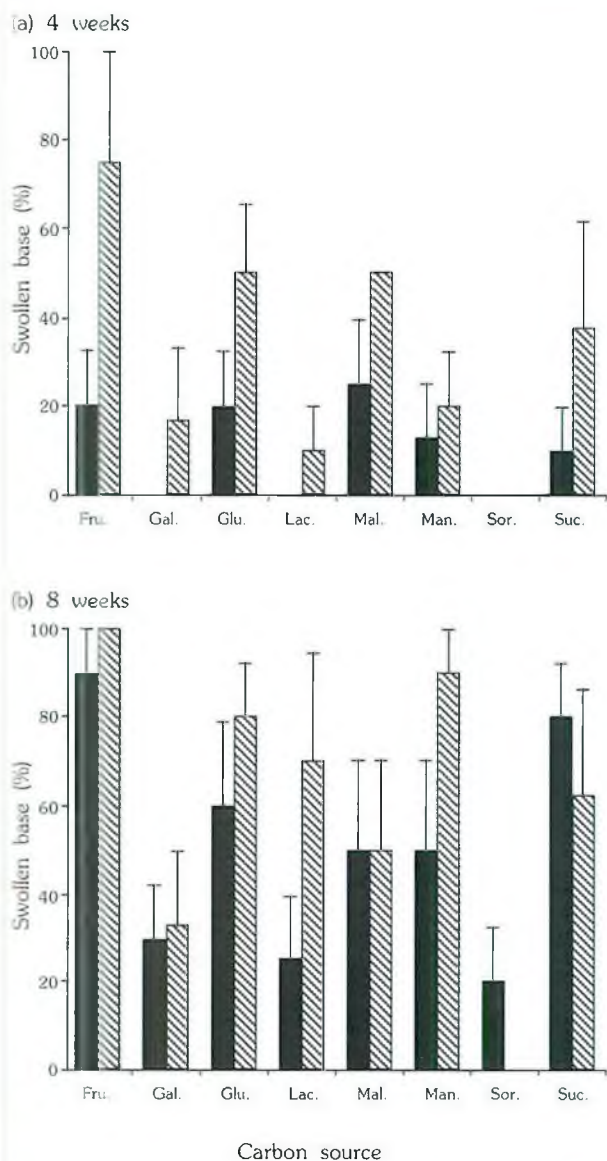
Results obtained at 4 and 8 WAC indicated that there were no significant differences in percentage shoot formation among the carbon source treatments. However, there were significant differences in percentage root formation

and percentage swollen base among the treatments. The percentage root formation for all treatments at 4 and 8 WAC is shown in Figure 1. At four weeks, treatments with 3 and 5% fructose (Fru.), mannose (Man.), and sucrose (Suc.), 5% lactose (Lac.), and 5% maltose (Mal.) gave significantly higher percentage root formation than treatments with 5% sorbitol (Sor.) and 3 and 5% galactose (Gal.). In treatments 3 and 5% sucrose, fructose, and mannose, and 5% maltose and 5% lactose, all the explants rooted, but 5% sorbitol gave a significantly lower percentage root formation at 8 WAC.

At 4 WAC, 5% fructose gave a significantly higher percentage swollen base formation than 3 and 5% lactose and sorbitol, and 3% sucrose and galactose. At this stage, none of the



**Figure 1** Response of yam nodal cuttings to different carbon sources: percentage root formation. Vertical bars = S.E. ■, 3%; ▨, 5%

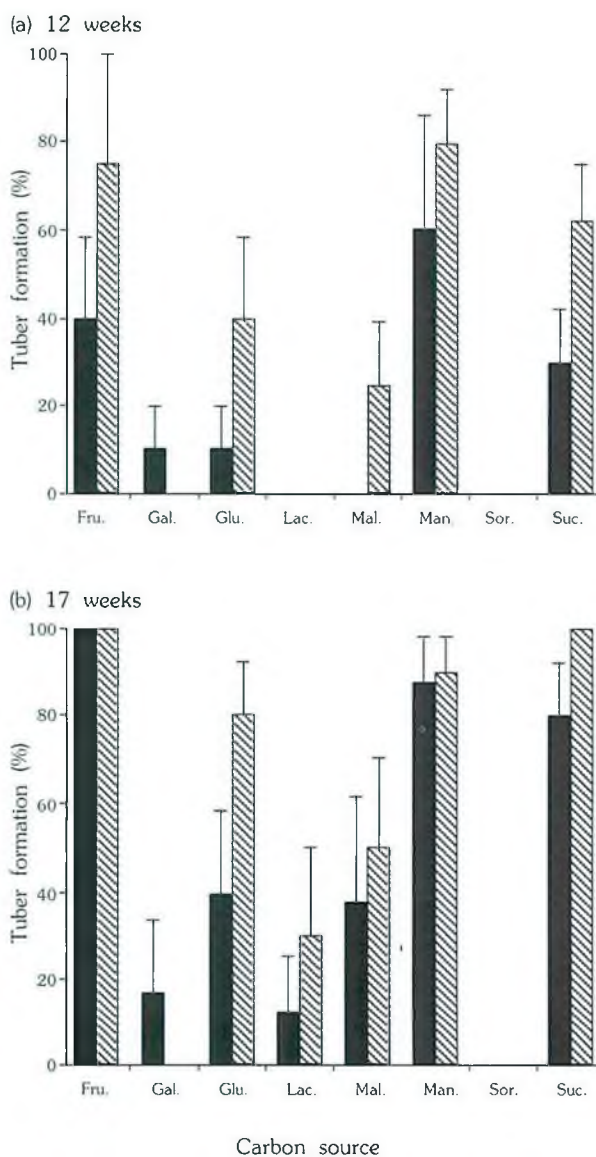


**Figure 2** Response of yam nodal cuttings to different carbon sources: percentage swollen base. Vertical bars = S.E. (■), 3%; (▨), 5%

explants in treatments 3% galactose, sorbitol, and lactose, and 5% sorbitol gave rise to a swollen base. Results also showed that the higher concentration (5%) of the carbon source gave a higher percentage swollen base than the lower concentration (3%). Eight weeks after culturing, treatments with 3 and 5% fructose, 5% mannose, 3% sucrose, and 3% glucose (Glu.) had significantly higher percentage swollen base than treatments with 5% sorbitol (Figure 2b). The lower percentage swollen base in treatment with 5% sucrose compared to fructose (3 and 5%), mannose (5%), glucose (3%), and sucrose (3%), although not statistically significant, was due to some of the cultures in 5% sucrose forming micro-tubers (25%). This indicated that micro-tubers can be formed even as early as 8 WAC under 5% sucrose treatment.

At 12 and 17 WAC, there were no signifi-

cant differences in growth parameters such as percentage shoot formation and percentage root formation. However, there were significant differences in percentage tuber formation among the treatments. The results of percentage tuber formation in all treatments at 12 and 17 WAC is shown in Figure 3. Percentage tuber formation was highest in 5% mannose, followed by 5% fructose, and 5% sucrose and 3% mannose at 12 WAC. At this stage, treatments with 3% maltose, 5% galactose, and 3 and 5% sorbitol and lactose did not form any tubers. However, at 17 WAC, treatments with 3 and 5% fructose and 5% sucrose gave 100% tuber formation, followed by 5 and 3% mannose. Tubers were not formed in treatments with 3 and 5% sorbitol and 5% galactose. Results obtained at 12 and 17 WAC showed that higher concentrations (5%) of all the carbon sources tested gave



**Figure 3** Response of yam nodal cuttings to different carbon sources: percentage tuber formation. Vertical bars = S.E. (■), 3%; (▨), 5%

higher percentage tuber formation than the lower concentrations (3%). This is in agreement with findings reported by Ng (1988b) where sucrose was used to induce *in vitro* tuberization.

Positive correlation was obtained between the percentage root formation and percentage swollen base ( $r = 0.27$ ,  $P < 0.05$ ), percentage tuber formation at 12 weeks ( $r = 0.41$ ,  $P < 0.01$ ), and percentage tuber formation at 17 weeks ( $r = 0.52$ ,  $P < 0.01$ ). There was also a positive correlation between percentage swollen base with percentage tuber formation at 12 weeks ( $r = 0.39$ ,  $P < 0.01$ ) and percentage tuber formation at 17 weeks ( $r = 0.44$ ,  $P < 0.01$ ). This indicated that it may be possible to use the percentage swollen base at 4 WAC to predict the percentage tuber formation and that early root formation contributed positively to tuber formation.

Nodal cuttings are source plant materials for further multiplication in yam. The number of nodes produced determines the multiplication ratio. Among all treatments, 5% fructose had the highest number of nodes per plant (NNP) and highest number of leaves per plant (NLP), followed by 3% fructose and 3% sucrose (Table 1). Galactose, in general, gave very poor growth. This indicated that both fructose and sucrose are better choices of carbon source for shoot multiplication. Although 3% glucose gave higher plantlet fresh weight, followed by 5% fructose, there were no significant differences among treatments on percentage DM content. Nevertheless, the percentage DM content was higher in 5% galactose, although it had a very poor shoot growth (Table 1).

Sucrose and glucose were found to be the best carbon sources for supporting the germination and growth of yam embryos (Okezie *et al.*, 1994), which is in agreement with the results of this study. Fructose, galactose, and mannose, however, induced germination of the embryos but with retarded growth which is in contrast with the results of this study where fructose supported better growth of the plantlets. A study on callus culture and suspension culture of sweetpotato showed that cultures can grow equally well in media containing sucrose, glucose, maltose, and starch (Handley and Locy, 1984). Fructose and saccharose also had positive effects on banana growth *in vitro* (Folliot and Marchal, 1992). This suggested that sucrose, glucose, and fructose are the most suitable carbon sources which can be efficiently utilized by plants in culture and is in agreement with the results of this study.

There were significant differences among the treatments in terms of number of micro-tubers per plant (TNP) and micro-tuber weight per plant (TWP). Five per cent and 3% sucrose and fructose, 5% mannose, and 5% glucose gave an average of more than one TNP; with 5% sucrose being the highest, whereas 3 and 5% sorbitol, and 5% galactose did not produce micro-tubers although some plants did produce a

**Table 1** Effects of different carbon sources on yam shoot growth and microtuber formation

Carbon source	NNP	NLP	PLW (g)	TNP	TWP (g)
Sucrose (5)	6.4 bc	10.6 abcd	1.8 ab	1.80 a	0.85 ab
Sucrose (3)	7.4 ab	15.9 ab	1.8 ab	1.50 ab	0.64 abcde
Fructose (5)	12.8 a	18.8 a	2.4 a	1.50 ab	0.74 abcd
Fructose (3)	8.4 ab	13.8 abc	1.3 abcd	1.60 ab	0.34 abcde
Mannose (5)	7.5 ab	11.7 abc	1.5 abcd	1.60 ab	0.79 abc
Mannose (3)	5.5 bcd	13.0 abc	1.6 abc	0.90 abc	0.48 abcde
Glucose (5)	7.4 ab	12.5 abc	2.5 a	1.30 abc	0.92 a
Glucose (3)	4.9 bcd	9.8 abcde	1.6 abc	0.90 abc	0.15 bcde
Maltose (5)	3.8 bcd	9.9 abcde	0.9 bcd	0.50 abc	0.06 de
Maltose (3)	3.5 bcd	8.0 bcde	1.0 bcd	0.50 abc	0.13 cde
Lactose (5)	6.1 bcd	11.9 abc	0.9 bcd	0.20 bc	0.13 cde
Lactose (3)	3.2 bcd	9.4 bcde	0.5 bcd	0.25 bc	0.03 e
Galactose (5)	0.3 d	1.0 e	0.2 d	0.00 c	0.00 e
Galactose (3)	1.3 cd	2.5 de	0.3 cd	0.33 abc	0.10 cde
Sorbitol (5)	4.3 bcd	10.6 abcd	0.3 cd	0.00 c	0.00 e
Sorbitol (3)	2.7 bcd	5.6 cde	0.3 cd	0.00 c	0.00 e

Figures in same column followed by same letters are not significantly different at 5% level by Tukey's Studentized Range Test. NNP is number of nodes per plant; NLP, number of leaves per plant; PLW is plantlet fresh weight; TNP, number of microtubers per plant; and TWP, microtuber weight per plant.

swollen base (Table 1). The results also showed that 5% glucose gave higher TWP followed by 5% sucrose. Treatments that had TWP higher than 0.5 g were 3 and 5% sucrose, 5% glucose, 5% mannose, and 5% fructose. Sucrose at 5% level which gave highest TNP as well as relatively higher TWP might be related to the early formation of micro-tubers in this treatment as mentioned earlier. It was also observed that the higher concentration (5%) of carbon sources gave higher TWP than the lower concentration (3%). Similar results were obtained in yam when sucrose was used as the carbon source (Ng, 1988b). Micro-tubers harvested from mannose, fructose, and sucrose treatments sprouted eight months after harvest.

Correlation analysis showed that there were significant correlations between TWP and plantlet fresh weight ( $r = 0.54$ ,  $P < 0.01$ ), plantlet fresh weight and NNP ( $r = 0.62$ ,  $P < 0.01$ ), and plantlet fresh weight and NLP ( $r = 0.58$ ,  $P < 0.01$ ). There were also significant correlations between TNP and plantlet fresh weight ( $r = 0.34$ ,  $P < 0.05$ ), and plantlet fresh weight and NNP ( $r = 0.39$ ,  $P < 0.05$ ). This suggested that carbon sources that supported better growth of the shoot also gave higher micro-tuber production.

## Conclusions

Fructose, sucrose, mannose, and glucose carbon sources produced greater plantlet growth than maltose, galactose, lactose, and sorbitol on

NNP, NLP, and plantlet fresh weight and were highest with fructose. These carbon sources also supported higher percentage tuber formation, TNP, and TWP. Sucrose promoted early micro-tuber formation and had higher TNP, whereas glucose gave higher TWP. Fructose seems to be the best choice of carbon source for shoot multiplication, followed by sucrose. However, it was the reverse for micro-tuber production.

## Acknowledgements

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# Multiplying taro and tannia planting material: Splitting of corm apices and the use of commonly available growth substances

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This study was undertaken to assess the effect of splitting of planting materials of taro (*Colocasia esculenta*) and tannia (*Xanthosoma sagittifolium*), and treatment with two commonly available growth hormones on plant establishment and yield. Conventional planting pieces of taro and tannia (called 'huli' made up of apical corm portions with attached petiole bases) were split longitudinally into halves or quarters and planted out in the field or treated with coconut milk (liquid endosperm) before being planted (in the case of taro). The field experiments for tannia were supported by greenhouse observations of split huli treated with coconut milk, or with acetylene generated from calcium carbide. In all cases, the split huli established more slowly in the field, produced a lower leaf area per stand, and produced lower yields than the intact huli. In both the field and in the greenhouse, the coconut milk treatment had a depressing effect on root growth and bud expansion. However, acetylene treatment promoted bud expansion in split tannia huli, and encouraged significant root development.

Keywords: Taro; Tannia; Corm apices; Growth substances

The shortage of planting materials is a problem common to tropical root and tuber crops (Onwueme, 1994), and is particularly severe for taro (*Colocasia esculenta*), tannia (*Xanthosoma sagittifolium*), and yams (*Dioscorea* spp.) where there is competition between material for planting and consumption.

Efforts to solve this problem have involved subdividing taro and tannia corms, and using the pieces as planting material. While the corm has numerous buds, each of which could theoretically grow into a shoot, experiments have shown that smaller sets resulting from subdivision have always resulted in lower yields (Bourke and Perry, 1976; Soto and Arze, 1984). The yield reduction per hectare has been attributed to a combination of low yield per plant, and high mortality in the field (Bourke and Perry, 1976). Attempts have not been made to improve the performance of the sectioned corms by exogenous applications of growth substances.

The objective of these experiments were to (i) multiply taro and tannia planting material by longitudinal sectioning of the corm apex, and (ii) attempt to improve the performance of the sectioned pieces by the use of two commonly available growth substances, coconut liquid endosperm and acetylene. In order to minimize competition with edible material, attention was confined to 'huli' (the inedible extreme apex of the corm plus the attached petiole bases; Onwueme 1978).

## Materials and Methods

The study was conducted in the 1995 and 1996 growing seasons, at the University farm in Lae, Papua New Guinea. The location was 6°41' S, 146°98' E at an altitude of 65 m above sea level. The plot was located on an alluvial plain, and the soil was sandy, mixed, isohyperthermic Typic Topofluvents (USDA Soil Taxonomy).

### Plant material

Taro (*C. esculenta* cv. Numkoi) and tannia (*X. sagittifolium*, local cultivar) were used for the experiments. Huli of each crop of relatively uniform size were selected. The huli were excised from the corm proper at the point of attachment of the oldest living leaf. The base of the huli had a diameter of 5–8 cm. The length of petiole left attached to the huli was 15–20 cm. Intact huli were sectioned longitudinally to give two halves or four quarters.

Liquid endosperm was obtained from immature coconuts and diluted 1:1 with water. Huli or huli sections to be treated with endosperm were soaked in the liquid for 15 h before being planted out in the field. The field layout was a randomized complete block design, with four replications and 20 plants per plot. Field spacing was 100 cm × 50 cm. The soil had remained fallow for the previous year. Rainfall during the season ranged between 183 mm and 533 mm per month.



Establishment counts were done on all plots of the field plants at weekly intervals for taro and at 3-weekly intervals for tannia. A plant was considered established when the first (oldest) lamina on it had fully unfurled.

At 14 weeks after planting (WAP) for tannia and at 20 WAP for taro, leaf numbers and leaf area were determined for the plants growing in the field. Leaf area for taro was determined according to the method of Ezumah and Plucknett (1981), where the distance from the leaf apex to the point of attachment of the petiole was measured. Then the leaf area was estimated by the equation  $Y = 1.5 X + 1.06 X^2$ , where:

- Y = leaf area; and
- X = distance from petiole attachment to leaf apex.

For the tannia, leaf area was estimated by the method of Goenaga *et al.* (1991). The crops were harvested at 30 WAP, at which time the main corm and the side corms or cormels of each plant were weighed separately.

In addition to the field experiments, greenhouse experiments were conducted to observe the effects of splitting and two growth substances on tannia huli. Intact, half, or quarter huli were treated as follows:

Control — immersed in water in 10 L buckets up to the bases of the petioles;

Coconut endosperm — immersed in half-strength coconut endosperm for five days and then transferred to water immersion;

Acetylene — fumigated with acetylene for two days and then transferred to water immersion.

For acetylene fumigation, the huli were placed in an enclosed bin. Pellets (about 40 g) of calcium carbide were placed in a watch glass in one corner of the bin. A few drops of water were added to the calcium carbide and the lid of the bin was closed. The resulting acetylene provided the fumigation for the next two days.

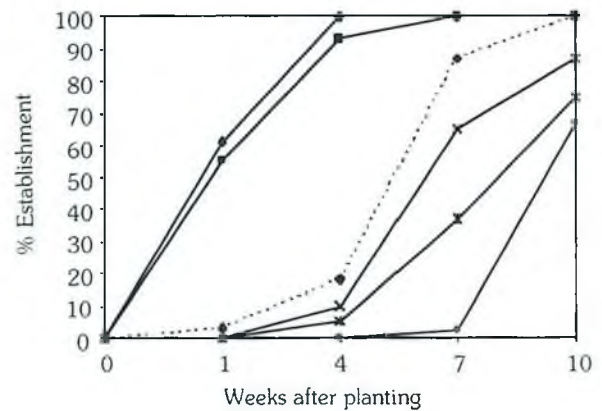
The root and bud development on the greenhouse huli were observed at weekly intervals and scored on a scale of 0–10, where 0 meant no development, and 10 maximum development.

The data were analysed using the analysis of variance for a 3 × 2 factorial experiment.

## Results

### Field establishment

The pattern of field establishment for tannia is shown in Figure 1. Splitting of the huli resulted in much slower field establishment, as well as a lower count of the plants that eventually established. Unlike the split huli treatments, the in-



**Figure 1** Tannia establishment in the field; (—◆—), intact; (—■—), intact coconut; (····◆····), half; (—▲—), half coconut; (—\*—), quarter; (—●—), and quarter coconut

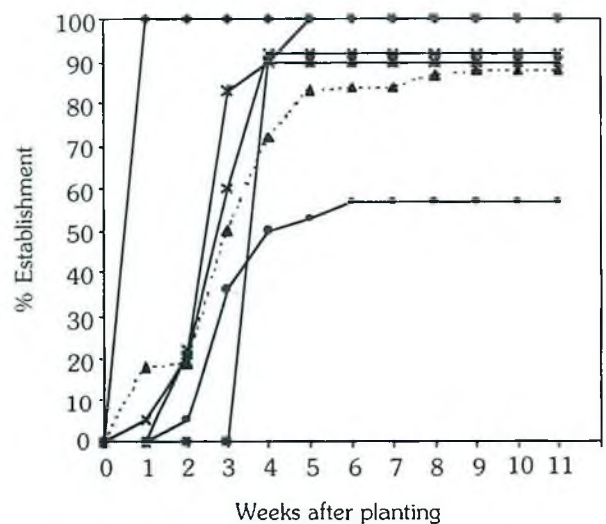
tact huli treatments were able to attain 100% establishment.

Coconut endosperm treatment of each type of huli resulted in slower establishment than those that were not treated.

The establishment pattern for taro (Figure 2) followed generally the same trend as that of tannia.

### Leaf and shoot

The data for leaf and shoot measurements in the field are shown in Table 1. For both taro and tannia, there were no significant differences between the treatments in terms of leaf number per stand and shoot number per stand. For each of the crops, leaf area per stand was significantly higher for the intact untreated huli than for any of the other treatments. Coconut endosperm treatment had a significantly depress-



**Figure 2** Taro establishment in the field; (—◆—), intact; (—■—), intact coconut; (····◆····), half; (—▲—), half coconut; (—\*—), quarter; (—●—), and quarter coconut

**Table 1** Shoot, leaf, root, and bud parameters for intact or split huli treated or untreated with growth substances

	Intact	Intact coconut	Half	Half coconut	Quarter	Quarter coconut	Intact acetylene	Half acetylene
Taro in field at 20 WAP								
Shoots per stand	1.50	2.00	1.77	1.08	1.32	1.88	—	—
Leaf no. per stand	4.34	3.81	3.55	4.05	3.24	3.48	—	—
Leaf area per stand (cm <sup>2</sup> )	1702	740	705	995	460	889	—	—
Tannia in field at 14 WAP								
Leaf no. per stand	5	5	4	4	4	4	—	—
Leaf area per stand (cm <sup>2</sup> )	5808	3284	1801	1676	1270	1125	—	—
Tannia greenhouse at 30 days								
Root development score	9	6	10	1	—	—	10	10
Side bud development score	0	0	4	9	—	—	0	5

ing effect on the leaf area of the intact huli, but its effects on the half and quarter huli were not significant.

### Yields

The corm and cormel yield data for taro and tannia are given in Table 2. For the control taro huli, splitting resulted in a significant reduction in the yield of main corm and total corm. With respect to side corms, quarters produced significantly lower yields than the intact or half huli.

In every category, coconut endosperm treatment resulted in lower yield than the corresponding untreated huli, but the differences were significant only for the main corm and total corm of the quarter huli.

### Greenhouse observations

Observation in the greenhouse showed that coconut endosperm treatment depressed root growth in both intact and half huli (Table 1). Acetylene, on the other hand, permitted significant root development in half huli, as well as maintaining

the level of root development in the intact huli.

Intact huli did not expand their side buds, whether it was left untreated or it was treated with coconut endosperm or acetylene. Apparently, apical dominance from the undamaged terminal bud was enough to inhibit side bud expansion. The untreated half huli, on the other hand, showed some indication of side bud development. This was further enhanced by coconut endosperm treatment, but not by acetylene.

### Discussion

These experiments have confirmed that it is possible to grow taro and tannia from longitudinally split halves and quarters of huli. Huli splitting resulted in reduced field establishment, confirming the findings of Bourke and Perry (1976) that smaller setts experience a high mortality rate in the field.

Huli splitting also resulted in a reduction in leaf area per stand, which probably was a contributory factor to the yield decline that resulted.

**Table 2** Yields (t ha<sup>-1</sup>) for intact and split huli of taro and tannia

	Intact		Half		Quarter	
	Control	Coconut	Control	Coconut	Control	Coconut
Taro						
Main corm	11.7±2.45	9.4±2.08	4.2±1.31	3.0±0.63	3.3±0.47	2.1±0.29
Side corms	2.1±0.29	1.9±0.58	1.9±0.63	1.4±0.48	0.8±0.32	0.6±0.44
Total	13.8±2.80	11.3±2.66	6.0±1.94	4.4±1.12	4.1±0.79	2.7±0.58
Tannia						
Main corm	7.9±1.62	6.9±0.38	3.2±0.82	2.6±0.52	1.6±0.13	3.3±0.68
Cormels	8.5±3.88	6.9±1.30	6.4±3.42	3.4±0.98	1.2±0.25	3.3±0.60
Total	16.3±5.50	13.8±1.24	9.5±3.98	6.0±1.49	2.8±0.38	6.6±1.14

Again, this finding is consistent with earlier reports where smaller sets resulting from subdivision of the corm resulted in lower yields (Bourke and Perry, 1976; Soto and Arze, 1984). Coconut endosperm or acetylene did not improve the performance of split huli. Although acetylene encouraged root proliferation in split huli, coconut endosperm seemed to have a generally depressing effect on both intact and split huli.

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# Improving traditional yam production systems: The case of yellow yams in Jamaica

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In 1992, a technological package based on minisett production techniques was introduced to assess the impact on the traditional system of production and its efficiency to provide a year-round supply of yellow yam (*Dioscorea cayenensis*) in Jamaica. After its introduction, a modified system was developed for local conditions. One of the major components of the system was the adjustment of the planting sett size to 200 g, which was reduced from the traditional sett size of approximately 1 kg and increased from the standard minisett of 30 g. In order to reduce variability in sprouting, setts that sprouted at the same time were selected from a base population and planted weekly throughout the year. Uniformity in sprouting was achieved in the first generation and yam types were selected which would produce mature tubers in each month of the year. After the first year, the level of adoption of the components of the technological package was assessed using a farmer survey. Results indicated that farmers' decisions to adopt the new practices were influenced largely by yield obtained from their plots. Ninety per cent of farmers demonstrated varying levels of adoption and 90% of these were willing to plant smaller setts. Ease of application and reduction in labour were the most frequently reported advantages, and less frequently given reasons were marketability and profitability. Farmers continue to apply the components resulting in increased levels of productivity. However, the selected yam types already have been lost, for the most part, and there is need for development of a seed yam production unit that will monitor the supply of correct selected material to farmers. There is need also for development and promotion of a specialized market for minisett yams in order to encourage greater levels of efficiency.

Keywords: Yellow yam; Minisett; Farmer adoption; Production; Jamaica

Jamaica is the largest producer of root crops in the Caribbean. Production of roots and tubers in 1996 was: yams (*Dioscorea* spp.) 253 371 tonnes, sweetpotato (*Ipomoea batatas*) 33 218 tonnes, dasheen (*Colocasia* spp.) 31 765 tonnes, coco (*Xanthosoma* spp.) 13 846 tonnes, bitter cassava (*Manihot esculenta*) 11 871 tonnes, and sweet cassava (*M. esculenta*) 8498 tonnes (Planning Institute of Jamaica, 1997). Yam is the most important root crop produced and 15 cultivars are available at different periods throughout the year. In 1996, export earning from yams was U.S. \$10.8 M making yam the most important non-traditional agricultural export. Comparison with earnings from traditional agricultural exports (1996: sugar U.S. \$109.7 M, banana U.S. \$44.1 M, and coffee U.S. \$33.5 M) (Planning Institute of Jamaica, 1997) justifies the importance placed on the export of roots and tubers in the national diversification programme as the island seeks to reduce its reliance on traditional agricultural export crops.

The Jamaican yellow yam (*D. cayenensis*), which is normally available throughout the year, accounted for approximately 56% of yam production and 81% of yam exports in 1996. Of the 253 371 tonnes of yams produced in 1996, only 6% (15 364 tonnes) was exported indicating a per capita consumption of approximately 95 kg yr<sup>-1</sup> (Planning Institute of Jamaica, 1997). Yam remains an important part of the diet of Jamaicans residing locally and overseas.

Despite decades of production, the traditional systems of yam cultivation continue to pose major constraints to increased production. These include the large volume and high cost of planting material needed for crop establishment; the increase in nematode populations in both soil and tubers; the high cost and labour intensity of the staking operation; the deforestation resulting from the removal of large numbers of hardwood trees for use as stakes; the loss of soil as a result of erosion of the yam hills through wind and water run-off and by cultiva-

tion on slopes in areas with loose bauxitic soils; and need to improve the marketability of the export yams by producing a smaller yam, better suited to conventional packaging with a reduced need for trimming or cutting to size. Consequently, there is a constant need to assess new methods of production to maintain the market position and remain competitive within the free market system worldwide.

The minisett system for producing yams, first developed in Nigeria, was introduced to Jamaica in 1985 under an IITA/UWI/Ministry of Agriculture initiative to improve the traditional system of yam production. In 1990, the technological package was modified to suit local conditions and a national programme to promote the technology was implemented. The programme was funded jointly by the United States Agency for International Development (USAID) and the Government of Jamaica. The programme was an integral part of the Government's Five Year Plan (1990-95) for the agriculture sector and its objectives were to:

- (i) increase production and productivity in order to increase and sustain the contribution of the agricultural sector to economic growth and development of Jamaica, by making a substantial contribution to meeting food and nutritional requirements of the population, and increase agricultural exports and foreign exchange earnings from agriculture;
- (ii) improve the quality of rural life and increase agricultural employment;
- (iii) reduce environmental degradation and pursue developmental strategies which incorporate long-term conservation objectives and promote the efficient use of natural resources; and
- (iv) foster the development of appropriate technology through research and development and to ensure the transfer of this technology to farmers.

The objective of this paper was to provide an overview of the efforts to improve the traditional method of production through the application of the minisett technology. The paper examines:

- (i) farmers' responses to the national programme to disseminate a new technological package which was based on minisett techniques;
- (ii) efforts to improve year round supply of yellow yam; and
- (iii) sustainability of the programme.

### The National Programme

The national programme was implemented by the Rural Agricultural Development Authority (RADA), the extension arm of the Ministry of Agriculture, with technical assistance from the Inter-American Institute for Cooperation on Agriculture (IICA) over three years (1990-93). It was envisioned that the project would improve the traditional system of production and in-

crease production and export of yams.

Under the programme, 1000 demonstration plots were established on farmers' holdings in the main yam-growing areas. Each plot was 0.04 ha. The Jamaica Agricultural Research Programme (JARP) which was also funded by USAID, supported research to address problems associated with the technology under local conditions. The research students were supervised by Staff of the Faculty of Agriculture, The University of the West Indies. At the farm level, an on-farm adaptive research methodology was applied to disseminate the technology. The farmers were provided with a technological package outlining the benefits of the different components and the agronomics of yam production. They were encouraged to make their own decisions on which components to adopt, based on their individual farm conditions. A number of modified systems emerged on farms that had varying levels of resemblance to the original technological package.

It was found that one of the main setbacks to adopting the original package was the poor rate and percentage germination of 25-g yellow yam minisetts. Under the best local conditions with supervision by trained personnel, only 5.6% of 25-g heads setts sprouted compared to 88% of 200-g heads 30 days after placing in the nursery. Eighty-eight per cent sprouting of 25-g heads occurred after 100 days (Campbell, 1994). Middle and tail setts sprouted even more slowly. Under African savannah conditions, Kalu *et al.* (1988) reported that fewer marketable tubers were produced from yellow yam minisetts than from minisetts of *D. rotundata* and *D. alata* supporting the use of a larger sett for this variety. Given this, the original objective of the production of seed yams to be used as planting material as was practised in Nigeria was abandoned and, instead, studies were initiated to improve uniformity in sprouting yellow yam minisetts. Further, the modified technology which was promoted to farmers sought to produce marketable tubers directly using the larger minisetts. Accordingly, the modified technological package that was to be validated on farmers holdings utilized 200-g setts, planted on continuous mounds spaced 1 m apart and mulched with polyethylene along with optional use of shorter stakes. The major components of the technological package and the constraints they would address are presented in Table 1.

The new system differed considerably from the traditional system of production which utilized large (1-3 kg) head setts, planted on individual mounds 2 m x 2 m to 3 m x 3 m with the vines staked with poles up to 3 m tall.

### Farmers' Responses to the Modified Technological Package

One year after farmers were introduced to the technological package, a survey was conducted

**Table 1** Components of the technological package and the corresponding constraint addressed

Component	Constraint
Treating sett pieces	Reduce the build-up of nematode populations
Use of smaller setts	Increase production efficiency
Reduce the need for stakes	Reduce the amount of forest trees required for staking
Continuous mounds	Reduce soil loss created by the traditional method of individual mounds
Mulching	Reduce surface run-off and soil loss accompanying the clean weeding system
Close planting	Increase production per unit area

to determine farmers' responses and the level of technology adoption. One hundred and ninety-one farmers were randomly selected from a list of programme beneficiaries to be interviewed. Ninety per cent of the farmers who had harvested their plot adopted between one and three of the components of the technology, and 90% of these were willing to plant smaller setts.

Forty-five per cent of farmers planted on continuous mounds, 38% treated setts, 35% used mulch, 25% planted closer, and 25% modified the use of stakes. Ease of application and reduction in labour were the most frequently reported advantages of the new system, while tuber size being too small was the most frequently reported disadvantage. Only nine farmers did not adopt any of the components. The level of sprouting of setts of those who adopted was similar to that of those who did not adopt, but those who adopted obtained higher yields per unit area. The most common reason for adoption was given as yield. Farmers who did not adopt obtained an average yield of 458 kg plot<sup>-1</sup> compared to 728 kg plot<sup>-1</sup> for those who did. Seven of those who continued to use the traditional system agreed that the new technology required less labour.

### Uniformity in Sprouting and Year Round Supply

Each of the 1000 farmers in the programme established his or her own nursery to sprout tuber pieces for establishment on 0.04 ha. Farmers were pre-sprouting tuber pieces for the first time as the traditional heads did not require nurseries for sprouting.

The survey showed that 16% of farmers obtained over 90% sprouting, with the majority of farmers (over 70%) obtaining between 76 and 90% sprouting. Sprouting within the small nurseries was spread over a period of three to four weeks with planting and harvesting of tubers similarly affected. Using the wide variability in time of sprouting obtained and the corresponding spread in harvest time observed on small plots, one large farm established a nursery to produce enough setts to plant a plot of 0.04 ha weekly.

It was found that the variability in sprouting could be manipulated to provide a regular supply of yellow yam throughout the year. The commercial indoor nursery had the capacity to hold 36 000 setts at one time. Planting was based on the selection of setts that sprouted at the same time regardless of the morphological size of origin of the sett. Large headless tubers were collected from several yam-growing areas in Jamaica to simulate the method normally used to secure planting material for a new farm establishment. Selection was conducted over two years with observations made on planting and harvest dates. All plots were established on farmers holdings under rain-fed conditions. The setts were transplanted 30 cm apart onto ridges spaced 1 m apart. The ridges were covered with plastic mulch. Each plot was harvested at maturity using the development of dark suberised tissue at the distal end of the tuber as the indicator of maturity.

Twelve plots were established on a monthly basis using setts that sprouted at the same time. For each of the populations selected, heads and whole tubers of the same size (200 g) were planted in field nurseries to observe sprouting. The nurseries were managed by farmers utilizing material obtained from their own holdings. An examination of harvest and planting dates showed an average growth period of approximately nine months and a dormant period of 11-12 weeks, so that a planting pattern was easily established in which setts could be so selected that they could be planted on a monthly basis and harvested likewise, resulting in a year-round supply of yellow yams.

Within plots that were established with setts that sprouted at the same time, 90% of the tubers were mature at the same time. The results indicated that the length of the growth period was not affected by the month of planting and demonstrated that it is possible to produce tubers of yellow yam throughout the year. This possibility of manipulating the growth period of some yam species had been already demonstrated by Buffard-Morel and Toure (1980) who found that plants from cuttings of *D. cayenensis* and *D. rotundata* could be planted year round. Further, Campbell *et al.* (1962) showed that the growth period of *D. rotundata* from planting to maturity was between 7.5 and 8 months irrespective of date of planting. On the other hand, Wickham *et al.*

(1984) demonstrated a cyclic pattern of growth in *D. esculenta* and *D. alata* with the length of the dormant period varying with the degree of maturation of the tuber at harvest. Reports on monthly plantings of *D. alata* from April to September showed leaf duration shortened with late planting (Clairon and Zinsou, 1980). Thus, in *D. cayenensis* there seems to be a lower level of environmental influence on the growth cycle than in other species of *Dioscorea*. This could be one explanation for the seasonal supply of *D. alata* varieties in Jamaica and the fact that yellow yam is available all year with periods of low supply between July and September.

The study supported the view that the middle and tail setts from different tubers which sprouted at the same time, were of the same physiological age which was associated with the development of the primary nodal complex (PNC) (Wickham *et al.*, 1981). The data supported the position that tuber maturity in *D. cayenensis* is linked to the development of the PNC and was not influenced by environmental conditions as observed for other species of *Dioscorea*. It was demonstrated that with careful selection of pre-sprouted setts, yellow yam can be planted year round and that tuber populations can be produced specifically for the period July to September to increase supply.

Uniformity in sprouting was shown to improve through use of setts of the same type and size from daughter tubers from the first selected setts. Among the 200-g whole tubers and head setts from the daughter tubers, 20% sprouting was observed two months after harvest and 80% sprouting was observed within three weeks of the first sprout. This was a significant improvement over the sprouting observed in small nurseries on farmers' holdings. Unless the farmer needed to expand production, the heads and whole tubers could be planted in nurseries to sprout uniformly using harvested material. There normally would be no need to continue to sprout tuber pieces after the first year.

## Sustainability

The results of the survey indicates that the technological change is sustainable. According to Hilderbrand (1985) the acceptable index for technology adoption is given by the formula,

$$\text{Acceptability Index, } AI = \frac{C \times A}{100}$$

where *C* = percentage of farmers who used at least part of the technology the following year; and

*A* = among the farmers using the technology, the percentage area to which the technology is applied.

Where *AI* is greater than 25 and *C* is greater than or equal to 50, it can be expected that adoption will follow. Based on the response after the first year, the minimum yam plot on which farmers need to apply this technology in the following year is  $A = 100 \times 25/90$  or approximately 30%, in order to sustain adoption.

Although the national programme has ended, the Rural Agricultural Development Authority is committed to continued promotion of the technology through their offices islandwide without providing additional financial assistance. Unfortunately, RADA's support targets small farmers and no provisions are made to support larger farms where adoption was more prevalent.

There is evidence to support the fact that the minimum tuber size exported decreased from 1.3 kg to 1 kg and the tubers exported are more attractive today than prior to the introduction of the technology. The main constraint to the rate of adoption, however, is the fact that the market for small tubers was not promoted and developed overseas. Although farmers were willing to plant smaller setts, they adjusted the sett size bearing in mind the need to produce a large enough tuber for the present export market.

The selected tuber populations which showed uniformity in sprouting were not maintained because there was no institution with the capability of preserving the population from year to year. It was observed that some of the farmers who were practising the technology since 1985, now have a year-round supply of yellow yams on their farms.

Since the inception of the national programme (1990), total production of yellow yam increased from 29 500 tonnes in 1984 to an average of 78 500 tonnes during 1985-90 and an average of 118 900 tonnes during 1991-94 and exceeding 142 000 tonnes in 1995 and 1996. Yam exports increased from 8000 tonnes in 1990 to an average of 10 600 tonnes during 1991-96 (Planning Institute of Jamaica, 1985-96). Production per unit area increased from 10-13 t ha<sup>-1</sup> making Jamaica the most efficient producer of yams in the Caribbean, which could be a result of the smaller setts and increased technical knowledge available to farmers (FAO, 1996).

## Lessons Learnt

In assessing the progress, it is important to appreciate that the technological package that was exposed to farmers differed significantly from the traditional system. There were short-term and long-term objectives that the project hoped to achieve which contributed to the complexity of the programme. Some of the long-term objectives were not grasped by the target groups, for example, (a) the need for environmental preservation is not traditionally appreciated by farmers, and (b) exporters did not see

the immediate need to export a whole tuber with head intact, thus, reducing the need for chemical postharvest treatment. A number of important lessons can be learnt from this experience:

The on-farm adaptive research approach was an effective tool to develop appropriate technology for farmers based on farmer participation in technology validation. The programme demonstrated the impact of introducing technological packages on traditional systems of production and the unforeseen benefits that can be derived; in this case the possibility to improve year round supply. The adaptation of the technological package by farmers to their own needs led to only partial adoption since the farmers adjusted the technology to suit their own condition, and this, probably more than anything else, contributed to the sustainability now demonstrated by increased productivity.

Although small farmers did not accept the complete package, improved yield per unit area, and increased levels of production and export suggest greater awareness at the farm level and that much was achieved through the introduction of the technology. More of the research data generated on stations could be better utilized if they were developed into technological packages and exposed to farmers allowing them to modify the packages to suit their needs.

Greater priority should have been given to market promotion and development. No financial provision was made for this activity.

Greater emphasis should be placed on supporting the private sector to develop mother farms and pioneer development of a specialized market. At least one large farm has fully adopted the technological package and has mechanized land preparation and harvesting operations.

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# Comparative output of calories from starchy food crops in sub-Saharan Africa

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The literature is replete with yield data, food consumption tables, and production data from various sources. These were used to derive the overall energy accruing from the production of the various starchy food crops in the African continent. In this study, the changes over time in the contribution of the principal starchy staples to food energy output in Africa were determined and their potential future roles are discussed. Country specific information was then considered for more specific action plans for the use of scarce research resources for the implementation of the themes identified by the researchers.

Keywords: Calories; Starchy foods; Staples; Sub-Saharan Africa

The high ratio of calories to other food nutrients required by humans is a critical factor in measuring the food adequacy of diets. On average, 2500 kilocalories (625 g of carbohydrates) are required to sustain an adult per day, but a 70 kg man only needs about 70 g of protein for normal body repairs and other maintenance processes (Godman and Gutteridge, 1979). Thus, the amount of food energy to be sourced for most sub-Saharan Africans must be carefully planned, both in designing research and in formulating development strategies. During the 1983-93 decade, the crop categories showed that cereals and root and tuber crops were the main crops grown. Africans ate more cereals (Table 1), but their production was more sensitive to bad weather, and poor soil fertility. In the areas where fertilizers are available, cereals receive fertilizers but the root and tuber crops do not. This is partly because the root and tuber crops give appreciable yields even on soils and in weather conditions where cereal crops give low or no yields.

This study compares the relative output of calories obtained from non-cereal starchy crops in tropical Africa.

## Starch Food Crops

There are many crops that provide carbohydrates in the form of starch. The main staples of Tropical Africa include: rice, sorghum, millets in savannahs, maize, cassava, yam, cowpea, groundnut, bambara nut, sweetpotato, potato, plantain and banana, and cocoyam. As staples that are principally produced in large and regular quantities, first for food, and also for cash income generation, their demand is well known and is regular and can be estimated. Their production, distribution, storage, and efficient utilization must be properly systemized. The suitability of agro-ecologies for their production and use has led to zonal specialization of their production and has influenced the relative emphasis placed on their research and development. For example, in very dry areas of the Sahel zone, millet growing is more common than is sorghum or maize. Millet is locally important but it cannot compete with sorghum or maize outside such agro-ecologies. In the humid equatorial forest zones, cocoyams are also more common. These two examples show that zonal specialization in crop selection and production is an important factor in deciding which crop to select for both research and development. Many rural farmers grow dozens of crop species, but specialize in a few staples. In research, the efforts should be to promote the efficient production, storage, and use of the more locally suitable crop species, irrespective of their global and continental importance. This is particularly so as research resources are becoming increasingly scarce. It is expected that continental projects would plan and undertake activities on continentally important crops, while national programmes would focus on locally grown crops species significant for assuring sustainable food security.

**Table 1** The relative output of food crop group in Africa

Food group	1994 output (M t)
Cereals (85% dry)	104.7
Root and tubers (33% dry)	122.5
Pulses	6.8
Vegetables	34.1
Fruits	51.8

Starchy crops are very important as a base for establishing a more intensive livestock industry. Livestock require calories which can be supplied from starchy crops, instead of cereal crops which tend to be preferably reserved for human consumption. Beside food, the industry needs starch for its manufacture of many items, and the use of cereals in industry is well established. The level to which non-cereal starchy crops can complement cereal supplies should be improved. In that way, more attention would then be given to the production, processing, storage, and utilization of non-cereal starchy crops. It is therefore essential that the starchy crop economy be structured to provide food and other products. The major starchy crops would, however, be such crops that: (i) are processed in the household for food; (ii) do not have a significant amount of other food classes such as protein and fats or oils as are found in the pulses and cereals, vitamins, minerals, and roughage as in pomological and olericultural crops; (iii) are directly used daily in large quantities by humans which excludes crops like sugar cane; and (iv) have traditional acceptance and preference in the local food systems.

### Starchy food crops of Tropical Africa

The major crops grown for food starch in Africa are cassava, yam, sweetpotato, cocoyams, potato, and banana and plantains. Many cereals are excluded because they have more protein and oils than is stipulated for the starchy crop category. These crops have less than 5% protein, and only a trace of oils after they have been processed and prepared for human food. They are generally low in vitamins and roughage. They are chiefly consumed to provide the energy component of the human diet. Although cereal grains have about 70% carbohydrates (Platt, 1962), they cannot be categorized as starchy crops, since they are much more nutritionally balanced with respect to their protein (8.15%), oils (5%), and fibre (24%) content. Their use as food may require much less supplementation compared to the use of non-cereal starchy crops when consumed by humans.

### Food energy supply from starchy crops

The ratio of the amount of starch crops to the cereal and pulse group of food crops differs for different countries in the sub-region. The age group distribution is also different but the overall structure of the population is not significantly so among countries of sub-Saharan Africa. The relative proportion of the population in each age group determines their need for calories. Younger people consume more calories for growth and play, whereas older people require fewer calories. The under 15-year group has lower energy requirements, thus the calorie needs of each country would depend, in part, on the distribution of the age groups apart from mere size of population. Nigeria, Africa's

most populous nation has a structure that is mainly dominated by the young, and 47.9% of its population is under 15 years of age (FOS, 1996). This has implications for the way local programmes for starchy crops are used for providing more calories. The extent of their production will thus dictate how well soil and weather conditions are used, as well as the role of other factors on the socio-economics of the production, marketing, and pricing of alternative sources of food calories.

### Cassava

Cassava roots are peeled before use. The peels comprise about 15% of the fresh weight harvested (Kay, 1973). After processing, between 25 and 33% of the harvested material recovered is usable as food (Onwueme, 1978). Other losses including storage and transport encountered before food is prepared may be another 10%.

### Yams

Yam tubers are usually peeled which results in a loss of about 10–15% and boiled, fried, or boiled and pounded. Post-harvest losses are 30–40% of the harvested crop (Akoroda and Hahn, 1995). Another 20% of the output is reserved as planting materials for the next season.

### Cocoyams

Cocoyams are also peeled before use resulting in about 15% loss. The amount of production kept for planting is around 15–20% of the output depending on the level of dehydration of preserved corms. Post-harvest losses vary around 25% before most of the annual crops is utilized. Consequently, the overall usable proportion of the annual harvest is about 36%. Dry corms contain about 20% carbohydrates which is mainly starch (Onwueme, 1978).

### Sweetpotato

Sweetpotato is often boiled with the peels and suffers lesser peeling losses (5%), but in storage, weevil damage increases as the storage period lengthens, reaching about 12.6% in Kenya (Gatumbi *et al.*, 1994), 40% in Cameroon (Akoroda *et al.*, 1992), and 12.5–58.7% in the late harvest in Nigeria (Lema, 1992).

### Plantain

The bunch of plantains and bananas constitute about 0.8 to 1.8 kg of stalk (peduncle). At plant populations of 1666 per hectare (3 m x 2 m), about 2166 kg is removed for the stalk or about 10% (Ferris *et al.*, 1996). Where yields are in the range of 5.2–16.0 t ha<sup>-1</sup> in south-east Nigeria (Arene, 1996), usable finger output would be around 80% of the gross harvest value. Losses of bunches due to over-ripening and spoilage cause 15–20% loss. Furthermore, the proportion of peels to total fruit weight depends on the stage of fruit ripeness and is about 33–42%. Thus, the used portion is about 64% of the finger weight.

## Potato

In Cameroon, 25–35% of the annual output is lost after harvest (Nzietchueng and Tchio, 1992), but 26.9–47.3% could be unmarketable according to Fontem and Aighewi (1994). About 15–20% of the output is kept for planting the next crop. Peeling losses are about 10% (Table 2).

The high percentage of the overall carbohydrate that is starch in each of these crops attests to the classification of these crops as starchy food crops. Notwithstanding that there is a small percentage of reducing sugars as they ripen or remain in storage, they are consumed primarily for their energy. Each of these crops has a small percentage of crude protein even after they have been processed and prepared for food.

The actual proportion of the daily food energy requirements of adults met from starchy crops produced within the country is presented in Table 3. The percentage of the total kilocalories per head per day (per cent of total KHD) is the overall food energy contained in the total production of a crop, expressing it as percentage of 518.26 KHD for all starchy crops for all 43 countries. Thus, cassava contributes 72.43% of 518.26 kilocalories, on average, to each of the 526 million sub-Saharan Africans. The amount of food energy obtained from imported amounts of this group of crops cannot be adequately estimated. Statistics of such movements of local cross-border trade are not available. It is significant to note that the ranking of countries by their sourcing of food energy from root and tuber crops and banana-plantains is quite different from the mere consideration of total outputs. Thus, while Nigeria is a major producer of most of the starchy crops, it is on a per capita basis less dependent on these crops. The ranking of the 12 most dependent countries, based on the per cent of daily food energy from starchy staples, are as

follows: Congo (DR) (54.19), Uganda (45.00), Ghana (36.76), Benin (34.28), Tanzania (30.80), Nigeria (30.44), Congo (Braz) (30.37), Gabon (30.31), Rwanda (29.71), Centrafrique (26.65), Cote d'Ivoire (26.62), and Equatorial Guinea (25.73).

All these countries obtain at least one quarter of their food energy needs from starchy staples. The ranking may shift slightly if the population structure is considered. The frequency distribution of the population by age can be adjusted to cater for lesser energy requirements for ages below 15 years and above 45 years. This will make it possible to raise the percentage of the daily food energy requirement that is satisfied. In Nigeria, the under-15 year group accounts for 47.9% of the population, whereas the 15–45 year, and the above 45 year groups account for 36.2 and 15.8%, respectively. The energy needs of the middle group are much higher, in that they are the most active. Therefore a declining scale that equates X in the younger group and Y in the older group to Z in the middle group can be computed. In this way, the percentage of food energy actually met from these starchy crops would be much higher. In all, the relative importance of the individual crops would depend, for each country, on its contribution to overall food energy needs.

The overall food energy provided by the seven starchy crops listed in Table 3, are mainly from: cassava (72.43%), plantain and (or) banana (12.10%), and yams (10.31%) which constitute the major starchy food crops to be emphasized in regional programmes. The seven crops together contribute about 20.72% of the overall daily food energy requirements, while cereals, pulses, and other food items provide nearly 80% of daily food energy. Focusing regional efforts is important, but the diversification of national efforts is recommended. In all, about 520 kcal are supplied to each of the 526 million people among the 43 countries of the

**Table 2** Effective food energy contribution from overall farm output (P) of starchy crops

	Cassava	Yams	Cocoyams	Sweetpotato	Potato	Plantain/ Banana
Post-harvest losses (%P)	10.0	35.0	25.0	40.0	33.6	15.0
Peeling losses (%P)	15.0	12.5	18.5	5.0	10.0	37.0
Stalk (%P)	—	—	—	—	—	10.0
Reserved for planting (%P)	—	20.0	20.0	5.0	17.5	—
Usable fresh food (%P)	75.0	32.5	36.5	50.0	39.0	38.0
Dry matter (%P)	34.0	33.0	27.2	34.5	25.0	34.5
Starch content (%P)	30.0	26.5	16.4	18.5	18.1	24.2
Carbohydrates (%P)	33.0	27.10	21.0	24.0	22.0	30.0
Available carbohydrate (%P)	24.75	8.81	7.67	12.0	8.58	11.4
1000 kcal t <sup>-1</sup> of P*	990.0	352.4	306.8	480.0	343.2	456.0

Sources: Ferris *et al.* (1996); Onwueme (1978); Lema (1992); Kay (1973); Nzietchueng and Tchio (1992); Akoroda *et al.* (1992); Gatumbi *et al.* (1994); Ortiz *et al.* (1996); Geertsema (1994); Fontem and Aighewi (1994).

\*Based on 4 kilocalories per gram of dry carbohydrate ingested

**Table 3** Farm output of starchy food crops in sub-Saharan Africa (in thousand tonnes) and the food energy contribution (in kilocalories head<sup>-1</sup> day<sup>-1</sup>) from these crops in each country in 1994 based on FAO (1994) figures

A	B	C	D	E	F	G	H	I	J	K
Country	Pop. (m)	Cassava	Yams	Potato	Sweetpotato	Taro	Plantain	Banana	KHD <sup>†</sup>	%DN <sup>††</sup>
Angola	10.67	986	—	35	82	—	860	275	409.05	16.36
Benin	5.25	1169	1287	—	50	4	—	13	856.88	34.28
Botswana	1.44	—	—	—	—	—	—	—	—	—
Burkina Faso	10.05	5	52	9	20	—	—	—	9.80	0.39
Burundi	6.21	471	6	37	507	103	—	1269	588.85	23.55
Cameroon	12.87	1300	95	32	170	—	—	100	310.52	12.42
Cape Verde	0.38	2	—	3	4	—	—	7	58.56	2.34
Centrafrique	3.24	620	235	1	—	45	73	96	666.18	26.65
Chad	6.18	195	245	8	48	38	—	—	140.46	5.62
Comoros	0.63	51	—	—	12	—	—	57	357.65	14.31
Congo (Braz)	2.52	630	13	2	22	—	85	44	759.24	30.37
Congo (DR)	42.55	19600	322	36	386	41	2300	580	1354.79	54.19
Cote d'Ivoire	13.78	1564	2824	—	36	337	1300	199	665.60	26.62
Equat. Guinea	0.39	48	—	—	36	62	—	17	643.30	25.73
Ethiopia	53.44	—	263	350	155	—	—	80	16.59	0.66
Eritrea	3.44	—	—	39	—	—	—	—	10.66	0.43
Gabon	1.28	200	110	—	2	—	246	9	757.71	30.31
Gambia	1.08	6	—	—	—	—	—	—	15.07	0.60
Ghana	16.94	4378	1000	—	—	1272	1322	4	918.88	36.76
Guinea	6.51	945	75	—	143	40	441	151	552.51	22.10
Guinea Bissau	1.05	15	—	—	—	18	34	5	99.56	3.98
Kenya	27.34	842	—	250	650	—	360	220	149.90	6.00
Liberia	2.94	390	15	—	18	—	33	80	420.79	16.83
Madagascar	14.31	2260	—	270	560	120	—	210	522.95	20.92
Malawi	10.84	200	—	350	—	—	200	91	113.94	4.56
Mali	10.46	73	11	—	55	—	—	—	26.86	1.07
Mauritania	2.22	—	3	1	2	—	—	—	2.91	0.12
Mauritius	1.10	1	—	19	1	1	—	6	27.48	1.10
Mozambique	15.53	3294	—	72	55	—	—	80	590.75	23.63
Namibia	1.51	—	—	—	—	—	—	—	—	—
Niger	8.85	225	—	—	35	—	—	—	74.16	2.97
Nigeria	108.47	21000	22000	75	40	1300	1447	1050	760.90	30.44
Reunion	0.65	1	—	15	1	—	—	4	35.58	1.42
Rwanda	7.75	350	4	200	1000	62	2600	—	742.79	29.71
Sao Tome e Princ.	0.13	1	1	—	—	6	—	12	182.41	7.30
Seychelles	0.07	1	—	—	—	—	—	2	74.44	2.98
Senegal	8.11	77	—	10	3	—	—	7	28.48	1.14
Sierra Leone	4.41	98	—	—	14	3	28	—	72.95	2.92
Sudan	27.36	9	126	15	7	—	—	20	7.10	0.28
Tanzania	28.85	7209	10	230	267	—	834	834	769.99	30.80
Togo	4.01	400	400	—	29	20	—	16	385.55	15.42
Uganda	20.62	3420	—	352	2151	—	8613	—	1124.94	45.00
Zambia	9.21	580	—	11	57	—	—	1	180.21	7.21
Zimbabwe	11.01	130	—	31	2	—	—	67	42.51	1.70
Total	525.65	72746	29097	2453	6720	3472	20776	5606	518.26	20.73
% of total KHD	—	72.43	10.31	0.85	3.24	1.07	9.53	2.57		

<sup>†</sup>KHD = kilocalories per head per day as the sum of the food energy contributions from all starchy crops. The contribution of cassava alone to this sum is = [(column C × 990 000 kcal per tonne/column E)/365 days of a year]

<sup>††</sup>%DN = percentage of daily food energy requirement supplied = column J/2500 kcal required daily per adult according to Godman and Gutteridge (1979)

zone in which these crops can be grown.

### Future potentials of starchy staples

The future of the starchy staples is very bright, given that they are easier to grow in adverse weather conditions with less fertilizer use, and can be easily processed for acceptable food, and can be stored. The need for improved planting materials to increase the planting area of the already cultivated farmlands (thereby replacing poor yielding varieties) is the greatest constraint. The sale of planting materials is an input that subsistence farming does not yet accommodate. The growing trend to grow root crops (especially cassava and yam) both for home and for the market has raised the demand for planting materials. The various strategies for a systematic multiplication and distribution of the planting materials of superior varieties should be considered as priority for each agro-ecological zone and demographic situation to optimize available resources and time spent on programmes for development.

The use of processed products from these crops is essential to their increasing role in the food security systems of Tropical Africa. The sun-dried or processed produce can be reconstituted into food after short preparation. All of these crops can be stored dry. The fresh form in which the bulk of the harvest is currently utilized should be supplemented with the use of dried products so as to cut down on wastes, as has been indicated in Table 2.

This study shows that the ratio of share of funds to be allocated to cassava, yam, and plantain and (or) banana, should reflect their abilities to provide food energies. Sweetpotato provides a much lesser percentage of all the energy intake in the region. But its suitability in drought-prone environments adds more weight to its use, especially as a supplement, where the short cropping seasons make the the cultivation of longer duration starchy crops more risky.

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# A review of progress in Trinidad on some processing technologies of three selected root crops

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Unavailability of food processing technologies is a major constraint to increasing the utilization of tropical tubers, both in the Caribbean and worldwide. Towards the formulation of such product and process technologies, progress in on-going investigations in Trinidad on production of dehydrated sweetpotato [*Ipomoea batatas* (L.)] flakes, the effects of freezing on the sensory characteristics of tubers of *Dioscorea cayenensis* cv. Round Leaf yellow yam, and the puffing characteristics of *D. alata* cv. White Lisbon yam tuber flour are reviewed. The physicochemical changes which occur on extrusion processing of cassava (*Manihot esculenta* Crantz) and the production of protein-fortified cassava farine and wafer products are also reviewed. Recommendations for continued research are discussed.

Keywords: Tropical tubers; Utilization; Technologies; On-going investigations

The food industry in the Caribbean is plagued by high post-harvest losses of indigenous crops (especially root and tuber crops), inadequate marketing systems which contribute to seasons of glut and scarcity, high and increasing food import bills, and food processing companies which depend heavily on importation of raw materials. One solution is to extend the shelf life of indigenous crops via processing.

Root crops are considered to be staple crops in developing countries and are found in the tropics, sub-tropics, and warm-temperature regions. They are high-energy foods, with a high concentration of carbohydrates in the form of starch, although low in protein content, and are consumed mainly in the fresh form. The tuberous roots of the plant are most important as a food source, but the leaves may also be eaten and they possess nutrient components comparable with those of the tubers. In developed countries, grain crops are important suppliers of foodstuff and are consumed in various processed forms. A great deal of research on white potato has taken place which has led to a wide range of products available to the consumer and in a readily acceptable form. In developing and undeveloped countries, importation of the sometimes cheaper or more prestigious grain crops, such as wheat, and the analogy of some of the root crops to 'poor man's food', have also led to a limited utilization of the root crops.

If root crops could be processed into readily acceptable forms, they would increase in acceptability and importance to food producers. Given their high energy content, root crops could be

used for food shortages, in developing and undeveloped countries, although some measure of fortification is necessary to combat their nutrient deficiencies. This paper reviews some processing technologies for three root crops: sweetpotato [*Ipomoea batatas* (L.)], cassava (*Manihot esculenta* Crantz), and yam (*Dioscorea cayenensis* and *D. alata*).

## Sweetpotato

### Manufacture of a convenient type breakfast food

Sweetpotato tubers were sliced, immersed in a 2% solution of sodium metabisulphite, and transferred to boiling water. The boiled slices were then blended with ground cinnamon to obtain a puree which was then drum-dried, the flakes cooled, and packaged into polyethylene bags.

The flakes did not retain their brittleness when milk was added for consumption. They crumbled and formed a mushy mass. It is possible that the flakes were too thin (25/1000 in) compared to commercial cornflakes (5/16 in).

Most of the adult and teenage sensory panelists liked the dry product (before milk was added). However, all of the children found the flakes acceptable, signifying the formulation could be used to develop a children's breakfast food. The other panelists preferred the product as a snack-type food.

Polyethylene bags used for packaging the flakes were inadequate for the moisture transfer characteristics of the film, resulting in a complete lack of brittleness of the flakes after four weeks of ambient temperature (32°C) storage.

### Manufacture of dehydrated sweetpotato flakes

Producing dehydrated flakes from Caribbean varieties of sweetpotato (Chicken Foot, Purple Top, Rasta, Black Vine, and Red Devil) was investigated, with the view of elucidating optimum peeling, cooking, and processing conditions. The peeling methods investigated were hand peeling, lye peeling, brine peeling, steam peeling (different pressure per time), baking, and boiling. The cooking methods were cooking in steam, oven-baking, and boiling in water. The processing conditions were the Inherent Enzyme Activation (IEA) and the Commercial Enzyme Addition (CEA) methods. The effect of temperature on the IEA method, the effect of enzyme-treated proportion on the CEA method, and the effect of sugar addition were also investigated.

As regards IEA, the purpose of this experiment was to determine the optimum temperature for the degradation of sweetpotato starch by the inherent amylases. This information was important since the IEA method involves the maintenance of the tubers at the optimum temperature for starch destruction. With respect to CEA, the purpose of this experiment was to assess the influence of the enzyme-treated proportion on the flake quality. Commercially prepared amylase (0.1% wet wt) was added to a portion (50%) of the comminuted puree and dissolved in the added water. This portion of the puree was then heated at 55–60°C for 30 min to activate the added amylase. The characteristics preferred in sweetpotato and the acceptability of the flakes made were also determined, in a consumer survey.

Steam peeling (10 psi for 3 min; 15 psi for 1 min) and steam (0 psi for 20–30 min) or water cooking (100°C for 20–30 min) were found to be good conditions for these processes. The optimum temperature for IEA was 76°C, the optimum proportion for CEA was 50%, and adding sugar up to a maximum of 20% sugar produced good quality flakes. The results of the consumer survey indicated that consumers preferred pale yellow, sweet, moist sweetpotatoes. The flakes made from Caribbean varieties were acceptable in all quality characteristics analysed; colour, taste, mouthfeel, and quality characteristics overall acceptability.

### Fried sweetpotato

Chips and frozen french fry products were prepared from Chicken Foot and Purple Top sweetpotato varieties.

### Chips processing

Chips were prepared from uncured roots cut into slices 1, 2, 3, and 4 mm thick, then fried in soya bean oil at 180°C. A consumer survey revealed that chips 1–2 mm thick produced from the Chicken Foot variety were found to be the most acceptable. Accelerated storage tests revealed that 0.15% butylated hydroxy anisole (BHA) was very effective in inhibiting rancidity.

### French fries processing

Purple Top frozen french fries were produced from strips blanched in water or 0.5% sodium acid pyrophosphate at 100°C, followed by 5 min dehydration at 60°C or in oil at 180°C, and subsequently stored at –18°C for eight weeks. Chemical and organoleptic properties of these stored fries were periodically evaluated. The oil-blanched fries were most favoured. Appearance was the most important factor in determining overall acceptability, followed by texture and taste. Chemical profiles showed a significant link between moisture content before frying and texture, and an interaction between sugars and taste.

Analysis of crude food components in both types of products showed fat enrichment and apparent concentration of protein, minerals, and carbohydrates due to frying.

### Cassava

Cassava is grown mainly in developing countries where it is a primary source of carbohydrates for millions (Coursey, 1978). Cassava roots consist mainly of water and starch. The high water content (60–70%) makes the unprocessed roots bulky and difficult to handle. Furthermore, the roots do not store well and begin to deteriorate within two to four days after harvest (Odigboh, 1983). The major post-harvest problems of cassava are physical injury, physiological and microbial deterioration, and pest injury (IICA, 1989). In the Caribbean, cassava is mainly consumed as a boiled or fried food and served as a boiled or fried food, with protein-rich foods such as oilseeds, pulses, and fish. The primary concern for food processors is to extend the storage life of the cassava, by the application of various processing technologies in the production of value-added products such as extruded products, cassava farine (fortified and unfortified), and cassava wafer (fortified and unfortified).

The objectives of this research were to produce cassava farine and cassava wafer of varying starch percentages and to investigate the effects of varying salt or sugar addition, and length of heat treatment on the acceptability of the products. Further research was performed to protein-fortify the cassava farine and cassava

wafer by adding soya bean flour and to evaluate the products based on composition, water activity, colour, and sensory attributes.

### Evaluation

The composition of the cassava farine (unfortified) and cassava wafer (unfortified) as determined by the methods of the AOAC (1980), No. 14.002 moisture; 2.057 crude protein; 7.056 crude fat; and 7.065 for crude fibre. Sensory evaluation of the unfortified cassava farine and cassava wafer was performed by a 13 member semi-trained panel to select the most acceptable products. The panel members were students and staff of the Food Technology Unit, Department of Chemical Engineering, The University of the West Indies, St Augustine. Packaged samples (30 g) with or without unsalted butter were presented to the panelists. A 5-point hedonic questionnaire (5 = like definitely, 1 = dislike definitely) was used for the selection of the most acceptable products and in the evaluation of the quality attributes as affected by the length of heat treatment.

The protein content of the cassava farine was determined by the AOAC (1990), No. 884.13. Colour was measured using a Minolta Chroma Meter, model CR-200B (Minolta Camera, Corporation, Osaka, Japan) and water activity using a Water Activity Meter Aqua Lab CX-2. Sensory evaluation was performed on the fortified products by untrained staff and student members of The University of the West Indies. The products were evaluated for acceptability using paired comparison tests, directional difference tests, and acceptability tests.

Expansion of the cassava extrudates was determined by the method of Mercier and Feillet (1975) on 10 randomly chosen pieces of extrudates, bulk densities by the method of Harper (1981), product moisture No. 13.003 (AOAC, 1965), water absorption index (WAI), and water solubility index (WSI) by the method of Anderson *et al.* (1969).

There was no significant difference for 'acceptability' and 'degree of likeness' between unfortified cassava products and the fortified cassava products. A cassava farine product fortified at the 20% level addition of soya bean flour and a cassava wafer fortified at the 10% level of addition of soya bean flour were selected by panelists as acceptable products. The protein content of the fortified farine was 16.6% (d.b.) which represented an increase which was 10 times that of the unfortified product and the protein content of the fortified wafer was 12.01% (d.b.) which represented a fivefold increase over the product. A limited shelf life study showed no significant difference ( $P > 0.05$ ) in the colour, water activity, and organoleptic characteristics of the fortified products.

Optimum expansion (2–82) of cassava extrudates was obtained at 11% feed moisture

d.b., 120–125°C, and screw speed 520 rpm with a feed rate of 250 g min<sup>-1</sup> using a Wenger X-5 laboratory extruder. The effect of feed moisture was most significant ( $P < 0.01$ ) on expansion, bulk density, and extrudate moisture. Increasing the temperature increased the expansion and water-solubility, but decreased bulk density, extrudate moisture, and WAI. The extrusion variable of screw speed was most significant ( $P < 0.01$ ) on WAI and WSI. The WSI of the extrudates correlated negatively ( $r = -0.83$ ;  $P < 0.05$ ) with extrudate moisture and WAI ( $r = -0.80$ ) and correlated positively ( $r = 0.80$ ;  $P < 0.05$ ) to expansion.

### Yam

Yams (var. yellow Round Leaf) were washed and peeled, the 'seed' material removed, and the remaining portion divided into three equal portions: the head, middle, and tail. The portions were sliced into 0.5-in thick pieces and dipped in 1% metabisulphite solution. The slices were then pre-cooked at 30–40°C for 15 min and frozen at -40°C to -46°C for 30 min. The frozen products were then stored in a walk-in freezer (-3 to -5°C) for three months, after which the samples were evaluated for colour, flavour, and texture using a triangle test. The control samples were slices dipped in 1% metabisulphite solution and cooked from a tuber which was stored for one month at ambient (29–30°C) condition.

The results showed that peeled raw and peeled pre-cooked frozen yam slices were both organoleptically acceptable products. An antioxidant such as sodium metabisulphite will greatly improve the colour of the frozen product. It was concluded that in cases where the fresh tuber was not severely discoloured on injury, a 1% sodium metabisulphite solution can completely prevent discoloration.

The texture, colour, or flavour of the raw or pre-cooked slices of the mature yam tubers were not significantly affected by the quick freezing process. Also, the freezing process was not affected by the variation in the maturity of the yam tuber. The texture and flavour of the products were affected by the presence of bark and cortex in the final product. Therefore, proper peeling techniques must be employed to remove all the bark and cortex for the production of an organoleptically acceptable product.

It was observed that the first 3–4 ins of the head section of the yam tuber were generally of very poor quality and, thus, should be discarded. Also, bitterness, toughness, and the extent of discoloration decreased from the head to the tail of the yam tubers. Once the first 3–4 ins of the head region were removed, the quality attributes in the different sections of the yam tubers were not significant.

In conclusion, the Round Leaf Yellow Yam



can be processed into organoleptically acceptable peeled, raw, and pre-cooked frozen yam slices. The frozen products can be stored at 3–5°C for a period of three months without any significant changes in quality. The pre-cooked frozen slices were the superior products because the raw frozen slices adhered strongly to each other and thus created difficulty in separation.

### Yam flour extrudate

The tubers were washed to remove dirt and weighed. The hand-peeled tubers were then immersed in a 0.05% metabisulphite solution for a few minutes to prevent browning. The tubers were sliced into thicknesses between 0.25 cm and 0.50 cm and dried to a moisture content of 8.5–9.0%. The dried chips were weighed and ground to allow 100% of the ground particles to pass a sieve with aperture 1.4 mm. The samples were then graded into fine (A), medium (B), coarse (C), or very coarse (D). These samples were then stored in sealed high density polyethylene bags (HDPE). The moisture content of the samples was then brought up to 9%. Batches of the yam flour and amylose mixtures were prepared by adding various weights of amylose to yam flour at levels of 5, 10, 15, 20, 25, and 30% of the mixture. The same procedure was followed for the amylopectin:flour mixtures.

The above mixtures were then extruded under experimental conditions: Die and Barrel temperatures [(80–85°C, 75–80°C); (90–95°C, 85–90°C); (100–105°C, 95–110°C); (110–115°C, 105–110°C); and (120–125°C, 115–120°C)]; screw speed of 538 rpm; number of extruder barrels to be used, 5; and diameter of the die, 5.0 mm. The resulting products were then collected, analysed within 15 min, and stored in sealed HDPE bags.

### Extrusion temperature on various flour grades

Grades A, C, and D yam flours fed well through the extruder at all the temperature ranges used. Extrusion of Grade B flour at temperatures below 95°C was not successful. On each occasion, the product was rapidly ejected as burnt pellets with no expansion. At temperatures higher than 100°C, however, acceptable products similar to those from Grades A, C, and D flours were obtained with Grade B flour. The results of analyses of the products revealed that when extrusion temperature was increased, product moisture, bulk density, and firmness decreased while expansion index and colour gradient (cream to golden brown) increased. This was noted for all grades of flour. At temperatures below 95°C, cooking was incomplete for all grades of flour, while at temperatures above 100°C, cooking seemed complete except for grades C and D products which had a few uncooked particles.

### Feed particle size and product characteristics

The results of analyses of products showed that, generally, small differences with no trend were observed in product moisture content, bulk density, and expansion index with increasing particle size range suggesting that particle size did not play a significant role. Increase in firmness with increasing particle size range was observed for each temperature range. There was no change in colour with particle size at each temperature range, except for the presence of a few uncooked particles in products obtained from coarser grades of flour (Grades C and D).

### Feed moisture content and product characteristics

Analyses of the products reveals that there was a progressive increase in product moisture content as the feed moisture content increased. An increase in expansion and decrease in firmness and bulk density with increasing feed moisture content was observed until an optimum feed moisture content of 9% was reached, after which the reverse occurred. A colour gradient (golden brown to cream) was observed as the feed moisture content increased from 8–17%. The product obtained at the 6% feed moisture level had a colour similar to that obtained at the 10% level, but, in addition, contained a few dark brown specks indicative of burning.

### Addition of various levels of amylose and amylopectin on product expansion

The results of analyses of these products revealed that increasing the levels of amylose in the feed material resulted in a decrease in expansion, regularity in product surface, and a slight lightening in colour. Variations in expansion were observed with increasing levels of amylopectin but there was no obvious trend. Products obtained at all six levels of amylopectin were fairly identical, with no differences in colour or product surface regularity.

It can thus be concluded that yam flour can be successfully extruded and expanded at low moisture content (8–10%) and temperatures between 100–115°C. Also, the products' characteristics are related to extrusion temperature, feed moisture content, and amylose content of the feed materials. Finally, the feed particle size and amylopectin content of the feed ingredient do not contribute significantly to variations in the products characteristics.

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# Market potential for cassava flours and starches in Ghana

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This study details the results of a baseline survey carried out in 1996 of the current starches and flours markets, and an assessment of the market potential for cassava starch and flour in Ghana. The market for starch comprises a number of end users who use maize, cassava, and potato starch in the textiles, pharmaceuticals, paper, food, and adhesive industries. The current market size is 4200 tonnes per annum which are mainly imported, and is likely to grow to 6000 tonnes per annum by the year 2000. Most users have very high quality specifications with 60% of the market being for modified starches. There would appear to be potential for the production of cassava starch within Ghana to meet some of these specifications. The market for flour in Ghana is currently dominated by wheat flour. In 1996, approximately 300 000 tonnes of wheat equivalents were imported. Most of this flour was used by the food industry in the preparation of bread and snack foods but some was also used as a glue extender by the plywood industry. Cassava flour has potential to substitute for wheat flour in wood glues. The food sector is an attractive market for cassava flour because it uses large amounts of imported wheat flour. However, to be successful, cassava flour needs to be made to a high standard to meet the stringent quality specifications of the potential users in the food industry.

Keywords: Cassava; *Manihot esculenta* Crantz; Starch; Flour; Ghana

Cassava (*Manihot esculenta* Crantz) is probably the most important root crop in Ghana. Annual production has been rising consistently over the last decade and currently is estimated to be about six million tonnes of fresh roots per annum. Cassava supplies a major source of daily carbohydrate intake to the majority of Ghanaians and also has an important role as a food security crop due to its ability to grow on poor-quality land and its tolerance of drought. In Ghana, cassava is eaten in the fresh state (boiled and pounded to make *fufu*, fried or roasted) or processed into a range of traditional products including *agbelima* and *gari*. Cassava is also cut into chips, sun-dried, and pounded or milled to prepare a flour known as *kokonte*. Consumption of *kokonte* flour reaches its peak during the 'hungry season' when maize is either unavailable or too expensive for many families to purchase.

Cassava is also used on a small scale for industrial purposes and as a source of carbohydrate in livestock feeds. The internal market for cassava in livestock feeds seems likely to expand. Recently, two companies in Ghana have

started to export dried cassava chips to the European Union for use in livestock rations. An assessment of the post-harvest needs in non-grain starch staple food crop systems in Ghana (Kleih *et al.*, 1994) highlighted the need to add value to cassava and improve producer prices, and the apparent interest of farmers in expanding the market for cassava. One approach for achieving this aim would be to improve the marketing opportunities for locally-produced cassava flour and starch. However, the potential market for industrial uses of locally-produced cassava starch and flour has not been explored.

The objectives of the current study were to examine the current markets for flour and starches in Ghana and to assess the potential future market for cassava flours (non-*kokonte*) and starches as partial substitutes for imported materials.

## Materials and Methods

For this study, a survey of producers and users of starches and flours in Ghana was made during the period February to April 1996 by the Natural Resources Institute (NRI) working in collaboration with the University of Ghana (UoG). For the first phase of the survey, visits were made to as many potential users and producers of starch and flours as possible to obtain a broad view, via a formal questionnaire. In the second phase, more detailed information was obtained by revisiting selected producers and

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users, and carrying out semi-structured interviews using the methodology reported by Kleih *et al.* (1997).

## Results and Discussion

### Market potential for cassava starch

The market for starch within Ghana comprises a number of end users (Table 1) who make use of maize, cassava, and potato starch which is mostly imported. The survey carried out by NRI/UoG indicated that the current market size is approximately 4200 tonnes per annum, which compares well with figures in a survey carried out by Glucoset Limited of Ghana (Anon., 1994). The Glucoset survey also predicts that demand will increase to 5600 tonnes by the year 2000. Both the UoG/NRI and Glucoset surveys found that most users have very high quality specifications with 60% of the market requiring modified starches.

Although the market for starch is small (approximately 5000 tonnes per annum) compared to the annual production of cassava at about six million tonnes per annum, there is still potential for a large-scale producer of cassava starch within Ghana to meet these high quality specifications and reduce Ghana's dependence on imported products. There is also potential for the export of cassava starch from Ghana to other countries in the sub-Saharan Region. South Africa would appear to be a particularly attractive possibility due to its large industrial base relying on imported starch.

The small-scale less sophisticated producers already operating in Ghana have little chance of competing against these imported products on the basis of product specification and scale of production. However, there may be scope for these producers to produce cassava starch for conversion into maltose and malto-dextrins for use as sweeteners by Ghana's expanding food industry. Malto-dextrin production has the advantage of producing a higher value product with relatively unsophisticated technology and it eliminates the need for drying which is always a problem for small-scale producers of cassava starch.

### Market potential for non-kokonte cassava flour

In Ghana, most people associate cassava flour with *kokonte* which is a relatively low grade traditional product. The product can be of poor quality because drying times are typically long allowing microbial growth. Non-*kokonte* cassava flour in this study is, however, a high-quality product.

The market for flour in Ghana is currently dominated by imported wheat flour. At the present time, Ghana imports approximately 250 000–300 000 tonnes of wheat equivalents (wheat grain and flour) per annum (Table 2). Most of this flour is used by the food industry in the preparation of bread and snack foods such as biscuits, cakes, pies, and doughnuts. However, food grade wheat flour is also used as a glue extender by the plywood industry in Ghana. Although the market for glue extenders is relatively small (approximately 1200 tonnes

**Table 1** Market for starch (maize, cassava, and potato) in Ghana 1996

Sector	Market share (%)	Metric tonnes per annum (estimated)	Requirements
Textiles	40	1680	High quality specifications in terms of purity and microbiological quality.
Pharmaceuticals	20	840	Medium specification; requires high level of purity and consistent product quality with respect to viscosity.
Paper	10	420	Low specification; requires low fibre and particulate contaminants.
Food	3	126	High quality specifications in terms of purity, microbiological quality and specialised pasting characteristics for particular products.
Plywood (glue extenders) + others	27	1134	Low specification; requires low fibre and particulate contaminants.
Total		4200 metric tonnes	

**Table 2** The Ghanaian wheat market: Key statistics

Year	Wheat imports (wheat equivalents) <sup>†</sup> tonnes	Estimates of current wheat milling activity and capacity <sup>††</sup>
	Miller	Milling activity (M t yr <sup>-1</sup> )
1980	131 000	Takoradi Flour Mills 80 000
1981	150 000	
1982	120 000	Golden Spoon 45 000
1983	111 000	Irani Brothers 40 000
1984	93 000	GAFCO 80 000
1985	77 000	
1986	75 000	
1987	140 000	Miller Milling capacity (M t day <sup>-1</sup> ) 1200
1988	170 000	Takoradi Flour Mills
1989	154 000	Golden Spoon 150
1990	225 000	Irani Brothers 750
1991	207 000	GAFCO 200
1992	164 000	

Source: FAO Trade yearbooks and GAFCO

<sup>†</sup>wheat and flour<sup>††</sup>Ghana Agro-Foods Company (GAFCO)

per annum) the quality specifications are quite low, which makes this market an attractive starting point for developing cassava flours. Previous research in Peru has shown that cassava flour can be used to substitute for wheat flour in plywood glues at a level of 46% (Jones, 1994). Cassava flour has been successfully adopted by plywood manufacturers in Peru who appreciate the cost saving derived from using locally-produced cassava flour. There is also potential for formulating water-resistant wood glues by mixing cassava flour or starch with urea or phenol formaldehyde resins. Glues of this type (containing cassava starch) have been successfully tested by the timber industry in the Philippines (Fidel *et al.*, 1992).

The food sector is an attractive market for high-grade cassava flour because of its high consumption of imported wheat flour. Previous research carried out in Ghana and many parts of the world has demonstrated that cassava flour can be used to substitute for a percentage of the wheat flour in many products. In the case of bread the maximum substitution level is around 15%. Above this level, technical problems are encountered due to a reduction in the amount of gluten present in the flour (De Ruiter, 1978; Dendy and Trotter, 1988; Satin, 1988). In the case of snack foods such as biscuits and cakes which are not gluten-sensitive products, the theoretical level of substitution is 100%. However, in practice, commercial producers of biscuits in Colombia found that the maximum level of substitution was around 30%.

In this case high levels of cassava flour made the products brittle. Cassava flour is also very hygroscopic which can be advantageous in cake formulations but will cause hard biscuits to go soft if too high a level of cassava flour is used. However, to be successful, cassava flour needs to be made to a high standard to meet the stringent quality specifications of the potential end users in the food industry.

## Conclusion

Previous research outside Ghana has shown that the technical problems associated with cassava flour can be overcome. Non-kokonte cassava flour has the advantage over cassava starch of being easier and cheaper to produce with lower capital requirements and less need for large volumes of high-quality water for processing. However, non-kokonte cassava flour and cassava starch face certain common constraints which will have to be overcome for production to be viable.

A major potential difficulty is cassava root supply. Successful processing units must have a reliable supply of high quality fresh roots of correct variety and maturity available at an affordable price for a minimum of six months a year (10 months for a modern starch factory). This will present difficulties in many parts of Ghana where production and harvesting techniques are primitive and transport infrastructure is in need of improvement. In addition, transportation costs are high, accounting for more than 40% of the wholesale price of fresh roots. There is also the need to achieve the correct balance between minimizing processing costs and producing products that meet end users specifications. Provided these issues can be overcome, the production of cassava flours offers the potential to provide new markets for cassava producers. Research is required to adapt these findings to meet the future requirements of the various industries in Ghana.

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# Development of alternative flavour types of sweetpotato as a means of expanding consumption

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The flavour of cooked root and tuber crops is a primary determinant in consumer acceptance. Flavour is composed of taste and aroma and can be substantially altered via plant breeding. Using sweetpotato [*Ipomoea batatas* (L.)] as a model, a sweetpotato line with culinary traits similar to baked white potatoes [*Solanum tuberosum* (L.)], was developed which has a much lower flavour impact, in keeping with other staple crops (e.g., cassava, potato, and rice). The successful alteration of the flavour of a staple crop requires a combination of sensory testing and chemical analysis of critical flavour components (i.e., identification and quantification of the impact of each component on the characteristic flavour). The volatile profiles and identity of odour-active compounds were determined for a traditional 'North American' sweetpotato ('Jewel') and a non-sweet, staple-type line with a white potato flavour (GA90-16). 'Jewel' had substantially higher levels of 2-furmethanol, 2-acetyl pyrrole, maltol, and geraniol, the latter three conferring sweet and (or) caramel notes to the aroma. GA90-16, in contrast, had low levels of volatiles critical to the aroma of 'Jewel', and substantially higher levels of 2,3-nonadecanediol, 2,4-decadienal, octyl ketone, and one unidentified compound with a distinct white potato aroma.

Keywords: Sweetpotato; Flavour profiles; Breeding; Organoleptic qualities; Volatile compounds

Most of the more widely grown staple crops of the world (e.g., cassava, rice, wheat, and potato) are relatively low in flavour intensity; the most notable exception is sweetpotato [*Ipomoea batatas* (L.)] (Kays, 1985). This has an exceptionally wide range of flavours present within its gene pool (McLaurin and Kays, 1992).

A primary deterrent to selection for flavour in conventional plant breeding programme is that flavour is very difficult to measure accurately. Using conventional sensory analysis techniques, a routine test can assess only five to eight samples at one sitting and due to the subjective nature of the analysis, large panels (i.e., >15 individuals) are required to obtain an accurate estimate of preference. Thus, to screen for flavour using conventional sensory methods, a large number of people are required while the number of lines that can be screened is low. This has resulted in a lower priority being given to flavour in the overall selection process (Kays, 1988) even though eating quality may be considered as a top breeding priority (Martin and Jones, 1986). The difficulty of selection for flavour is additionally compounded when the breeding programme is in one location (e.g., South America) and the target population is in another part of the world. Since flavour preferences differ with location, sensory panels must comprise members of the target population. Therefore, either the progeny to be assessed, or the panel members must be transported to the test location.

In typical breeding programmes for potato and sweetpotato, selection generally occurs ini-

tially in the greenhouse where a large number of lines can be tested under controlled conditions. With each reduction in the population size as desirable lines are discarded, the chance of selecting previously unselected traits diminishes. In typical potato (Mackay, 1987) and sweetpotato (Jones *et al.*, 1976) breeding programmes, up to 98% (potato) to 99% (sweetpotato) of the lines are eliminated by the end of the first year and screening for flavour usually occurs after a major portion of the progeny has been discarded. The lower priority for flavour in the selection sequence arises from the difficulty of measuring flavour using conventional sensory analysis techniques.

If flavour could be measured analytically, the number of lines that could be screened could be increased to approximately 40 to 50 a day per gas chromatograph while greatly increasing precision (Sun *et al.*, 1993). This in turn would allow moving flavour to an earlier position in the selection sequence. An analytical method does not, however, completely eliminate the use of sensory panels, although it does allow moving large numbers of progeny through several cycles prior to final selection (Kays and Horvat, 1983). Using an analytical selection protocol requires an understanding of the basic chemistry of the flavour traits desired, but allows imposing a substantially increased selection pressure for the desired trait. Several advantages of an analytical rather than a subjective approach to selection of flavour include: (1) the trait is well

defined; (2) accurate parent line selection; (3) increased sample population; (4) accuracy of progeny selection; (5) the ability to simultaneously select for multiple consumer groups with distinctly different flavour preferences; (6) the ability to select new unique flavour types; (7) the potential for a centralized analytical programme; and (8) a database for future use (Kays, 1988). Studies have already been undertaken to (1) identify the major positive and negative flavour components; (2) assess the range in flavour within the genepool (McLaurin and Kays, 1992); (3) develop an analytical procedure for rapid screening of large numbers of parent lines and progeny (Sun *et al.*, 1993); (4) characterize the chemistry of flavour preference of target consumer populations; and (5) identify desirable clones using chemical analyses interfaced with sensory analyses.

The objective of this study was to compare the volatile profiles emanating from a traditional 'North American' baked sweetpotato (cv. 'Jewel') and a non-sweet, staple type line (GA90-16), identifying and quantifying critical compounds.

## Materials and Methods

### Sample preparation

Two sweetpotato lines ('Jewel' and GA90-16) were grown at The University of Georgia Horticultural Farm using standard sweetpotato production practices (Granberry *et al.*, 1990). 'Jewel' is a standard commercial sweetpotato grown in the United States and is characterized by its soft orange flesh, intense sweetpotato odour, and sweet taste. In contrast, the breeding line GA90-16 has a flaky, dry white flesh, an odour that much more closely resembles a baked white potato [*Solanum tuberosum* (L.)], and is essentially non-sweet. Roots cured for seven days at 29.4°C and 95% RH and stored at 13°C and 85% RH were used for the subsequent tests. Five individual roots, 7–9 cm in diameter, of each line were washed and peeled. Cross-sectional cores (1.7-cm diameter × 3 cm) were removed, giving samples of equivalent weights (350 g) and approximately equivalent surface areas. Individual samples were placed inside a specially constructed 1-L glass container in a electric convection oven. The temperature was increased from ambient (25°C) to 204°C at approximately 4.5°C min<sup>-1</sup> and held at 204°C for a total baking and volatile collection time of 70 min.

### Volatile collection

The volatiles evolving during baking were collected using a solvent trap system (glass container 3 cm diameter × 75 cm high) containing

redistilled methylene chloride (J.T. Baker, Phillipsburg, N.J.) as the solvent. Helium carrier gas transported the volatiles from the sample container within the oven to the ice-cooled solvent trap. The collection apparatus and procedures were previously described by Sun *et al.* (1993). One hundred and forty millilitres of methylene chloride containing 2.25 µg ethyl benzoate as the internal standard were used to trap the volatiles. An additional 10 mL of methylene chloride were used to rinse the trapping chamber at the end of the collection period. The solvent containing volatiles was concentrated using a Kuderna-Danish evaporator/concentrator in which the sample flask was heated with a steam bath. The samples were reduced to 5 mL and then were concentrated to 0.2 mL by slowly flushing them with purified nitrogen gas. An amount of 5 µL aliquots were immediately chromatographed.

### Gas chromatography

Analyses were performed using a HP-5890 gas chromatography (Hewlett Packard, Avondale, PA) with a split-splitless injection port temperature of 200°C and flame ionization detector (FID) temperature of 250°C. Separations were made using a wide bore (15 m × 0.53 mm i.d.) fused silica capillary column coated with DB-1 (Alltech Associates, Deerfield, IL). The samples were injected in the splitless mode (35°C) with a purge time of 0.5 min and held at that temperature for 2 min, after which the oven temperature was increased to 220°C at 3°C min<sup>-1</sup> followed by holding at 220°C for 10 min. The chromatograph was connected to an HP 3396 Series 111 integrator to quantify the area under the peaks.

### Gas chromatography-mass spectrometry (GC-MS)

An HP-5985B GC-MS system (GC-MS) was used to identify the volatile compounds. Mass spectrometer conditions were: ion source (200°C); ionizing voltage (70eV); multiplier voltage (220V); GC-MS interface zone (300°C); and scan range (40–400). Identities were in most cases confirmed using authentic standards for verification of retention time, mass spectrum, and odour.

### Gas chromatography olfactometry (GCO)

The aroma extracts were also analysed by GCO evaluation using a thermal conductivity detector (TCD) and the same column and chromatographic conditions as with FID analysis. The aroma of individual peaks eluting from the exit port was ascertained independently by panelists. By using standard compounds (four), the retention times for individual compounds were shown to be identical between TCD and FID analysis.



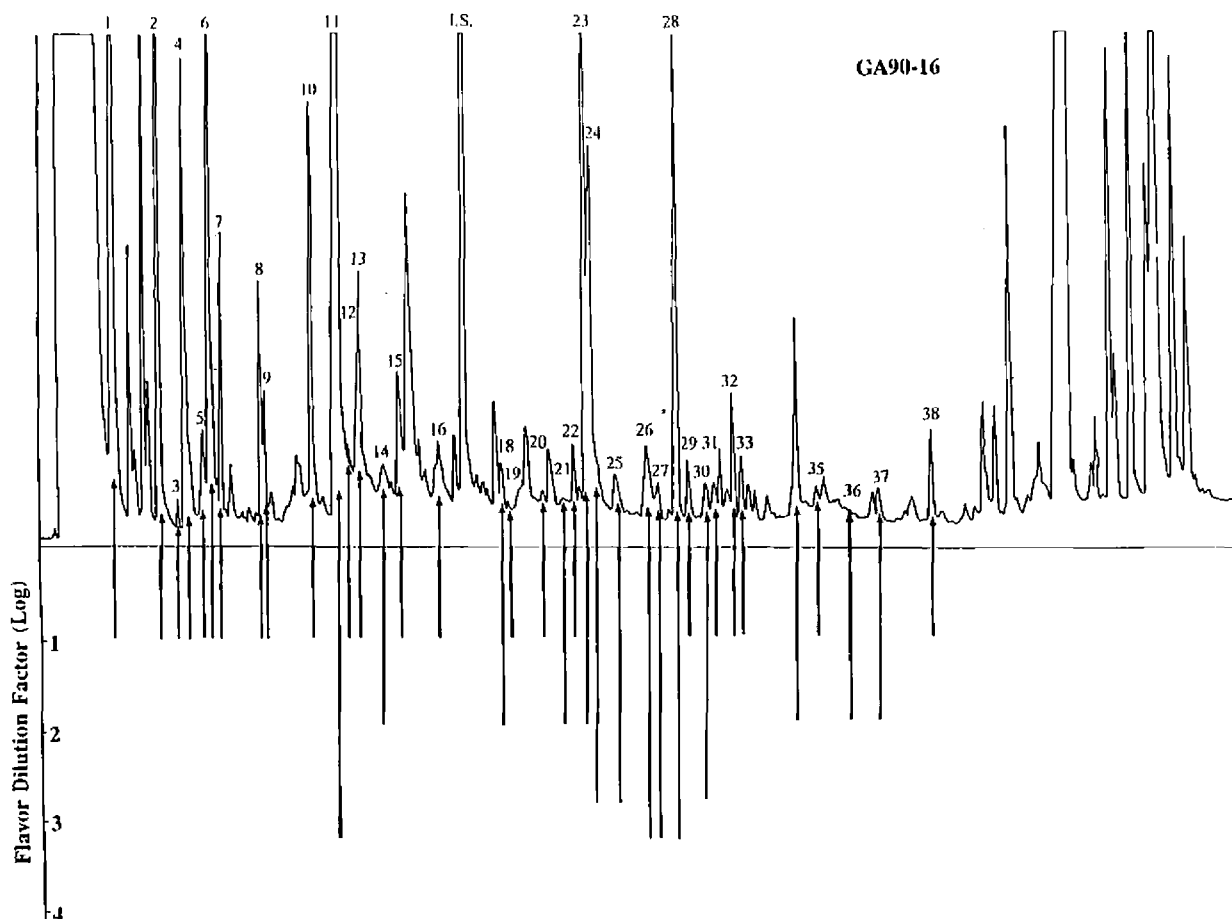
## Results and Discussion

The two lines displayed distinctly different odours (i.e., 'Jewel' having a traditional 'North American' sweetpotato odour, and GA90-16 more closely resembling a baked white potato). These differences were reflected in their respective GC volatile profiles (Figures 1 and 2). Chemically, the volatiles included heterocyclic compounds, aldehydes, alcohols, ketones, aromatic hydrocarbons, monoterpenes, sesquiterpenes, and acids (Purcell *et al.*, 1980; Kays and Horvat, 1984; Horvat *et al.*, 1991; Sun *et al.*, 1995).

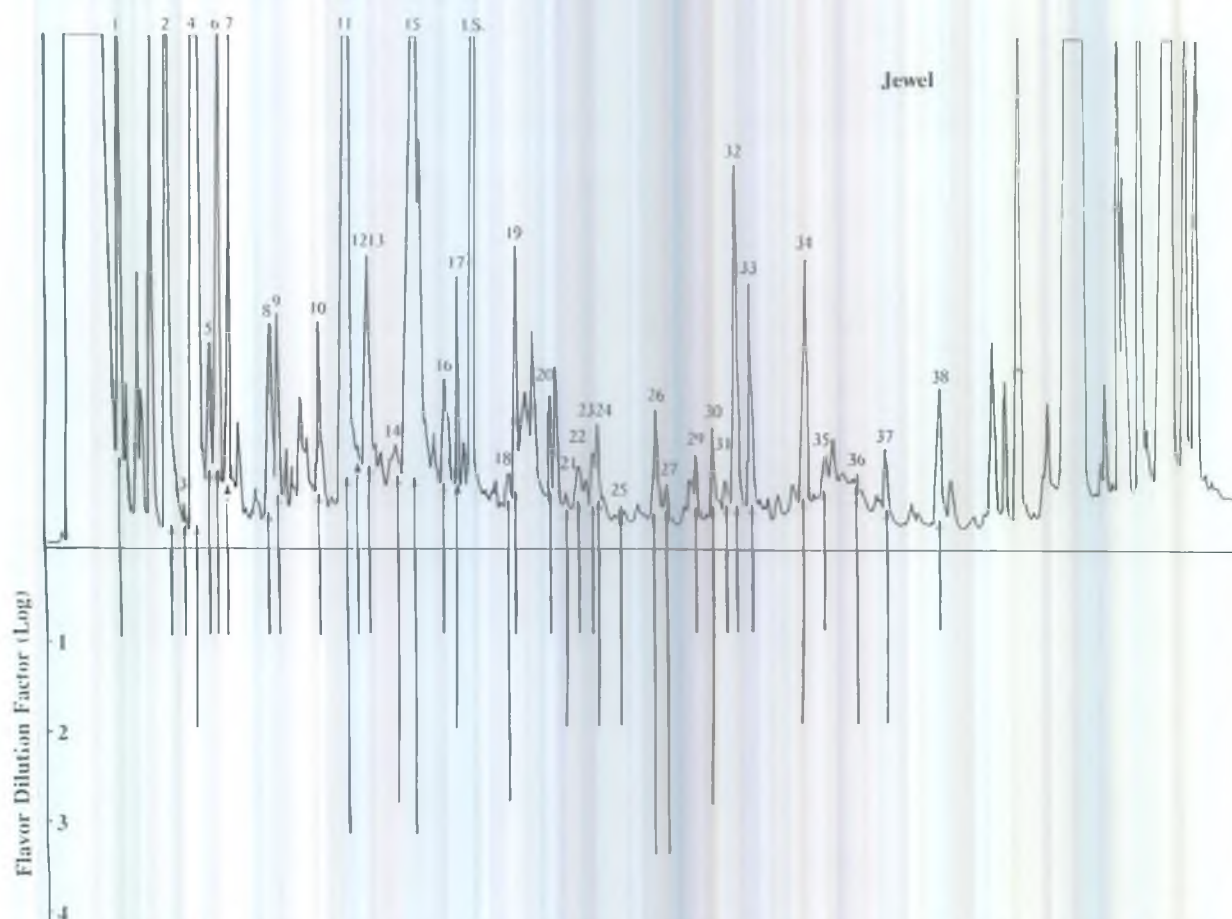
A total of 38 odour-active compounds were found between the two lines. Olfactory analysis via a dilution series allowed assessing the contribution of each volatile to the overall odour. The majority of the odour active compounds were minor contributors to the characteristic aroma. Compounds that contributed the most to the characteristic odour are indicated with an asterisk in Table 1. While the volatiles present in both lines were qualitatively similar (except that peak 17 was not found in GA90-16 and peak 28 was not found in 'Jewel'), there were major quantitative differences. Thus differences in concentration of critical odour volatiles resulted in the distinct aroma of each line (Table 1). 'Jewel' had substantially higher levels of 2-

furfural, 2-acetyl pyrrole, maltol, and geraniol, the latter three conferring sweet and (or) caramel notes to the aroma. Maltol was a critical odour compound, with a strong caramel aroma (Sun *et al.*, 1995) and maltose is an efficient precursor of maltol when heated in the presence of amino acids (Tressl *et al.*, 1989; Kays and Wang, 1998). Maltose also represents a primary precursor for several of the other volatiles (Sun *et al.*, 1994). Sun *et al.* (1995) concluded that Maillard reactions appear to be the operative means of synthesis of the characteristic aroma of baked 'North American' sweetpotato.

GA90-16, in contrast, with an aroma similar to baked white potato, had low levels of the volatiles critical to the aroma of 'Jewel', and substantially higher levels of 2,3-nonadecanediol, 2,4-decadienal, octyl ketone, and one unidentified compound. The latter compound has a distinct white potato aroma but appeared to be spectrally unique from compounds previously identified from baked white potato with potato-like odours (Buttery *et al.*, 1973; Pareles and Chang, 1974; Coleman and Ho, 1980; Ho and Coleman, 1980; Coleman *et al.*, 1981). The remaining three compounds conferred musty, cooked starch, and cereal odours to the line. The very low levels of sugars, especially



**Figure 1** Chromatographic profiles of the volatiles emanating from cured 'Jewel' sweetpotato during baking (peak numbers correspond to compounds in Table 1; I.S. = internal standard) and relative intensity (flavour dilution factor) for each compound



**Figure 2** Chromatographic profiles of the volatiles emanating from cured sweetpotato cultivar 'GA90-16' during baking (peak numbers correspond to compounds in Table 1; I.S. = internal standard) and relative intensity (flavour dilution factor) for each compound

**Table 1** Odour-active volatiles emanating from baked 'Jewel' and GA90-16 sweetpotato cultivars

Peak	Volatile compound	Relative concentration				Peak	Volatile compound	Relative concentration			
		$\mu\text{g kg}^{-1} \text{ fwt}^1$ and FD factor <sup>2</sup>						$\mu\text{g kg}^{-1} \text{ fwt}^1$ and FD factor <sup>2</sup>			
		Jewel		GA90-16				Jewel		GA90-16	
1	Pyridine	5.6	(10)	1.7	(10)	20	Cyclohexanol	5.4	(10)	tr	(10)
2	3-furaldehyde	14.5	(10)	2.4	(10)	21	n-decanal	tr*	(100)	tr*	(100)
3	Xylene	0.3	(10)	0.1	(10)	22	2,2-dimethyl-1,3-cyclohexanediol	0.4	(10)	0.2	(10)
4	2-furmethanol	14.1* <sup>3</sup>	(100)	1.8	(10)	23	2,3-nonadecanediol	0.5	(10)	2.5*	(100)
5	Furfuryl alcohol	1.3	(10)	2.8	(10)	24	2,4-decadienal	0.6*	(100)	1.9*	(1000)
6	2-acetyl furan	4.4	(10)	2.8	(10)	25	Octyl ketone	tr	(100)	0.3*	(1000)
7	Benzaldehyde	2.1	(10)	0.5	(10)	26	Germacrene D	0.9*	(2000)	0.6*	(1500)
8	5-methyl-2-furfural	0.9	(10)	0.7	(10)	27	Caryophyllene	0.3*	(2000)	0.2*	(1500)
9	2-pentyl furan	1.2	(10)	0.1	(10)	28	Unknown 1	—	—	1.8	(1500)
10	2,3-pentanedione	0.7	(10)	1.5	(10)	29	$\beta$ -farnesene	0.3	(10)	0.3	(10)
11	Phenylacetaldehyde	29.7*	(1500)	20.9*	(1500)	30	$\alpha$ -copaene	0.3*	(1000)	0.2*	(1000)
12	Limonene	tr <sup>4</sup>	(10)	tr	(10)	31	$\alpha$ -bisabolene	0.3	(10)	0.2	(10)
13	3,4-dihydropyran	2.1	(10)	1.0	(10)	32	Bohlmann 176	1.5	(10)	0.3	(10)
14	2-acetylpyrrole	0.3*	(1000)	0.1*	(100)	33	2(4H)-benzofuranone	1.3	(10)	0.3	(10)
15	Maltol	30.8*	(1500)	0.7	(10)	34	$\beta$ -ionone	1.6*	(100)	0.8*	(100)
16	Linalool	0.8	(10)	0.2	(10)	35	Nerol	0.2	(10)	0.1	(10)
17	Isopulegone	0.8	(100)	—	—	36	4-decanolide	tr	(100)	tr	(100)
18	Geraniol	0.4*	(1000)	0.1*	(100)	37	Unknown 2	1.0*	(100)	0.2*	(100)
19	2,4-nonadienal	1.2	(10)	tr	(10)	38	Tetradecanoic acid	4.2	(10)	0.5	(10)

<sup>1</sup>fwt is Fresh weight

<sup>2</sup>FD = flavour dilution factor

<sup>3</sup>\* = important contributor of odour

<sup>4</sup>tr = less than 1.0% of volatile fraction based on area of GC peak

maltose, in this line resulted in very small amounts ( $0.7 \mu\text{g kg}^{-1}$  fwt) of maltol being formed.

The non-sweet, staple-type line (GA90-16) has several important traits that make it or similar lines valuable. It has a low flavour impact, much more in keeping with other staple crops grown world-wide. Thus, the potential range in flavour of dishes prepared with it is substantially greater. In addition, while a potato-like sweetpotato has only minimal utility, due to higher production costs, in areas of the world where white potato can be readily grown, it can be grown in hot, humid regions where the white potato is not adapted.

The results indicate that it is possible to distinguish differences in the aroma among sweetpotato lines using GC analysis. Likewise, compounds such as sugars and organic acids that modulate taste, can also be readily quantified (Chapman and Horvat, 1989). By identifying the chemistry of preference of specific target populations of consumers, it is therefore possible to use an analytical means of making selections decisions for flavour. This, in turn, allows assessing each progeny surviving initial selection for a minimum of agronomic traits (e.g., yield), matching each clone's flavour profile to one or more of a number of potential consumer groups around the world. After a sufficient number of backcrosses and selection cycles, a small population of superior clones can then be transported to designated countries for sensory analysis by potential consumers. The advantages to a breeding programme are: (1) accurate parent line and progeny selection; (2) a tremendous increase in the number of lines that can be screened for flavour; (3) greater accuracy in selection for flavour; (4) elimination of the need for specific consumer population sensory panels in the initial selection cycles; (5) the ability to simultaneously select for a large number of consumer populations around the world; (6) the ability to identify and utilize unique, new flavour types; and (7) a much more rapid improvement in the flavour quality of sweetpotato consumed worldwide.

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# Processing technology and safety of 'Kpukpuru': An indigenous weaning food in Nigeria's rural communities

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*Kpukpuru* is a fermented cassava staple which is gaining attraction as a weaning food in the rural communities of the riverine areas of Ondo State of Nigeria. Cassava is harvested, peeled, washed, fermented, dehydrated, moulded into large balls, and kept over a fireplace to smoke-dry in order to preserve the product for months or years. This cassava product is consumed by both young and adults but its novel use as a weaning diet for babies from four months upwards is intriguing. The fermentation and pressing remove considerable hydrocyanic acid (HCN) and lowers pH while the smoking completely eliminates HCN and gelatinizes its starch granules rendering it easily digestible by babies. Mothers in these villages scrape off the smoky part, break the dried moulded balls, pound them, and sieve out the powdered cassava flour. This is stirred with hot water (just as custard or maize pap). Some mothers feed babies with this preparation alone and support with breast milk. Elite ones fortify it with baby milk. The improved technology incorporating soya bean powder or paste into the *Kpukpuru* gruel will go a long way to alleviate the malnutrition that is occasioned by feeding *Kpukpuru* alone to children.

Keywords: Cassava; *Kpukpuru*; Fortify; Hydrocyanic acid; Utilization; Digestible

In Nigeria, cassava is processed mainly into various human foods using varying traditional processes. Popular products are *gari*, *fofoo*, *akpu*, starch, cassava chips, *abacha*, and *ampesi*. *Kpukpuru* is a product of cassava tubers largely consumed by adults. It is popular in the riverine areas of the south-western zone of Nigeria. It is a fermented cassava staple that is presently gaining acceptance as a weaning food in the rural riverine communities. The attributes of this cassava product are that unlike most others, it is highly digestible. Since many women in these communities are resource-poor farmers, they cannot afford high calorie foods like rice, maize, bread, or baby food for their children. Feeding of this product has, therefore, been found to be a cheap source of energy. It is, however, very low in proteins and minerals and vitamins and therefore results in malnutrition, since it is not adequately fortified. Its consumption, however, helps to alleviate problems of hunger and carbohydrate intake deficiency and, thus, its importance in terms of food security in these areas cannot be overemphasized.

Adults often consume this cassava food with vegetables, legumes, and meat or fish. This provides the necessary protein and vitamins. For babies from four to eight months old, *Kpukpuru* is occasionally fortified with soya bean flour, crayfish, or groundnut powder to make a weaning diet.

## *Kpukpuru* Processing Technology

Traditional processing techniques have been practised over many generations to achieve innocuous cassava products. Processing of cassava into *Kpukpuru* is one of the most efficient methods of cyanide removal, enhancement of carbohydrate digestion, and preservation of the tubers.

In the traditional method, unpeeled cassava tubers are soaked in jute bags placed in a flowing stream or in earthenware pots filled with water. They are then soaked for a period of 5-7 days.

Fermentation takes place under this condition of soaking (Westby and Choo, 1994). Disintegration of the tubers takes place during this process and is described as retting. Microbial growth takes place resulting in a reduction of pH as in typical lactic acid fermentation. According to Jones *et al.* (1993), fermentation is known to offer a wide range of potential advantages. These include the extension of shelf life of a perishable commodity, the addition of variety to the diet, and the improvement of taste and flavour.

After soaking, the peel is removed by hand (since unpeeled roots are soaked) after which the retted pulp is mashed and placed underneath heavy stones for a maximum of one day to express water from the pulp. During the

process of soaking and subsequent water extraction, considerable cyanide is lost. As described by Tewe (1992) tissue disintegration in cassava brings into contact extra-cellular linamarase enzyme and intracellular cyanogenic glucosides linamarin and lotaustralin which become hydrolysed to the poisonous hydrocyanic acid (HCN). Microbial action plays an important role in this process through enhancement of tissue disintegration. As contained by studies at the Natural Resources Institute, London (Westby, 1991), reduction of cyanogens in soaked roots exceeded 90% after three days, whereas in the absence of microbial growth, reduction of cyanogens was less than 50% after four days of soaking.

Hydrocyanic acid also being soluble, gets dissolved in the water medium and when the jute sack containing the soak root is placed in a flowing stream, the cyanogens are washed away. In the process of pressing to dehydrate, cyanogens are also removed with extraction of the water from the retted pulp.

After fermentation and dehydration, the fibre in the cassava pulp is sifted manually and the pulp moulded into balls. This is kept over the fireplace to dry in order to preserve it for months. This process not only further removes the HCN through volatilization, it also gelatinizes the starches thus making the carbohydrates more digestible when consumed. The possibilities of carcinogens in smoked foods have been indicated by IARC (1973). It is, however, noteworthy that the outer cover of the dry balls is removed when prepared for consumption.

A number of modifications have been introduced in modern-day processing of cassava into *Kpukpuru*. These are as follows:

Cassava tubers are peeled, washed, and grated. They are then soaked for 3–5 days in water placed in a container. The grated pulp is subsequently placed in a jute sack and water pressed out using a hydraulic jack. Fibre is sifted out of the semi-dry pulp which is then moulded and placed on the fireplace as earlier described.

The product obtained from the traditional method has a more unpleasant odour than that from the modernized method. It might also be possible that the modernized method is more efficient at the removal of cyanogens even with the reduced soaking time, because the material is pre-peeled thus enhancing disintegration of the roots. Moreover, use of a hydraulic jerk in the modernized method suggests better dehydration of the crushed roots and extraction of cyanogens prior to smoking.

### Utilization of 'Kpukpuru' Food Product

This food product can be consumed by adults and babies. The latter is advantageous as most traditionally produced cassava foods are unac-

ceptable for baby feeding. This is so because smoking of *Kpukpuru* enhances HCN loss and gelatinizes its starch granules rendering it easily digestible by babies.

For adults, the outer cover of the ball is scraped with a knife to remove the smoky surface, broken, and pounded in a mortar into a powdery form. This is sieved, cooked in boiling water, and later pounded again and turned in a mortar until a smooth consistency is derived for consumption with either vegetable or okro soup.

As a weaning diet, *Kpukpuru* balls are scraped, broken, pounded, and sieved into a powdery form. The powder is then mixed in hot water with soya bean flour or other food ingredients like egg or crayfish to make various weaning diets. However, in most cases, due to the level of poverty and ignorance, the powder is just mixed in hot water to make a gruel that is fed solely to the babies with only breast milk as supplement.

### Safety Aspects of *Kpukpuru*

While toxicity associated with *Kpukpuru* consumption is not reported in the riverine areas of Ondo State where the indigenous processing is widespread, there is a strong indication from the literature that consumption of *Kpukpuru* without adequate supplementation with proteins might trigger some neuro-toxic responses in man and animals. Circumstantial evidence has been provided in this regard by Osuntokun (1968). He reported the endemicity of tropical ataxic neuropathy in adults particularly at 40–50 years of age in south-western Nigeria situated adjacent to the area of traditional processing of *Kpukpuru*. This area of the old Ijebu province of Ogun State named Ososa is also riverine. *Purupuru* but not *Kpukpuru* is reportedly consumed in the area. This is probably a variant of the earlier described process. Unfortunately, Osuntokun (1968) did not describe the processing technique for the *Purupuru*. He, however, reported that *Purupuru* contains 4–6 mg HCN 100 g<sup>-1</sup> of dry weight, while *gari* which is also commonly consumed, contained 1–2 mg HCN 100 g<sup>-1</sup> of dry weight.

The disease rarely occurs in children under 10 years, but its mode of onset in most cases, is reported to be gradual, characterized by a typical ataxic gait. Interestingly, the biochemical studies showed low plasma concentrations of some essential amino acids. These observations were subsequently confirmed in studies with the albino rat where sole consumption of the *Purupuru* diet showed similar neurological symptoms. The low or non-existent incidence of this disease in the Ososa area presently, and its absence from the Okitipupa division of Ondo State where *Kpukpuru* is most popular, suggest that the consumption of adequate protein plays a major role as has been clearly elucidated in mechanisms for cyanide detoxification in man

and animal (Tewe, 1975). This will, therefore, underscore the need to ensure adequate supplementation with proteins particularly in weaning diets.

The processing of cassava to *Kpukpuru* also needs to be modified in order to remove the need for smoking on open wooden fireplaces as smoke emanating might contain some carcinogenic substances.

Nonetheless, with proper processing of cassava to *Kpukpuru* and adequate supplementation with protein, a *Kpukpuru*-based indigenous food can be easily prepared by mothers in rural communities since cassava is one of the major staples produced. *Kpukpuru* weaning diet can, therefore, go a long way to alleviate malnutrition problems among children if proper processing and protein fortification is guaranteed.

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# Evaluation of *Xanthosoma violaceum* acetylated starch performance for creams, sausages, and ice cream production

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Malanga (*Xanthosoma violaceum*) starch acetates were prepared from malanga tubers cultivated in southern México. Native starch and starch acetates with 0.03 and 0.06 degree of substitution at two concentration levels were used. Modified starches were evaluated as ingredients for the production of creams, sausages, and ice creams. Products were analysed on their sensory and nutritional properties. The results of the sensory evaluation indicated that sausages manufactured with starch acetates at 9% starch concentration were preferred by the panelists. For the creams, no difference ( $P > 0.05$ ) was found among the different treatments. However, the stability of the starch acetates at low temperature for a 20-day period was better than native starch. For ice creams, there was no difference ( $P > 0.05$ ) among treatments. As in the case of the creams, this means that any of the starches assayed could be used for this kind of product. Nevertheless, for long storage periods under refrigeration or freezing temperatures, starch acetates are highly recommended because they do not present syneresis problems. All nutritional characteristics of the food products were in accordance with the Mexican Food Legislation.

Keywords: *Xanthosoma violaceum*; Acetylated starch; Food Products; Sensory and nutritional properties

Aroids are mostly herbaceous plants, often with enlarged root stocks that act as storage organs, which are mostly eaten. The family Araceae is a large family comprising about 110 genera and over 2000 species widely distributed in all parts of the tropical and sub-tropical regions of the world.

The important edible aroids which are commercially cultivated are: *Colocasia esculenta* (taro, eddoe, and dasheen); *Xanthosoma* spp. (tannia, new cocoyam); *Alocasia macrorrhiza* (giant taro) and *Cyrtosperma chamissomis* (Swamp taro) are also known to be consumed in some parts of the world; and *Amorphophallus campanulatus* (elephant foot yam).

Among these, the most important and more extensive in their cultivation are *Colocasia* and *Xanthosoma* which are together called cocoyams and differentiated as taro for *Colocasia* and tannia for *Xanthosoma*.

In some countries such as Hawaii, *Xanthosoma* flour is produced at a commercial level (Onwuene, 1978; Ustimenko-Bakumovski, 1982; Tindal, 1983).

*Xanthosoma violaceum* is a tuber rich in starch, containing about 94% in d.b. (Yamaguchi, 1983). In Mexico, this tuber grows well in the south of the country in the states of Chiapas, Tabasco, Oaxaca, and Veracruz. It is known locally as 'malanga' or 'tequescamote'

and generally is consumed boiled, baked, or fried (Miranda, 1975; Martínez, 1979).

In Mexico, the main source of starch is maize, but the production is not enough and therefore this commodity has to be imported. During the first months of 1996, maize was imported from the U.S.A. at a cost of \$368 million (Mexican Secretary of Agriculture, pers. commun.). Therefore, the option for countries like Mexico is to use non-conventional sources of starch. In Mexico, there are no reports for the commercial use of *X. violaceum* starch.

In a previous work, the isolation of the starch and also some chemical modifications such as hydrolysis, oxidation, and acetylation were carried out by the authors and the chemical and functional properties of these types of starches were determined.

The aim of this research was to evaluate the performance of *X. violaceum* native and acetylated starch for food production.

## Materials and Methods

### Sample preparation

*Xanthosoma violaceum* tubercles grown in Tabasco, México, were cut and placed in a ma-

chine for washing and peeling. The samples were cut into small pieces of approximately 1 cm<sup>3</sup> and milled in a meat mill for 2 min. The pulp was mixed with sodium bisulphite solution (1.48 g L<sup>-1</sup>) and passed through a colloidal mill. The slurry was filtered through a cheese-cloth and through a fine mesh in order to reduce the fibre content. The starch solution was settled for 4 h at 7°C. The supernatant was discarded and the starch was dispersed in water and washed in centrifuge three times. The purified starch was dried in an oven for 24 h at 50°C for subsequent analyses and uses.

### Acetylation

The acetylation procedure was done according to the method described by Nieto (1993). A 30% starch was heated at 30°C and pH 8.0 for 30 min. The acetylation was done at 3.6 and 7.2% acetic anhydride.

In order to maintain the pH value during the reaction, a dilute sodium hydroxide solution was added at a moderate rate. After completion of reaction and adjusting the pH to 4.5, the starch was recovered by filtration and washing to remove salts and by-products formed in the course of reaction. The starch acetate was dried in a forced convection oven at 60°C for 24 h and ground. The powdered sample was kept in sealed jars at room temperature for further analysis.

### Food products

Sausages, creams, and ice creams were prepared with six different formulations, using three types of starch (native and acetylated at 3.6 and 7.2% acetic anhydride) at two concentration levels (7 and 9% for sausages and creams and 1.0 and 1.5% for ice creams). The formulations reported below correspond to the lowest starch concentration used for each food product.

#### Sausages

##### Formulation

Pork meat: 50.61%, ice: 25.38%, pork fat: 12.85%, chicken seasonings: 1.01%, onion: 0.81%, phosphates: 0.40%, curing salt: 0.40%, white paper: 0.30%, nutmeg: 0.30%, salt: 0.21%, garlic: 0.20%, monosodium glutamate: 0.10%, sodium erythorbate: 0.10%, liquid smoke: 0.10%, and colour agent: 0.016%.

##### Preparation

The meat was ground in a meat mill (Cutter Koch, model C-3527) with one third of the ice, the salt, and the curing salt for 7 min. Then, another third of the ice and the starch were added and mixed for 2 min. The condiments were added and mixed for 1 min. Finally, the last third of the ice, the phosphate, and the colour additive were added. The ingredients were mixed for 2 min. The sausage

dough was transferred to a stuffer for extruding the mix into casings and cooked at 80°C for 30 min. The sausages were then immersed in cold water for 5 min and finally sealed in vacuum packages and stored at 4°C for subsequent analysis.

#### Creams

##### Formulation

Milk: 72.00%, sugar: 15.80%, egg yolk: 2.80%, and vanilla extract: 2.40%.

##### Preparation

The milk was heated at 40°C and the starch was added without stirring. The heating was continued until it reached 90°C and then the egg yolks, sugar, and vanilla extract were added. The heating was continued for a further 10 min and finally the deserts were placed in plastic vessels and stored at 7°C for subsequent sensory and nutritional analysis.

#### Ice creams

##### Formulation

Water: 58.21%, sugar: 14.32%, milk cream: 10.64%, powder milk: 9.90%, yellow cocoa: 2.80%, chocolate flavouring: 2.72%, and egg yolk: 0.41%.

##### Preparation

The powder milk was reconstituted with water, and the sugar and starch were added. Once these were dissolved, the egg yolk, the cream, the cocoa, and the flavouring agent were added. The mixture was heated until it reached 60°C. It was then passed through a colloidal mill (Siemens model G-91T085-18) for homogenizing the particle size of the mixture. A pasteurization procedure was then carried out at 75°C for 30 min and, finally, the mixture was stored at 4°C for 48 h. After the maturation period, the mixture was blended in a blender for 45 min at -10°C using ice and salt to maintain this temperature.

#### Physicochemical characterization of the food products and sensory evaluation

The physicochemical composition of the food products were analysed according to the standards of the Mexican Food Legislation following the AOAC methods (1990). For sausages, the moisture, ash, fat, protein, nitrites, and carbohydrates (by difference) were analysed. For creams, moisture, ash, proteins, and total reducing sugars, were analysed. For ice creams, total solids, protein, acidity (expressed as lactic acid) and pH were analysed.

The performance of the starches in the finished products was assessed by a panel of untrained panelists through an unstructured scale;



in this scale, the panelists expressed their level of satisfaction for each product (Pedrero and Pangborn, 1989).

### Experimental design and statistical analysis

The statistical design was a one factor balanced incomplete block design (Montgomery, 1991). In this design the blocks were the panelists. The result of the sensory tests were analysed through the appropriate analysis of variance to determine significant treatment differences ( $P < 0.05$ ), then Duncan's Multiple Range Test was applied for the mean multiple comparison. The statistical package used for the computations was Statgraphics 5.1.

## Results and Discussion

The physicochemical characteristics of *X. violaceum* native and acetylated starches used in the food products are given in Table 1. The swelling power and solubility increased with the increase in degree of substitution. This normally occurs in the acetylation reactions due to the weakness of the granules (Smith, 1982). The degree of substitution was 0.03 and 0.06 for the starches acetylated at 3.6 and 7.2% acetic anhydride, respectively. It was also observed that the syneresis problem decreased as an indication that the percentage of acetyl groups increased. This is very important for food products that will be stored for long periods under refrigeration or freezing temperatures.

**Table 1** Physicochemical characteristics of *Xanthosoma violaceum* native and acetylated starches used in the food formulations

Physicochemical characteristics	Native starch	Starch acetate (3.6% acetic anhydride)	Starch acetate (7.2% acetic anhydride)
% Moisture	10.13	9.35	11.59
% Crude protein <sup>1</sup>	1.75	1.60	1.46
% Ash <sup>1</sup>	0.65	0.30	0.25
% Crude fat <sup>1</sup>	0.26	0.23	0.26
% Crude fibre <sup>1</sup>	0.23	0.05	0.15
N.F.E. (by difference) <sup>1,2</sup>	97.11	97.82	97.88
Colour (L*a*b*)	78.8, -0.9, 1.7	77.6, -0.9, 2.1	78.4, -1.1, 1.8
Gelatinization temperature (°C)	75	72	70
Swelling power (90°C)	20.70	21.87	27.44
Solubility (90°C)	18.15	30.44	32.40
Size of the granule (microns)	4.2	—	—
Shape	poligonal	—	—
% Amilose	15.6	—	—
% Acetils	—	0.80	1.56
Degree of substitution	—	0.03	0.06

<sup>1</sup>Data reported in dry matter

<sup>2</sup>N.F.E. = 100 - (% moisture + % crude protein + crude fat + % crude fibre + % ash)

**Table 2** Physicochemical characterization of sausages elaborated with *Xanthosoma violaceum* native and acetylated starches

Type of starch	Percentage of starch	Moisture (%)	Proteins <sup>1</sup> (%)	Fat <sup>1</sup> (%)	Ash <sup>1</sup> (%)	Carbohydrates (by difference) (%)	Nitrites (ppm)
Native starch	7	77.25	11.23	3.66	1.38	6.48	24.20
Native starch	9	71.95	12.77	4.02	2.18	9.08	16.39
Starch acetate (3.6%)	7	77.80	10.43	3.77	1.44	6.56	13.76
Starch acetate (3.6%)	9	78.47	8.73	1.77	2.02	9.01	26.63
Starch acetate (7.2%)	7	77.13	11.11	3.71	1.54	6.51	22.98
Starch acetate (7.2%)	9	76.08	10.39	2.17	2.32	9.04	31.81
Standards <sup>2</sup>	—	70 max	9.5 min	30 max	—	10 max	156 max

<sup>1</sup>The results are expressed in wet basis in order to be compared with the standards and are the average of two determinations

<sup>2</sup>Standards reported by the Mexican Food Legislation

## Physicochemical characterization and sensory evaluation of the food products

## Sausages

The physicochemical characteristics of the sausages prepared with *X. violaceum* native and acetylated starch are given in Table 2. The only parameter that was out of specifications was the moisture content which should have been 70% and this was probably due to a slight excess of ice used in the formation and the water probably was held by the phosphates present in the formulation.

The fat, protein, ash, carbohydrates, and nitrite contents were in accordance with the Mexican Food Standards.

The analysis of variance of the qualifications of the panelists indicated that there was a significant difference among the treatments ( $P < 0.05$ ). Sausages produced with starch acetates of 3.6 and 7.2% acetic anhydride at 9% concentration level had the highest qualifications. These samples were not significantly different and, therefore, for economic reasons it is highly recommended to use starch acetates with 3.6% acetic anhydride at 9% concentration level for sausage formulation. The samples with the lowest qualifications were those produced with native starch at 7 and 9% starch concentration.

## Creams

The Mexican Food Standards used for the comparison of these products correspond to powders for preparing desserts (Table 3). The total reducing sugars of the present products were above 60% and were in accordance with the standards. The ash content was half of the minimum required by the Mexican Food Legislation, but the standard corresponded to products very similar to the one assayed and, therefore, the formulation could vary slightly from one formulation to another.

There was no significant difference among the treatments, which meant that the panelists did not detect any difference among the desserts prepared with the different types of starch. Therefore, the sensory characteristics for this sort of product could be achieved with any of the starches assayed. However, all products were stored for a 20-day period at 7°C in order to observe the stability of the starch in terms of water expulsion (syneresis), and it was noticed that the desserts prepared with starch acetates (acetylated at 7.2% acetic anhydride) at 7 or 9% concentration, did not present syneresis during the storage period and, therefore, starch acetates acetylated with 7.2% acetic anhydride and 7% starch concentration are recommended for this type of product.

**Table 3** Physicochemical characteristics of the creams prepared with different type and starch concentration

Type of starch	Percentage of starch	Moisture (%)	Proteins <sup>1</sup> (%)	Ash <sup>1</sup> (%)	Total reducing sugars <sup>1</sup> (%)
Native starch	7	64.38	3.99	1.71	64.09
Native starch	9	65.02	4.32	1.77	66.15
Starch acetate (3.6%)	7	65.61	4.51	1.66	65.51
Starch acetate (3.6%)	9	64.39	4.69	1.57	61.81
Starch acetate (7.2%)	7	65.69	4.87	1.66	60.80
Starch acetate (7.2%)	9	65.38	4.88	1.56	64.90
Standards <sup>2</sup>	—	2 min	—	3 min	60 min

<sup>1</sup>The results are expressed on dry basis and are the average of two determinations

<sup>2</sup>Standards from the Mexican Food Legislation

**Table 4** Physicochemical characteristics of the ice creams elaborated with *Xanthosoma violaceum* native and acetylated starch

Type of starch	Percentage of starch	Proteins <sup>1</sup> (%)	Total solids <sup>1</sup> (%)	Acidity (%; expressed as lactic acid) <sup>1</sup>	pH <sup>1</sup>
Native starch	1	2.70	47.76	0.224	6.25
Native starch	1.5	2.83	43.41	0.226	6.21
Starch acetate (3.6%)	1	2.66	44.60	0.222	6.28
Starch acetate (3.6%)	1.5	3.77	43.43	0.217	6.22
Starch acetate (7.2%)	1	3.71	43.24	0.220	6.27
Starch acetate (7.2%)	1.5	3.71	41.49	0.221	6.24
Standards <sup>1</sup>	—	2.5-4	28 min	0.126-0.224	6.28-6.40

<sup>1</sup>Standards from the Mexican Food Legislation

The desserts prepared with native starches presented syneresis after 13 days of storage.

#### Ice cream

The protein content, total reducing sugars, acidity, and pH of the ice creams are given in Table 4. All the parameters analysed were in accordance with the Mexican Food Legislation. There was no significant difference in the degree of satisfaction among the different types and concentrations of the starches used in the formulations. As in the case of the desserts, this means that any of the starches assayed could be used for these kinds of products. Nevertheless, for long storage periods under refrigeration or freezing temperatures, starch acetates are highly recommended because they do not present syneresis problems.

#### Conclusions

The majority of the physicochemical characteristics of the processed products were in accordance with the Mexican Food Legislation.

The evaluation of *X. violaceum* native and acetylated starch performance for sausages, creams, and ice creams production by sensory evaluation, demonstrated that the best sausages chosen by the panelists were those produced with starch acetates at 3.6 and 7.2% acetic anhydride at 9% starch concentration.

For creams and ice creams the adequate sensory characteristics can be achieved with any of the starches assayed. However, for commercial uses, starches acetates are highly recommended for those products that will be stored for long periods at low temperatures, because they do not present syneresis problems.

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# Development of sweetpotato cultivars for new processing use in Japan

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Sweetpotato [*Ipomoea batatas* (L.)] is one of the most important upland crops in southern Japan. Although its processing use has been mainly directed towards the starch industry, the starch from imported corn is replacing sweetpotato starch because of its lower price. Sweetpotato, known as an environmentally friendly crop, can grow under many unfavourable conditions such as drought and typhoon. It is important to identify new processing fields in order to upkeep sweetpotato production. Cultivar 'Joy White' was released in 1994 for the alcohol industry. This has a high starch content and no beta-amylase activity. Cultivar 'Ayamurasaki' was released in 1995 for the colorant industry. This has a high anthocyanin content and high dry matter content. Another new cultivar 'J-Red' was released in August 1997 for the juice industry. This has a high beta-carotene content and low dry matter content. Coloured powder or paste from sweetpotato will become new materials with natural colour and rich nutrition for processing food. In the near future, the use of the sweetpotato top will become more important from the view of nutrition and function. Research has just begun to select new lines for use in Japan.

Keywords: Sweetpotato; Beta-amylase; Anthocyanin; Beta-carotene

Sweetpotato [*Ipomoea batatas* (L.)] is one of the most important upland crops in southern Japan (Kyushu) and Central Japan (Kanto), because of its high dry matter content and great tolerance to inferior environmental conditions, such as drought, typhoon, and concentrated rainfall, which have been damaging the other main summer crops. Moreover, sweetpotato is recognized as an environmentally friendly crop because of the low input of fertilizers and agricultural chemicals in its cultivation.

Sweetpotato has been used in the starch industry for a long time, especially in Kyushu, but the agreement to the free trade of agricultural products was detrimental to sweetpotato starch, because it cost twice as much as corn starch.

Since no alternative crops can be found to take the place of sweetpotato which can guarantee stable production to the farmer, it is necessary to release new sweetpotato cultivars in order to develop new products or new processing techniques.

## 'Joy White' for 'Shochu' Alcohol Production

In Kyushu, 'Shochu' alcohol production is one of the largest markets for sweetpotato processing. The favourite characteristic of sweetpotato 'Shochu' is a rich and sweet flavour, but the sweetpotato scent which is retained in the alcohol is not liked by consumers who live in the

large city. Therefore, new cultivars were developed with only favourite flavours in 'Shochu' alcohol together with the brewing company's cooperation in 1990.

'Joy White', a new cultivar for sweetpotato 'Shochu' alcohol production, was released by the Sweetpotato Breeding Laboratory (KSBL) of Kyushu National Agricultural Experiment Station (KNEAS) in 1994 (Yamakawa *et al.*, 1995). It was tested throughout the south-western part of Japan as a breeding line 'Kyushu No. 108' and officially registered as 'Sweetpotato Norin No. 46' by the Ministry of Agriculture, Forestry and Fisheries (MAFF).

'Joy White' is a progeny from a cross between 'Kyushu No. 76' and 'Kyushu No. 89' conducted in 1983 at the Ibusuki Branch of the station. Both parents have well-shaped roots, high starch content, resistance to root knot nematode (*Meloidogyne incognita* Chitwood), and root lesion nematode (*Pratylenchus coffeae* Goodey).

'Joy White' has medium sprouting ability and is a slightly prostrate plant type. The top leaves are light green. The mature leaves are green, ribbed, and triangular. The vines are slightly slender with medium internode length. There is slight anthocyanin accumulation on the veins, but no accumulation on the nodes. Storage roots are uniformly fusiform and well-shaped with white skin and flesh colour.

As shown in Table 1, the yield of 'Joy White' is 16 t ha<sup>-1</sup> and lower than that of 'Koganesengan', but similar to that of

**Table 1** Yield and other characteristics of 'Joy White' on standard culture from 1991 to 1993

Characteristics	Name of cultivars			LSD <sub>0.05</sub>
	Joy White	Satsumahikari	Koganesengan	
Root yield (t ha <sup>-1</sup> )	16.1	17.1	19.4	3.02
No. of roots per hill	2.5	2.4	2.8	0.30
Root size (g)	165.0	191.0	184.0	31.20
Dry matter content (%)	37.2	32.6	33.7	0.78
Starch content (%)	24.9	20.9	22.7	0.39
Colour value of starch (L*)	92.7	91.3	91.7	0.90
After steaming roots				
Water soluble solid (Brix %)	2.7	2.3	4.7	0.53
Polyphenol content (mg 100 g <sup>-1</sup> )	57.0	56.0	51.0	19.60
Resistance				
Root knot nematode	Strong	Strong	S. weak	
Root lesion nematode	S. strong	Medium	S. weak	
Black rot	Medium	Medium	S. weak	
Storage ability				
	S. high	Medium	S. low	

In resistance and storage ability, "S" is "Slightly"

'Satsumahikari'. The starch content of 'Joy White' is 24.9% and higher than that of 'Koganesengan'. The steamed roots have a low sugar content of 2.7% and they are supposed to be deficient in beta-amylase activity. The polyphenol content in steamed roots was 57 mg 100 g<sup>-1</sup> FW which is a good characteristic for food processing. The starch of 'Joy White' has an L\* value of 92.7 and a brighter colour than that of 'Koganesengan'.

'Joy White' possesses medium resistance to black rot (*Ceratocystis fimbriata* Ell. & Halst), high resistance to root knot nematode, and strong resistance to root lesion nematode. The storage ability of the roots is good throughout the winter.

'Shochu' alcohol produced on the small (5 kg roots), medium (100 kg roots), and large (1000 kg roots) scale were evaluated for their qualities by sensory test. 'Shochu' alcohol from 'Joy White' gained the best mark because of more fruity and lighter taste than the usual sweetpotato alcohol (data not shown).

Recent studies about starch ageing revealed several interesting characteristics of the starch from 'Joy White', and special noodles will be made from the white powder of this cultivar in the near future.

### 'Ayamurasaki' for Anthocyanin Production

The colorant industry expects that a natural red colour market will have greater market appeal compared to the artificial red, because generally, consumers prefer natural food ingredients to artificial ones. Natural red colour is extracted from

insects, micro-organisms, and plants such as red cabbage and red beet. About 10 years ago, sweetpotato ('Yamagawamurasaki') with anthocyanin pigment in roots was found in Japan. Although the colour quality and stability of this sweetpotato anthocyanin is as high as that of red cabbage, the anthocyanin content in roots of 'Yamagawamurasaki' is not high enough for industrialization and its yield performance is extremely low. Therefore, new cultivars with high anthocyanin content and high yielding ability were developed by the author and colleagues in cooperation with a colorant company in 1990.

'Ayamurasaki' is a newly released cultivar with extremely a high anthocyanin content, developed by KSBL in 1995 (Yamakawa *et al.*, 1997). It was tested throughout Japan as a breeding line 'Kyushu No. 113' and officially registered as 'Sweet Potato Norin No. 46' by MAFF.

'Ayamurasaki' is a progeny from a cross between 'Kyushu No. 109' and 'Satsumahikari' conducted in 1988 at Ibusuki Branch of the station. 'Kyushu-109' originates from the indigenous cultivars 'Yamagawamurasaki' and 'Chiranmurasaki', both producing roots with purple flesh. 'Satsumahikari' is a non-sweet cultivar lacking beta-amylase activity.

'Ayamurasaki' has medium sprouting ability and prostrate plant type. The top leaves are purplish brown. The mature leaves are green and lobed. The vines are slightly slender with slightly short internode length. There is slight anthocyanin accumulation on the veins and deep accumulation on the nodes. Storage roots are elongated fusiform with uniform good shape and dark-purple skin. The flesh is uniformly deep purple.

As shown in Table 2, the yield of

'Ayamurasaki' is 16 t ha<sup>-1</sup> and higher than that of 'Kokei No. 14' and 'Kyushu No. 109'. The starch content is 20.9% and higher than that of 'Kyushu No. 109'. The steamed roots have a normal sugar content of 3.9%. The colour value based on the absorption coefficient (Odaka, 1994) is 9.2 and is about 1.5 times as high as that of 'Kyushu-109' and about 4 times that of 'Yamagawamurasaki'.

'Ayamurasaki' displays intermediate resistance to black rot and root lesion nematode, and is slightly resistant to root knot nematode. The storage ability of the roots is slightly good throughout the winter.

In KNEAS, the function of purple coloured sweetpotato has been studied (Suda *et al.*, 1997). This research suggests much promise for sweetpotato.

Anthocyanin extracted from roots with purple flesh is used for confectionery and various foods as a natural colorant. A deep purple powder and paste made of this cultivar is also used as materials for bread, noodles, and as a snack food.

### 'J-Red' for Sweetpotato Juice Production

Carrot juice which is rich in beta-carotene has acquired more popularity in the last 10 years than tomato juice because of its better taste. There are several sweetpotato cultivars which contain more beta-carotene than carrot (Takahata *et al.*, 1993). In order to produce juice with high quality from orange-coloured

sweetpotato, it is necessary to reduce the sweetpotato scent and colour degradation which is called browning. New cultivars with high beta-carotene content, low colour degradation, low dry matter content, and high-yielding ability were therefore developed by the author and colleagues in cooperation with the university and a juice company in 1992.

'J-Red' is a newly released cultivar with a low starch and high beta-carotene content, developed by KSBL in 1997 (Yamakawa *et al.*, in press). Its performance was evaluated in the prefectural agricultural experiment stations, food processing companies, and universities as a breeding line 'Kyushu No. 120', and officially registered as 'Sweetpotato Norin No. 49' by MAFF for food processing use, especially for sweetpotato juice.

'J-Red' is a progeny from a cross between 'Shiroyutaka' and '86J-6' conducted at the Ibusuki branch of the station in 1988. 'Shiroyutaka' has a high yield and high starch content. It is resistant to black rot and root knot nematode. '86J-6' is a breeding line with a high carotenoid content, which originated from an open-pollinated population in the U.S.A. (Jones's population).

'J-Red' has a slightly inferior sprouting ability and prostrate plant type. The top leaves are light green. The mature leaves are light green and lobed. The vines are slightly slender with medium internode length. There is no anthocyanin accumulation on the veins and nodes. Storage roots are uniformly fusiform with good shape, orange skin colour, and orange flesh colour.

As shown in Table 3, the yield of 'J-Red' was 30 t ha<sup>-1</sup> and was much higher than that of

**Table 2** Yield and other characteristics of 'Ayamurasaki' on standard culture from 1991 to 1993

Characteristics	Name of cultivars			LSD <sub>0.05</sub>
	Ayamurasaki	Kyushu No. 109	Kokei No. 14	
Root yield (t ha <sup>-1</sup> )	16.5	12.4	13.8	1.81
No. of roots per hill	2.5	2.4	2.8	0.27
Root size (g)	150.0	191.0	167.0	39.20
Dry matter content (%)	33.7	30.0	31.1	1.24
Starch content (%)	24.9	20.9	22.7	0.88
Anthocyanin content (colour value of FAO standard)	9.2	5.8	0.0	2.82
After steaming roots				
Water soluble solid (Brix %)	3.9	4.6	4.6	0.48
Resistance				
Root knot nematode	S. strong	S. strong	S. weak	
Root lesion nematode	Medium	Strong	S. weak	
Black rot	Medium	Medium	Medium	
Storage ability	S. high	High	S. low	

In resistance and storage ability, "S" is "Slightly"

**Table 3** Yield and other characteristics of 'J-Red' on standard culture from 1993 to 1996

Characteristics	Name of cultivars			LSD <sub>0.05</sub>
	J-Red	Benihayato	Koganesengan	
Root yield (t ha <sup>-1</sup> )	29.7	17.1	25.8	1.37
No. of roots per hill	4.0	2.7	3.5	0.23
Root size (g)	187.0	165.0	193.0	10.50
Dry matter content (%)	25.5	25.1	35.4	0.92
Starch content (%)	15.1	13.1	24.3	0.68
Beta-carotene content (mg 100 g <sup>-1</sup> )	38.6	41.2	0.0	9.48
After steaming roots				
Water soluble solid (Brix %)	3.9	4.2	4.5	0.48
Resistance				
Root knot nematode	Strong	S. strong	S. weak	
Root lesion nematode	S. strong	Medium	S. weak	
Black rot	S. weak	Medium	Weak	
Storage ability				
	S. high	Medium	S. low	

In resistance and storage ability, "S" is "Slightly"

'Benihayato' and comparable to 'Koganesengan'. The dry matter content was 25.5% and the same as that of 'Benihayato', but the starch content was slightly higher than that of 'Benihayato'. The steamed roots had a slightly low sugar content of 3.9%, and, therefore, 'J-Red' is not suitable for table use. The beta-carotene content of roots was 38.6 mg 100 g<sup>-1</sup> DW and slightly lower than that of 'Benihayato'.

'J-Red' exhibits slightly weak resistance to black rot, but slight strong resistance to root lesion nematode, and strong resistant to root knot nematode. The storage ability of the roots is very good throughout the winter.

In the sensory test for juice, flesh roots were peeled and cut into dices. They were soaked in water for a few minutes before squeezing by an electric juicer for home use. The extracted juice was formulated with 50% water, 0.2% citric acid, and 5% sucrose. The sweetpotato juice made from 'J-Red' had better quality with a clear orange colour and good taste, compared with carrot juice (data not shown). 'J-Red' will be also used as a material for snack food or as a vegetable for cooking like potato because of its low starch content and relatively low sugar content.

### Using Sweetpotato Top as Processing Material

The sweetpotato top is used as livestock food or incorporated into the soil as green manure when sweetpotato is harvested. Many data showed that the sweetpotato top is rich in nu-

trition such as protein, vitamins, and minerals. New technologies to use the top more efficiently and economically as materials for food processing have to be developed.

In KNEAS, a new research project has started to develop new cultivars, new production methods, and new processing techniques for sweetpotato top, and to estimate the possible effects on health.

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## Poster

# Polysaccharides of lotus root

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The lotus (*Nelumbo nucifera* Gaertn.) is a crop of local importance in south-east Asia, both for the starchy roots and for the nut-like seeds. The monosaccharide composition of whole lotus root and extracted polysaccharide fractions were analysed by anion exchange high performance liquid chromatography (HPLC). The major polysaccharide was starch but the lotus root also contained appreciable quantities of other non-starch polysaccharides. Lotus root flour was fractionated by sequential extraction with cold water, hot water, and 3% NaOH. Analysis of the fractions suggested the probable presence of a water-soluble arabino-galactan, mannan, and fructan. Lotus root is a useful raw material for starch production in China although commercial starch tested was found to contain arabinogalactan. In a model cooking for lotus root, the polysaccharides were extracted and those remaining in the tissue were quantified. The texture of lotus roots was analysed and the fibrous cellulosic nature of the root limited softening after cooking. Lotus seeds were also tested and found to have a predominantly starchy endosperm with the presence of pectic type polysaccharides being indicated.

Keywords: Lotus; *Nelumbo nucifera*; Non-starch polysaccharides; Arabinogalactan; Texture

The lotus (*Nelumbo nucifera* Gaertn.) is a versatile dicotyledonous plant widely cultivated in southern China and also in south-east Asia. The plant has many uses. The starchy root is a useful food and a raw material for commercial starch production in China. The seeds are used in soup and sweet desserts. Both the dried root and the seeds are sweetened for sale as confectionery. The seed pod is used for medicinal purposes and the leaves are used in food preparation for wrapping rice before steaming. Lotus grows submerged and, therefore, cultivation is naturally restricted to areas with a plentiful supply of water.

The lotus root presents an unusual anatomy containing large voids which serve to assist gas exchange in the underwater tissues. These are supported by a rigid and fibrous wall which softens during cooking. The main nutritional component of the root is starch which has been the subject of previous studies (Lii and Chang, 1991; Suzuki *et al.*, 1992). There have been no reports on the non-starch polysaccharides of lotus and this study examines their occurrence with a view to continuing research on their nutritional and functional significance.

## Materials and Methods

Lotus roots and lotus seeds of Chinese origin, were purchased in Hong Kong. Roots for extraction were washed, sliced transversally, and homogenised in a blender. Roots for direct testing were washed and sliced. Seeds were dry-milled to flour for extraction.

## Polysaccharide extraction

Roots or seeds (approximately 100 g fresh weight) were extracted sequentially by first stirring for 6 h in 4°C water (400 mL). The homogenate was centrifuged at 6000 × g and the supernatant retained. This was called the cold-water soluble fraction (CWX). The pellet was then stirred for 6 h in 60°C water (300 mL), centrifuged as before, and the supernatant retained. This was called the hot water soluble extract (HWX). The pellet was extracted with 3% (w/v) NaOH (300 mL) giving a third extract which was alkaline (3NX) and a final residue. The 3NX pellet was then neutralised with hydrochloric acid (HCl) and dialysed overnight at 4°C. Polysaccharides were then collected from the aqueous extracts by ethanol precipitation. Ethanol was added to the extracts to a final concentration of 80% and the polysaccharides recovered by centrifugation. The dry weights of samples were obtained after oven-drying to constant weight at 80°C.

Further purification of root CWX was performed by centrifugation at 20 000 × g for 20 min and dialysis against water overnight at 4°C.

## Texture analysis

The texture of the whole root sections was analysed using a Stevens QTS tensile testing machine. Sections were tested transversely to the long axis of the root. A plate probe 4 mm thick and 25 mm long, was used for compression testing with a speed of 30 mm min<sup>-1</sup>.



## Polysaccharide composition

Samples were hydrolysed in 3% (w/w) H<sub>2</sub>SO<sub>4</sub> at 120°C, 103 kPa for 1 h. Hydrolysates were analysed by anion exchange high performance liquid chromatography (HPLC) using a Dionex HPLC system with a CarboPac PA-1 (4 mm × 250 mm) column, 4 mM isocratic elution, 1 mL min<sup>-1</sup>. An ED40 electrochemical detector fitted with a pulsed amperometric cell was used.

## Results and Discussion

The quantity of polysaccharides recovered during the extraction of the roots and seeds is given in Tables 1 and 2. The monosaccharide composition of the various fractions is given in

**Table 1** Recoveries of polysaccharides extracted from lotus tissues

	Root	Seed
Fresh wt (g)	96.04	100.60
Water (%)	79.60	10.35
Dry wt (g)	19.59	90.14
CWX dry wt (g)	4.06	34.13
Yield dry wt (%)	20.74	37.87
HWX	6.10	23.76
Yield dry wt (%)	31.14	26.36
3NX	0.21	6.83
Yield dry wt (%)	1.06	7.57
Residue	9.22	25.42
Yield dry wt (%)	47.07	28.20

**Table 2** Recovery of polysaccharides extracted during soup preparation

Soup	2	3	4
Fresh wt (g)	126.78	92.09	124.08
Dry wt	25.86	18.79	25.31
Soup dry wt	0.35	0.43	2.68
Yield Dry wt (%)	1.33	2.28	10.57

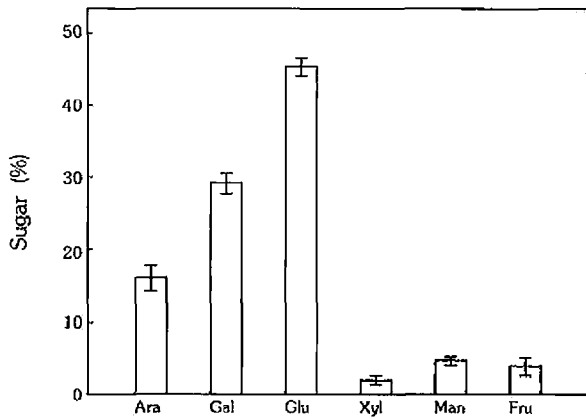
**Table 3** Relative per cent monosaccharide composition of polysaccharides extracted from lotus tissues as specified

		Fucose	Arabinose	Galactose	Glucose	Xylose	Mannose	Fructose
Root	CWX	0.00	4.83	2.69	88.29	0.46	0.69	3.05
	HWX	1.70	0.19	0.02	98.44	0.54	0.81	0.00
	3NX	1.05	0.64	1.51	97.86	0.00	0.00	0.00
Soup	2H	1.52	2.02	0.20	95.39	0.55	0.80	1.04
	3H	0.98	1.19	0.15	98.05	0.17	0.25	0.19
	4H	1.18	1.25	0.15	97.05	0.35	0.52	0.68
Seed	CWX	0.00	12.65	25.96	52.37	3.65	5.37	0.00
	HWX	0.00	4.22	7.94	80.61	1.33	5.90	0.00
	3NX	0.00	12.39	9.75	63.06	4.30	10.50	0.00

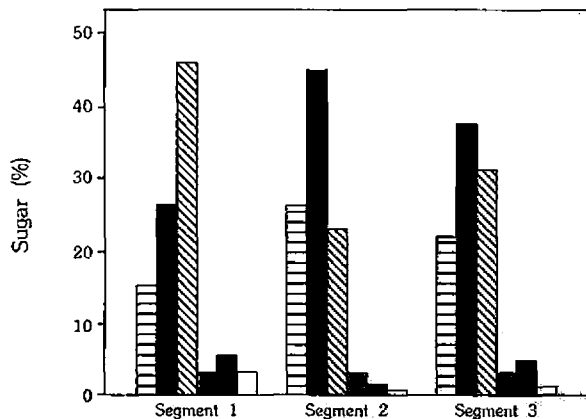
Table 3. The CWX contained appreciable quantities of arabinose and galactose, indicative of the presence of a mucilage-forming arabinogalactan proteoglycan as has been found in some monocotyledonous tubers. The occurrence of fructose suggests that fructans are present. These are low-molecular weight polymers used as reserve polysaccharides in a number of species but not previously known to occur in lotus. Mannose is probably derived from soluble galactoglucomannans and the xylose from soluble pectic polysaccharides but these are only a minor fraction in the root. The glucose present in the CWX can be attributed to readily soluble starch, but the bulk of the starch was removed in the HWX. The 3NX removed remaining starch and cell wall hemicellulosic polysaccharides, but in the root this did not reveal the presence of any further polysaccharides. In the preparation of soup from lotus roots, the readily soluble mucilage, polysaccharides, and fructans were extracted first with increasing amounts of starch being solubilised as cooking time was extended.

As the CWX fraction contains most of the root non-starch polysaccharides this fraction was investigated further. Purification of CWX by dialysis confirmed the presence of high molecular weight fructans (d.p. >10) as fructose was still present in the retained fraction (Figure 1). The further purification by high speed centrifugation also removed much contaminating starch and the final composition (Figure 1) indicates that the main component is an arabinogalactan. Three segments of a lotus root (with segment 1 being the oldest) were extracted separately to determine any change in the polysaccharides composition with the age of the root tissues (Figure 2). Overall, there was no profound difference among the different segments, although the younger tissues did appear to have proportionately more of the arabinogalactan present.

The seeds were characterized by much higher levels of non-starch polysaccharides with a great deal of soluble arabinogalactan being found in the CWX. Further work will be required to determine if there is any structural similarity between the seed and root CWX arabinogalactans. The alkaline fraction contained high levels of



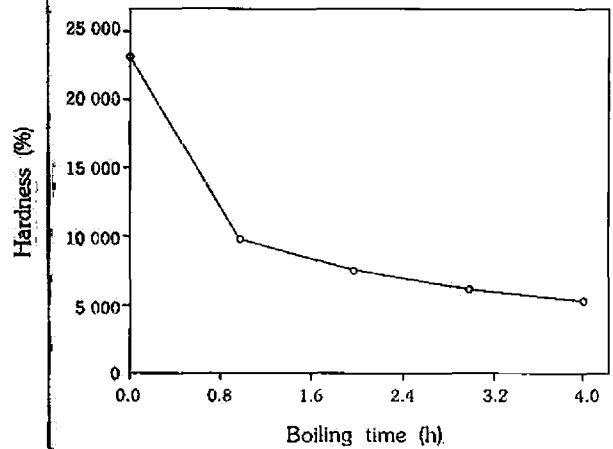
**Figure 1** Monosaccharide composition as a percentage of total sugar of purified cold water soluble fraction (CWX) from lotus roots. Values are the means of three samples



**Figure 2** Monosaccharide composition as a percentage of total sugar of different segments of a lotus root. Segment 1 is the oldest and segment 3 the youngest; (▨), arabinose; (■), galactose; (▩), glucose; (▧), xylose; (■), mannose; and (□), fructose

both xylose and mannose suggesting that glucomannans and xyloglucans may both be implicated as endosperm reserves in lotus seed. No fructose was recorded which is consistent with the role of fructans as easily mobilised short-term reserves found in root tissues.

The lotus root was softer around the periphery and had a harder central core, the periph-



**Figure 3** Change in texture of lotus root during cooking

eral tissues gave an average hardness of 202 g mm<sup>-2</sup>, and the central tissues 258 g mm<sup>-2</sup>. On cooking the root became much softer (Figure 3) with the greatest loss occurring in the first hour which was associated with the loss of the soluble polysaccharides. Extraction of the mucilage and pectic polysaccharides may facilitate rearrangement of hydrogen bonds within the cellulosic wall structure thus causing a loss of wall rigidity.

From this preliminary study, it is evident that the lotus has an interesting and varied non-starch polysaccharide composition. Further analysis will be required to confirm the structures of the polysaccharides postulated here and this will be combined with functional analysis of the physicochemical properties of the refined components individually and in mixed systems.

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## Poster

# Variability of non-starch polysaccharides in taro

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Although cultivated primarily for their starch content, the nutritional and functional properties of taro [*Colocasia esculenta* (L.) Schott] corms can be modified by the presence of non-starch polysaccharides. Twelve varieties from the Philippines, 14 varieties from the Solomon Islands, and 3 varieties from Hong Kong were assessed for their polysaccharide content other than starch. Several non-starch polysaccharides were present and a major fraction was the water-soluble arabinogalactan proteoglycan. Monosaccharide analysis of the whole flour from the corms also indicated the presence of xylose and mannose indicative of the presence of xyloglucan and glucomannans. High levels of arabinogalactan contributed to the mucilaginous character of taro corms. The functional consequences of the xyloglucan and glucomannan fractions were not established and are currently under investigation.

Keywords: Taro; *Colocasia esculenta*; Non-starch polysaccharides

Taro [*Colocasia esculenta* (L.) Schott] is primarily cultivated for the nutritional value of its starchy corm. Apart from starch the corm also contains non-starch polysaccharides which are usually identified as dietary fibre (Bradbury and Holloway, 1988). Based on their properties, these indigestible polysaccharides can be separated into soluble and insoluble dietary fibre. The soluble polysaccharides are found in the water-soluble mucilage which imparts a characteristic texture to taro corms. Insoluble polysaccharides are present as cell wall components. Taro corms and whole flours contain both these classes of polysaccharides which can appreciably modify their physical properties (Tagodoe and Nip, 1994). Taro products such as starch may also be contaminated with, or modified by, these polysaccharides (Moorthy *et al.*, 1993). Finally, the non-starch polysaccharides may themselves represent useful food additives (Lin and Huang, 1993).

The variation in non-starch polysaccharide content and distribution in samples of different taro varieties was examined. The purpose was not, however, to distinguish between different cultivars, but to establish the range of non-starch polysaccharides that can be found in taro. For the further development of taro, it is important to have more information on the distribution of different non-starch polysaccharides that will influence the functional properties of tuber products.

## Materials and Methods

### Production of taro flour

Taro corms were washed, peeled, sliced, and homogenized using a blender. The homogenate was suspended in 80% ethanol for 3–4 h to remove low molecular weight carbohydrates. Subsequently, the suspension was centrifuged at  $4000 \times g$  for 10 min and the pellet was dried at 40°C. The dried material was then milled into taro flour.

### Composition analysis of taro flour

Monosaccharides were obtained by total hydrolysis of polysaccharide samples in 3% (w/w)  $H_2SO_4$  at 120°C, 103 kPa for 1 h. Analysis of monosaccharides was performed using a Dionex high performance liquid chromatography (HPLC) system fitted with a CarboPac PA-1 (4 mm  $\times$  250 mm) column, and a 10  $\mu$ L sample loop, 4 mM NaOH isocratic elution, 1 mL  $min^{-1}$ . An ED40 electrochemical detector fitted with a pulsed amperometric cell was used.

### Varieties used

Varieties Liposnay (LP), Doho (DH), Lampagan (LA), Ngadaw (NG), Mindanao (MD), Arabian Gabi (AG), Aklaw (AK), Balet (BT), Paagaaga (PA), Rabok (RK), and Idchina (IC), from the Northern Philippines Root Crops Research and Training Centre and grown in Hong Kong, were used. Varieties A to N were supplied

from the Agriculture Research Division, Dodo Creek, Solomon Islands. Varieties HK, PL, and PS were collected in Hong Kong and grown at the Kadoorie Agricultural Research Centre in 1996 and 1997.

## Results and Discussion

The results of the monosaccharide composition analysis showed that the major sugar present was glucose derived from starch reserves and from cell wall cellulose. The other sugars were from the non-starch polysaccharides present in the corm. The most abundant non-starch polysaccharides are found in the cold water soluble mucilage of taro (Harris *et al.*, 1992; Jiang *et al.*, 1997) which contains an arabinogalactan proteoglycan that contains most of the galactose and all the arabinose in the corm. Galactose may also be found as a component of galactomannans, which together with glucomannans are found as reserve polysaccharides in several monocotyledon species and these may also represent a reserve in taro corms. These are cell-wall-related polysaccharides, and another cell wall polysaccharide xyloglucan, is the probable source of any xylose present. Xyloglucans are found as reserve polysaccharides in many legume seeds but have not been reported in monocotyledons.

The levels of non-starch polysaccharides, derived from the content of sugars other than glucose, varied from 7 to 23% of the total sugar composition. This represents an appreciable proportion of the corm reserves. The differences in levels of non-starch polysaccharides could arise from three scenarios: (i) they may represent genetic variation between the cultivars which have different capacities for polysaccharide synthesis and deposition; (ii) they may be a consequence of different environmental and field conditions which may favour the deposition or synthesis of particular sets of polysaccharides; and (iii) the differences may be developmentally related arising from different rates of growth of corms before harvest. It is possible that the cell-wall-associated polysaccharides indicated by xylose and mannose contents, may be related to the proportion of cell wall material present in the corm. Tissues which had smaller cells with thicker walls would contain less starch and

this may be found in younger tubers or those which had a slower rate of growth. Nutritionally, maximization of the starch content is the principal aim, however, the presence of other non-starch polysaccharides can have consequences for the value of the crop by influencing the cooking quality and mouthfeel as well as the handling of processed tuber products. The cell-wall-associated polysaccharides may also affect the texture and storability of the corms.

Future research will attempt to distinguish amongst these possibilities by studying the changes in non-starch polysaccharides contents of taro corms grown in controlled conditions and examined at different ages. The effects of non-starch polysaccharides on starch rheology and texture are also currently under investigation in the authors' laboratory.

## Acknowledgements

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## Poster

# Measurement of amylose content in sweetpotato starch by near infrared analysis

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Amylose content of sweetpotato [*Ipomoea batatas* (L.)] was predicted by near infrared analysis method using dry powder made from fresh sweetpotato roots. After smoothing treatment of 1000–2500 nm spectrum, the fittest calibration equation was obtained from selecting three wavelengths. A standard error of prediction (SEP) and a correlation coefficient ( $r$ ) of this calibration equation were 1.110 and 0.8528, respectively. The predicted amylose content ranged from 11.1 to 16.9% in 173 breeding lines. Ten lines with low amylose and high amylose content were each selected.

Keywords: Sweetpotato; Starch; Amylose; Sweetpotato powder; Near infrared analysis

Sweetpotato [*Ipomoea batatas* (L.)] has been used for table use as well as for starch materials in Kyushu, Japan. Most of the sweetpotato starch has been used as food materials and syrup after chemical or enzyme treatment. However, sweetpotato starch has been decreasing in production since the importation of corn starch, which is relatively cheaper. Therefore, new uses of sweetpotato have to be developed. There are some processed forms of sweetpotato in Japan such as 'karinto', which is fried sweetpotato and 'hoshi-imo', which is dehydrated steamed sweetpotato. In addition, paste, flakes, and granules made from the mashed sweetpotato have been used in Japanese cakes. Recently, sweetpotato powder was developed by Kyushu National Agricultural Experimental Station (Yakushido *et al.*, 1996) and the powder is beginning to be used for making noodles, breads, and cakes.

Starch mainly consists of amylose and amylopectin, and sweetpotato starch contains about 20% amylose (Noda *et al.*, 1992). It is generally said that a low amylose content of starch enhances the taste of foods and increases viscosity, for example, rice with desirable taste has a low amylose content and waxy rice (no amylose) has a higher viscosity. On the other hand, starch with a high amylose content is necessary for producing biodegradable plastics, because of its easy gelatinization and formation. Using starch for biodegradable plastic has been studied in some countries (Noguchi, 1992; Iwanami and Vemura, 1993). Sweetpotato amylose has a larger molecular weight and a higher viscosity

than corn starch (Takeda *et al.*, 1986, 1988). This characteristic would make it suitable for use in the manufacture of biodegradable plastic (Shiotani, pers. commun.). Amylose content in sweetpotato varieties was examined by the authors and researchers at Kagoshima University (Kagoshima prefecture, Japan) and a relatively low amylose variety was obtained. Kyukei 89376-12 is a variety with the lowest amylose content [11.3%, in the blue value (BV) adsorption method] among sweetpotato varieties examined so far (Kitahara *et al.*, 1996a).

Near infrared (NIR) analysis was developed to measure the important components in various botanical sources (Starr *et al.*, 1981). There are few reports about NIR analysis on sweetpotato (Lu and Sheng, 1990; Katayama *et al.*, 1996), but amylose content has not yet been researched. Adsorption of iodine-starch complex (BV method) (Takeda *et al.*, 1986) and gel filtration chromatography (Kitahara *et al.*, 1996b) have been used to examine amylose content, but those methods are not convenient for use in breeding programmes. Near infrared analysis was adopted, to predict amylose content of sweetpotato starch using sweetpotato powder and selected varieties with low or high amylose content.

## Materials and Methods

The sweetpotato cultivars used in this experiment were grown at Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki

prefecture in Japan in 1996. The planting density was 71 cm × 35 cm (approximately 40 000 plants ha<sup>-1</sup>) and chemical fertilizer (N:P:K) in the ratio of 6:8:12 at 600 kg ha<sup>-1</sup> and compost at 20 t ha<sup>-1</sup> were applied in the experimental field. A preliminary yield trial was carried out in two test fields (A and B). These were transplanted on 14 May and harvested on 2 October. Breeding lines in the third year of selection were transplanted on 10 May and harvested on 24 September.

### Sweetpotato powder

Sweetpotato powder was used for NIR analysis. Twenty-five breeding lines from the preliminary yield trial and 148 breeding lines in the third year of selection were prepared for sweetpotato powder. The powder was made as follows: five tubers per variety were cut into small rectangles and dried at about 50°C overnight, milled by a blender, and sieved in a 200-µm mesh.

### Starches

Sweetpotato starch from 25 lines in the preliminary yield trial was prepared for amylose content analysis. Starch was isolated as follows: an amount of 100 g of 4 sweetpotato roots cut into small rectangles was crushed with 160 mL of water for 90 s and sieved by 150-µm mesh. Isolated starch was dried for two days at room temperature and was further dried at 100°C. Starch characteristics such as amylose content, average granule size, digestibility, onset (T<sub>o</sub>) and peak temperature (T<sub>p</sub>), gelatinization heat (H), and X-ray diffraction patterns were also measured.

### Amylose content

Amylose content in the starch of 25 breeding lines from the preliminary yield trial were measured by BV method. The BV at 680 nm of the absorption spectra of iodine-starch complexes was determined (Noda *et al.*, 1992). The amylose content in the starch was estimated from the BV, using the BVs of sweetpotato 'Koganesengan' amylose and amylopectin (Takeda *et al.*, 1986).

### Near infrared analysis

A few grams of sweetpotato powder was packed into the sample holder in a sample unit (MPC 3100, SHIMADZU) and the spectrum of sweetpotato powder was measured using a spectrophotometer (UV 3100S, SHIMADZU) with the range of 600–2500 nm, a step of 2 nm, a slit of 6 nm, a middle speed, and one repeat. Multiple regression analysis software (multiple regression analysis system of UV 3100 series) was used for establishment and statistical analysis. Calibration equations were made for the spectra of powders from 25 breeding lines in the preliminary yield trial (grown in

field A) with the range of 1000–2500 nm, and accuracy of the calibration equations was confirmed using the same varieties grown in the Field B. Amylose content of 148 breeding lines in the third year of selection was predicted by using the selected calibration equation from their powder.

## Results and Discussion

### Calibration

In order to set up a calibration equation, the spectra for sweetpotato powders from 25 breeding lines grown in Field A were measured. Calibration equations were calculated by multiple regression analysis using 1 to 4 wavelengths to their amylose content examined by the BV method on pre-treatments to spectra, that is, non-treatment, a linear differentiation, second differentiation, and smoothing (Table 1). The amylose content for the 25 breeding lines ranged from 9.5 to 17.1 in Field A and from 9.2 to 18.6 in Field B. The results were consistent in ranking in both fields (data not shown). As seen in Table 1, a calibration equation selecting wavelengths of 1006, 1182, 1356, and 1892 nm on non-treatment showed the highest value of multiple correlation coefficient (R) of 0.9521 and the lowest value of standard error of calibration (SEC) of 0.576.

**Table 1** Multiple correlation coefficient (R), standard error of calibration (SEC), and selected wavelength for calibration equations after pretreatment of spectra

Pretreatment	R	SEC	Selected wavelength (nm)			
<b>No-pretreatment</b>						
Wavelength no.						
1	0.5831	1.428	1006			
2	0.8516	0.942	1006	1182		
3	0.9199	0.721	1006	1182	1356	
4	0.9521	0.576	1006	1182	1356	1892
<b>A linear differentiation</b>						
Wavelength no.						
1	0.5909	1.418	1012			
2	0.7512	1.186	1012	2204		
3	0.8548	0.954	1012	2204	1964	
4	0.9086	0.787	1012	2204	1964	1892
<b>Second differentiation</b>						
Wavelength no.						
1	0.5884	1.421	2254			
2	0.7689	1.149	2254	2352		
3	0.8604	0.937	2254	2352	1942	
4	0.9183	0.746	2254	2352	1942	2046
<b>Smoothing</b>						
Wavelength no.						
1	0.5557	1.461	1004			
2	0.8469	0.955	1004	1164		
3	0.8840	0.860	1004	1164	1312	
4	0.9222	0.729	1004	1164	1312	2066

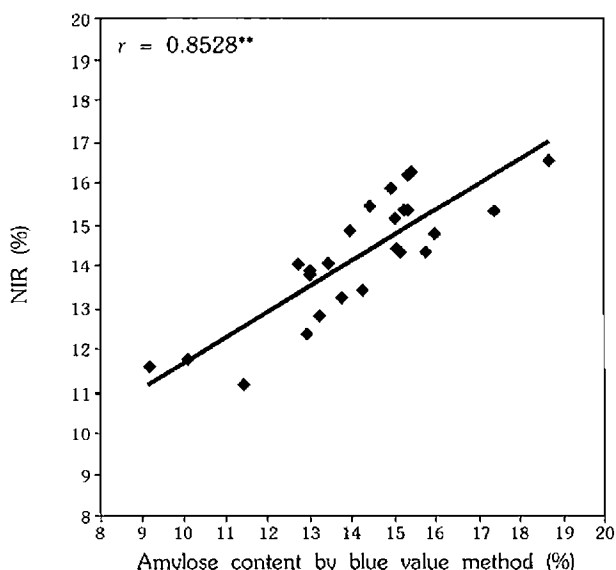
The accuracy of calibration equations was assessed using powders from the same sweetpotato lines grown in Field B. Some calibration equations showed better correlation coefficients (*r*), standard errors of prediction (SEP), and the mean difference between quantitative values and predicted values (BIAS) than that mentioned above (Table 2). The calibration equation using three wavelengths (1004, 1164, and 1312) after smoothing treatment, showed the highest value of the simple correlation coefficient (*r* = 0.8528) and the lowest value of SEP (1.110), and BIAS (0.084) (Table 2, Figure 1). Therefore, this equation was selected as the best calibration equation for prediction of amylose content. This calibration equation was: amylose (%) = -440.18 × (A1004) + 751.23 × (A1164) - 297.82 × (A1312) + 8.52.

### Prediction and selection

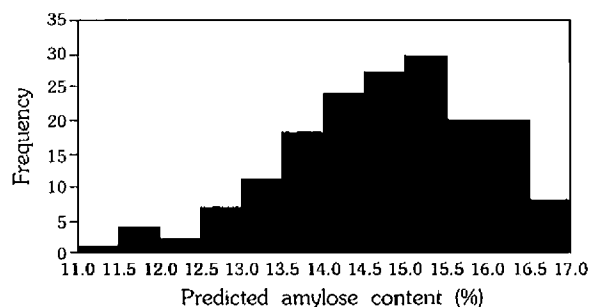
The amylose content of the additional 148 breeding lines was predicted using the selected calibration equation by NIR. The frequency distribution for amylose content of 175 breeding lines (containing those of 25 breeding lines for calibration) are shown in Figure 2. Predicted amylose content ranged from 11.1 to 16.9% and overall average was 14.7%. Ten varieties having low amylose content and 10 varieties having high amylose content were selected (Table 3).

**Table 2** Simple correlation coefficient (*r*), standard error of prediction (SEP), and mean difference between actual values and predicted values (BIAS) for calibration equations

Pretreatment	<i>r</i>	SEP	BIAS
No-pretreatment			
Wavelength no.			
1	0.7132	1.631	0.327
2	0.7741	1.303	-0.239
3	0.7288	1.478	-0.438
4	0.7126	1.638	-0.495
A linear differentiation			
Wavelength no.			
1	-0.0334	1.995	-0.428
2	-0.0672	1.919	-0.657
3	0.0560	2.041	-1.094
4	0.1495	1.867	-1.125
Second differentiation			
Wavelength no.			
1	0.3226	2.176	-0.575
2	0.4446	2.401	-0.608
3	0.4322	2.436	-0.398
4	0.5323	2.384	-0.254
Smoothing			
Wavelength no.			
1	0.6995	1.654	0.339
2	0.8520	1.096	0.015
3	0.8528	1.110	0.084
4	0.8141	1.196	0.156



**Figure 1** A simple correlation between predicted amylose content by near infrared analysis using powder and by quantitative amylose content blue value method using starch



**Figure 2** The frequency distribution for predicted amylose content of 175 breeding lines by near infrared analysis

**Table 3** Lines with higher amylose and lower amylose content selected from 173 breeding lines

High amylose lines	Predicted amylose content (%)	Low amylose lines	Predicted amylose content (%)
Kyukei 91162-21	15.3	Kyukei 92065-6	11.1
Shiroyutaka	16.5	Kyukei 92071-13	12.3
Koganesengan	16.9	Kyukei 92072-1	11.6
Kyukei 93099-3	16.8	Kyukei 92072-18	11.8
Kyukei 93099-6	16.4	S179	12.3
Kyukei 93104-6	16.7	Kyukei 93082-3	12.7
Kyukei 93165-20	16.6	Kyukei 93093-6	11.5
Kyukei 93118-6	16.3	Kyukei 93139-2	11.6
Kyukei 93119-4	16.9	Kyukei 93139-5	12.8
Koukei 14	16.5	Kyukei 93141-4	12.8

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## Poster

# Some evidences for involvement of jasmonic acid in storage root formation in sweetpotato

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The relationship between jasmonic acid (JA) a plant growth regulator and the formation of storage root in sweetpotato [*Ipomoea batatas* (L.)] was investigated. First, JA was identified from sweetpotato using electron-impact mass spectrometry (EI-MS). The levels of JA were high in sweetpotato compared with those in related wild species (*I. trifida*) that formed no storage root. *In vitro* application of JA from the surface of sweetpotato roots induced swelling of the cortical cells but it did not induce storage root formation. However, when JA was applied from the root end *in vitro*, the formation of storage root was promoted. Jasmonic acid application for top organs of wild species that formed no storage roots also induced root swelling. The involvement of JA in storage root formation in sweetpotato is discussed.

Keywords: *Ipomoea batatas*; Jasmonic acid; Storage root formation; Sweetpotato

In sweetpotato [*Ipomoea batatas* (L.)], as well as in the other root crops, the formation of storage roots is one of the most important phenomena determining yield and dry matter production. Therefore, it is important to know the physiological factors involved in the process of storage root formation. Several studies were conducted and a relationship between some plant hormones, especially cytokinins and the storage root formation was suggested (Matsuo *et al.*, 1983; Nakatani and Komeichi, 1991a, b). The primary stimuli for storage root formation are, however, still obscure.

Recently, jasmonic acid (JA), which is distributed widely among higher plants and plays important physiological roles, has been recognized to be a plant growth regulator (Meyer *et al.*, 1984; Parthier, 1990; Koda, 1992). In some tuber crops, especially in potato, it has been shown that JA and its related compounds play primary roles in the tuberization process of potato (Yoshihara *et al.*, 1989; Koda *et al.*, 1991), yam (Koda and Kikuta, 1991), and some other tuber crops. On the other hand, information is still limited about the involvement of JAs in storage root formation in sweetpotato.

The objective of this study was to demonstrate the relationship between JAs and the development of storage root in sweetpotato.

## Materials and Methods

### Identification of jasmonic acid from sweetpotato

Sweetpotato cultivar, Koganesengan, was used as the material. The cuttings grown in a nursery bed were subjected to extraction. Cuttings were also planted to the experimental field and grown under conventional cultural condition for 67 days. The top organs of these plants were also used for extraction. A related wild species, *I. trifida* (H.B.K.) Don., 4X clone that formed no storage roots were grown under the same conditions, and the top organs were also used as the materials.

Plant materials were homogenized in 80% methanol (4°C) and extracted for 12 h in the dark (4°C). After filtration, methanol was removed *in vacuo*. The aqueous solutions were acidified by 1N HCl and partitioned with ethyl acetate. The acid ethyl acetate fraction was subjected to purification. In the purification process, JA activity was assayed by potato tuber-inducing activities using potato-stem-segment culture according to the method described by Koda and Okazawa (1988). The active substance was separated from the extract of sweetpotato cuttings by high performance liquid

chromatography (HPLC). First, a silica gel ODS column was used with a solvent system which consisted of 60% methanol and 0.1% acetic acid. The resulting active fraction was further purified by a NOVA PAK C18 column eluted by 50% methanol and 0.1% acetic acid. Finally, the active substance was isolated by a NOVA PAK C18 column eluted by 30% acetonitrile and 0.1% acetic acid. The isolated substance was identified by electron impact mass spectrometry (EI-MS).

### Effects of JA application on root development of sweetpotato *in vitro*

Cultivar Beniazuma (red skin colour) was used as the material. Two different tissue culture systems were used. One was designed as a tissue culture system in which JA was applied from the root surface. The stem segments grown *in vitro*, were placed on the top of mountain-shaped filter papers that were soaked in a medium containing half concentration of Murashige and Skoog medium ( $1/2$  MS) (Murashige and Skoog, 1962), 6% sucrose, and (or)  $10^{-4}$  M JA. The experiment was replicated six times. After three months culture, the root number, length, and diameter, and shoot length were measured, and the thickest parts of roots were fixed by formaldehyde-acetic-acid alcohol (FAA). The fixed root segments were embedded into Acritron and cut into 5- $\mu$ m sections. The anatomical observations of roots were done after staining by safranin and fast green FCF.

In the other tissue culture system, JA was applied from the root base end. After sterilization of stem segments, they were incubated on a medium containing 3% sucrose and 0.2% gellan gum at 28°C in the dark for one week. The roots from these segments were cut and cultured in a liquid medium containing  $1/2$  MS, 5% sucrose, and 0.2% casamino acid at 25°C in the dark with shaking. After 4–5 weeks, roots with a length above 15 cm were transferred into a culture system. The cut-end of a root was inserted into 14 mL of medium solidified by 0.2% gellan gum in a 10-mL Erlenmeyer flask and the root tip was dipped into 50 mL of liquid medium in a 300-mL bottle. The basic composition for both solid and liquid medium consisted of  $1/2$  MS, 6% sucrose, and 0.2% casamino acid. Indole acetic acid (IAA) and (or) JA was added to the solid medium. Benzyladenine (BA) and (or) abscisic acid (ABA) was added to the liquid medium. They were placed at 25°C in the dark. Four replications were prepared per treatment. After six or seven months culture, the maximum root diameter was measured. Anatomies of the thickest parts of the roots were observed by the same method described above.

### Effects of JA treatment on root development of wild *Ipomoea*

The same clone of *I. trifida* used for the JA extraction was used as the test material. The cuttings were planted into porous pots (25 cm  $\times$  20 cm H) on 6 June 1991. Jasmonic acid treatments were done at the concentration of 0, 0.5, 5, 50, 500, and 5000 ppm. Fifty-five and 70 days after planting, the JA solutions containing 0.04% of Tween 20 and small amounts of DMSO were sprayed for the top organs.

One hundred days after planting, the diameters of the thickest roots and top weight were measured. The thickest parts of roots were fixed, prepared, and observed in the same way as described above.

## Results

### Identification of jasmonic acid from sweetpotato

Potato-tuber-inducing activities that were indicative of the presence of JAs of the extracts from sweetpotato and a related wild species are shown in Table 1. The activities were found in aqueous (AQ) and in acid ethyl acetate (AE) fractions from top organs of sweetpotato. In the extract from the storage roots of sweetpotato, activity was found only in the AE fraction. In the extract from the top organs of the wild species, the activity was found only in the AQ fraction.

Acid ethyl acetate fraction from sweetpotato cuttings was subjected to further purification by HPLC. A single peak showed potato-tuber-inducing activity that had the same retention time with JA. This substance showed the same profile with authentic JA in EI-MS (Figure 1) and was therefore identified as JA. The JA content of top organs of sweetpotato was estimated about 200 mg kg<sup>-1</sup> by the peak area in HPLC.

**Table 1** Jasmonic acid activities in the extracts of sweetpotato and a related wild species assayed by potato-tuber-inducing activity

Plant	Organ	Ratio of tuberization (%)		
		Aqueous fraction	Acid ethyl acetate fraction	Neutral ethyl acetate fraction
Sweetpotato	Top organs <sup>1</sup>	50	46	0
(cv. Kogane- sengan)	Top organs <sup>2</sup>	23	17	0
	Storage roots <sup>2</sup>	8	36	7
<i>I. trifida</i> (4X)	Top organs <sup>2</sup>	13	0	0

<sup>1</sup>Cut-sprouts; <sup>2</sup>67 days after planting

Values are presented as the percentage of tuberized potato segments to total segments

The assay was done with adding 0.3 g equivalent of top organs and 1 g equivalent of storage roots to 1 mL assay medium

Effects of JA application on root development of sweetpotato *in vitro*

The effects of JA applied from root surface *in vitro* on root development after three months of culture are shown in Table 2. Root length was decreased by JA to about 20% of the control. On the other hand, root number was not changed and average root diameter was increased twice by JA treatment. The colour of roots in JA treatment was light red, and that in the control was white. In the roots of both treatments, primary cambia were not differentiated and the steles were highly lignified. Although the stele diameters did not differ, the root cortex width was larger in the JA treatment than in the control. Thus, the difference in root diameter between treatments was due to the swelling of cortical cells in the JA treatment.

The effects of some growth regulators applied from the cut end and (or) the tip of the root *in vitro* on the root development are shown in Table 3. Although the effects of growth regulators added to the medium were not so apparent throughout the experiments, the highest frequency of root swelling and the thickest diameter of the root were observed in the treatments of JA  $10^{-5}M$  in solid and BA  $10^{-6}M$  in liquid medium. The vagueness of the effect of growth regulators may be derived partly from the variation in physiological conditions of roots used as the materials.

Effects of JA treatment on root development of wild *Ipomoea*

The effects of JA treatment on the top weight and maximum root diameter of wild *I. trifida* are shown in Table 4. Top weight was highest in the treatment of 50 ppm JA and lowest in those of 500 ppm JA. Although the average maximum root diameter did not differ among the control and JA treatment of 0.5, 5, and 5000 ppm, JA treatment of 50 and 500 ppm increased the maximum root diameter significantly.

Cross sections of the thickest parts of the roots in control and JA treatments of 50 and 500 ppm are shown in Figure 2. In JA treatment of 50 ppm, the degree of lignification of

root xylem was lower and the size of parenchyma cells was larger than those in the other treatments. In JA treatment of 500 ppm, the degree of lignification of root xylem did not differ much from that in the control; the activ-

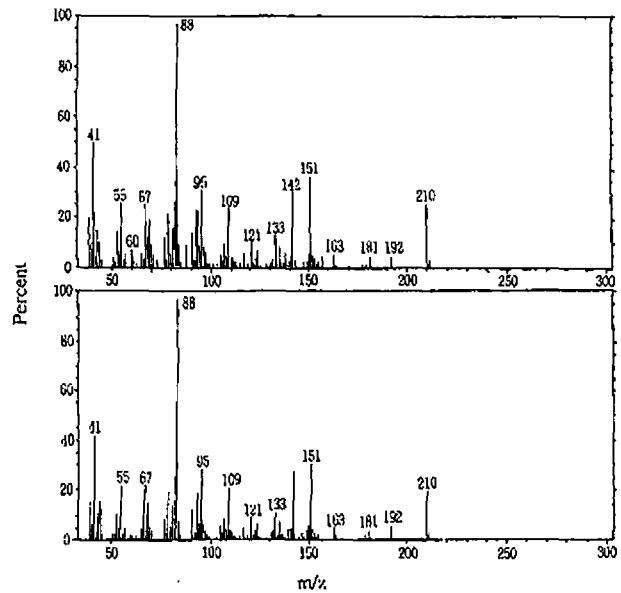


Figure 1 Electron impact mass spectrum of the potato tuber-inducing substance isolated from top organs of sweetpotato (lower) and authentic jasmonic acid (upper)

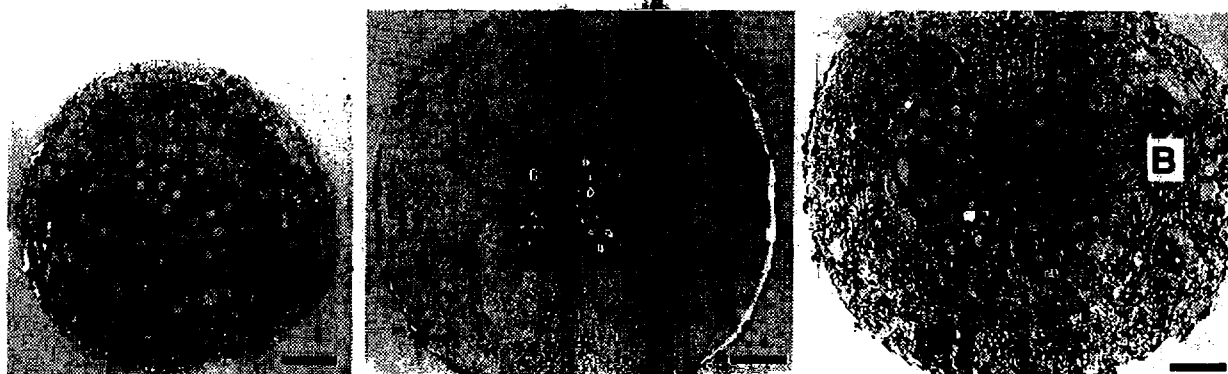
Table 2 Effects of jasmonic acid (JA) applied from root surface, on the growth and development of sweetpotato root after three months culture

Root characteristics	JA-free	JA $10^{-4} M$
Number of roots per segment	9.97±4.5	3.6±1.8
Total root length (cm segment <sup>-1</sup> )	50.5±28.3	9.8±5.3
Average root length (cm root <sup>-1</sup> )	30.3±20.1	5.5±3.6
Root diameter of the thickest root (mm)	0.66±0.12	0.95±0.10
Root stele diameter (mm)	0.22±0.08	0.25±0.05
Cortex width (mm)	0.19	0.35
Primary cambium	not observed	not observed
Stele lignification	highly	highly

Values are presented as the average ± standard deviation of the six stem segments

Table 3 Effects of growth regulators applied from root base end and tip on the growth and development of sweetpotato root after seven months culture

Solid medium (applied from root base end)	Liquid medium (applied from root tip end)	Number of thick roots (>2 mm φ)	Maximum root diameter (mm)
Hormone free	Hormone free	1	2.1
JA $10^{-5}M$	Hormone free	1	2.6
JA $10^{-5}M$	BA $10^{-6}M$	3	5.0
JA $10^{-5}M$ + IAA $10^{-8}M$	Hormone free	1	2.2
JA $10^{-5}M$ + IAA $10^{-8}M$	BA $10^{-6}M$	1	2.3
JA $10^{-5}M$ + IAA $10^{-8}M$	BA $10^{-6}M$ + ABA $10^{-7}M$	1	3.2



**Figure 2** Cross section of the thickest root of *Ipomoea trifida* (4X) treated with 0 (left), 50 (middle), and 500 (right) ppm jasmonic acid  
 B: phloic bundle  
 Bars in Figure show 0.5 mm

**Table 4** Effects of jasmonic acid (JA) application on top weight and maximum root diameter of *Ipomoea trifida* that forms no storage root normally

JA concentration (ppm)	Top weight (g plant <sup>-1</sup> )	Maximum root diameter (mm)
0	56.7 a	2.70 a
0.5	75.2 ab	2.61 a
5	52.3 a	2.66 a
50	82.6 b	3.31 b
500	41.5 a	3.30 b
5000	61.0 ab	2.79 a

Values are presented as means  
 Means within a column with similar letters are not significantly different according to Duncan's Multiple Range Test

ity of primary cambium was higher, and the size of phloem and cortical cells was larger than that in the control.

**Discussion**

Recently it has been shown that JAs are widely distributed among higher plants and play important roles as growth regulators (Meyer *et al.*, 1984; Parthier, 1990; Koda, 1992). In this study, JA was identified in sweetpotato top organs. It appears that the JA level observed here is sufficient for some physiological activity and is comparable with the JA level reported in other plants (Meyer *et al.*, 1984; Parthier, 1990; Koda, 1992). Although the potato tuber-inducing substances in the AE fraction from storage roots of sweetpotato were not identified, it seems likely that the active substance is JA or its related compounds. On the other hand, no activities were observed in the AE fraction from wild species that formed no storage roots. From these results, it may be surmised that JAs are involved in the formation of storage root in sweetpotato. Since intact sweetpotato plants form storage roots easily under normal condi-

tions, it is difficult to show the effects of JA on the formation of storage roots.

When JA was applied from root surface *in vitro*, the root diameters increased and root pigmentation was observed. Lowe and Wilson (1974) recognized that root pigmentation occurred at the same time as the formation of storage root during normal development. Anatomically, however, the swollen roots in JA treatment from root surface were different from the storage roots. Storage root thickens due to the increase in stele diameter by the active division of cambia (Togari, 1950; Wilson, 1982), but in this study, the roots in JA treatment thickened due to the swelling of cortical cells. The anatomical difference between them may have been as a result of the JA treatment.

The most apparent swelling was observed in the roots of the culture with JA and BA. Anatomical characteristics of these roots, i.e., the active primary cambium, low stele lignification, and differentiated periderm were identical with those of normal storage root (Togari, 1950; Wilson, 1982). It is concluded, therefore, that these swollen roots were storage roots.

When JA was applied to the top organs of wild species which do not form storage roots, an increase in root diameter was observed in the concentration of 50 and 500 ppm. It appears unlikely that the increase in root diameter was as a result of total growth, because the weight of these materials was the lowest in 500 ppm JA treatment. The anatomies of the wild species in these treatments were not the same as those of storage roots of sweetpotato. In these roots, the swelling was due to the swelling of the cortex and phloem. The activity of primary cambium, however, was higher in these swollen roots. It is also noteworthy that phloic bundles were observed in the swollen roots of 500 ppm JA treatment. Phloic bundles are observed in some sweetpotato cultivars that have high potential for dry matter accumulation (Wilson and Lowe, 1973). From these facts it was thought that the thickened roots were not storage roots but similar to them.

From these results, it is apparent that JA

has activity for inducing swelling of certain cells in root of sweetpotato and related wild species. It is known that JAs can induce swelling of cells in potato and it is suggested that the primary process of potato tuberization is swelling of stolon cells induced by JAs and cytokinins (Koda, 1992). Therefore, it is possible that endogenous JA may be the primary stimuli for the storage root formation in sweetpotato through the induction of cell swelling in root stele and this swelling may induce the accumulation of cytokinins. It is well known that cytokinins, especially zeatin riboside is associated with the formation of storage root in sweetpotato (Matsuo *et al.*, 1983; Nakatani and Komeichi, 1991a, b; Nakatani and Matsuda, 1992). Application of cytokinins, however, did not induce any root swelling in wild species used here (unpubl. data). It is also possible that this wild species cannot form storage root or swollen root due to the lack of JA. However, the information about JA in sweetpotato is not enough to form a conclusion. Further investigations are needed that include the interaction between JAs and other plant hormones and localization of JAs in sweetpotato root.

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## Poster

# Physicochemical characterizations of three unconventional sources of starch from the Andean region in Ecuador

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The physicochemical aspects of three native Andean starches from cultivars *Arracacha xanthorriza*, *Canna edulis*, and *Oxalis tuberosa* were investigated. Scanning electron microscopic investigations showed that the granular sizes were remarkably different among the three starches. The starch granules of *A. xanthorriza* were irregular, with granular sizes between 7–23  $\mu\text{m}$  whereas the granules of *O. tuberosa* and *C. edulis* both were oval-shaped with granular sizes between 22–25  $\mu\text{m}$  and 35–101  $\mu\text{m}$ , respectively. All three starches revealed an X-ray diffraction pattern of the B-type that also is reported to be typical for most kinds of tubers. The gelatinization enthalpies were  $14.6 \pm 0.2 \text{ J g}^{-1}$  for *O. tuberosa*,  $15.7 \pm 0.2 \text{ J g}^{-1}$  for *C. edulis*, and  $17.6 \pm 0.3 \text{ J g}^{-1}$  for *A. xanthorriza*, and the peak temperatures of endothermic differential scanning calorimetry (DSC)-transitions were  $55.9 \pm 0.2^\circ\text{C}$ ,  $61.2 \pm 0.1^\circ\text{C}$ , and  $60.1 \pm 0.1^\circ\text{C}$ , respectively. There were no direct correlations between the gelatinization parameters and the amylose content that was found to be  $4 \pm 1\%$  for *A. xanthorriza*,  $18 \pm 1\%$  for *O. tuberosa*, and  $24 \pm 0.4\%$  for *C. edulis*.

Keywords: Native starches; *Arracacha xanthorriza*; *Canna edulis*; *Oxalis tuberosa*; Starch granules; X-ray diffraction; Gelatinization enthalpies; Peak temperatures

Starch is a very useful hydrocolloid and its applications range from gelling systems of foods to manufacturing of paper and pulp. The sources of starch vary all over the world depending on local traditions and climatic conditions. In North America, various genotypes of maize are used for the production of starch and starch derivatives, whereas the main part of such production is based on potato in northern Europe. To be applied to specific industrial processes, most starches are chemically modified by degradation, substitution, or by cross-bonding. There are also some possibilities of using genetically modified crops. However, there is some uncertainty in using modified starches for food production, and the demand for natural starches is constantly increasing. Many developing countries also lack their own production of starch. It is, thus, of interest to characterize new and unconventional sources of native starch to replace some of the modified ones. Potato that is commonly used as a source of starch and starch derivatives, originates from the Andean region in South America but the use of other Andean crops as sources of starch has not yet been fully studied. This is most likely

as a result of only partial knowledge about the characteristics of such starches. In the present study, the physicochemical characteristics of three Andean starches extracted from *Arracacha xanthorriza*, *Canna edulis*, and *Oxalis tuberosa*, were investigated.

## Materials and Methods

Tubers of *A. xanthorriza* cv. FB-001, *C. edulis* cv. MH-1173 identified by International Potato Center (CIP), Quito, Ecuador, and *O. tuberosa* bought on the local market in Quito were used for starch extraction.

## Differential scanning calorimetry (DSC)

The gelatinization properties of the different starches were analysed on a Seiko SII 6200 DSC (Seiko, Japan) equipped with a standard software. The investigations were made in the temperature range of 17 to 97°C, at a scanning rate of 10° min<sup>-1</sup>. The samples were investigated in excess of water at a starch to water ratio of 1:3 (w/w) in coated pans from TA Instruments (TA Instruments, U.S.A.). Double-distilled and deionized water was used for all experiments, and an empty pan with double lids was used as a reference. The enthalpies are presented as J g<sup>-1</sup> and calculated on a dry matter basis, that was determined by puncturing and drying the pans in an oven at 105°C for 2 h. The endothermic DSC-transition temperatures, given in °C, are given as the onset tem-

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perature ( $T_o$ ), the peak temperature ( $T_m$ ), and the final temperature ( $T_c$ ).

### Scanning electron microscopy (SEM)

A thin layer of starch was mounted on an aluminium specimen holder by double-sided tape. The specimen holder was loaded in a Balzers SCD 004 Gold Sputter Coater for 120 s at 15 mA. During these conditions approximately 20 nm thin cover of gold will be obtained. The specimen holder was then transferred to a JEOL JSM-840A SEM and examined at 20 kV.

### Determination of the chain length distribution of amylopectin

Amylopectin was isolated and debranched with isoamylase. The chain length distribution was analysed with high performance anion exchange chromatography on a Dionex DX 500 (Sunnyvale, CA, U.S.A.) equipped with a pulsed amperometric detector (ED 40), a CarboPac PA-100 anion exchange column (250 mm  $\times$  4 mm) in combination with a CarboPac PA-100 guard column, and an autosampler (Spectra-Physics, Fremont, CA, U.S.A.).

### Wide-angle X-ray scattering technique (WAXS)

A flat-film camera arrangement according to Stenhagen was used for the wide-angle X-ray scattering determinations. The X-ray camera was equipped with a Philips PW 2273/20 LFF copper anode X-ray tube (Philips, The Netherlands), giving an average radiation of 1.54 Å and operated at 40 kV and 20 mA. Characteristic  $d$ -spacings in the wide-angle area for the  $b$ -form of tristearine were used for the calibrations.

The starch samples were weighed into small tubes and mixed with water to a water:starch ratio of 0.9:1 (w/w). The samples were equilibrated at room temperature for 1 h before they were mounted in designed X-ray cassettes with O-rings and mica windows. The X-ray scattering was registered on medical X-ray films, CEA Reflex 25 (CEA AB, Strängnäs, Sweden), and processed according to recommendations of the manufacturer.

### Determination of the amylose content

The amylose content was determined according to the method of Dubois *et al.* (1956).

## Results and Discussion

The SEM investigations showed that the starch granular sizes were remarkably different for the three starches of *A. xanthorrhiza*, *C. edulis*, and *O. tuberosa* (Table 1). The granules of *C. edulis* appear to be among the largest ones ever reported in the literature and can partly be compared with those of potato starch granules,

**Table 1** Physicochemical properties of *Arraccacha xanthorrhiza*, *Canna edulis* and *Oxalis tuberosa*

Starch	Shape	Size ( $\mu\text{m}$ )	X-ray pattern	Amylose content (%)
<i>A. xanthorrhiza</i>	Irregular	7-23	B	4 $\pm$ 1
<i>C. edulis</i>	Oval	35-101	B	24 $\pm$ 0.4
<i>O. tuberosa</i>	Oval	22-55	B	18 $\pm$ 1

whereas granules of both *O. tuberosa*, and *A. xanthorrhiza* had more modest sizes. The SEM investigations also showed the presence of pores along the equatorial region of granules of *A. xanthorrhiza* with approximated diameters around 2  $\mu\text{m}$ . No such pores were observed on granules from *C. edulis* or *O. tuberosa*. However, a similar arrangement of pores like those of *A. xanthorrhiza* has also been observed on potato starch granules, and granules from wheat, rye, and barley. The pores are thought to support the initial attack of enzymes during the germination process.

The X-ray diffraction pattern was identified as type B for the three starches (Table 1), and this is reported to be typical for almost all kinds of root and tuber starches, like potato (Zobel, 1988). The B pattern is also characteristic for retrograded starches independent on botanical source.

The DSC results of the starch samples showed that  $T_m$ , often referred to as the gelatinization temperature, was 55.9  $\pm$  0.2°C for *O. tuberosa*, 60.1  $\pm$  0.1°C for *A. xanthorrhiza*, and 61.2  $\pm$  0.1°C for *C. edulis* (Table 2). The gelatinization enthalpies was 14.6  $\pm$  0.2 J g<sup>-1</sup> for *O. tuberosa*, 15.7  $\pm$  0.2 J g<sup>-1</sup> for *C. edulis*, and 17.6  $\pm$  0.35 J g<sup>-1</sup> for *A. xanthorrhiza* (Table 2). The amylose content was the highest for *C. edulis* (24  $\pm$  0.4%) followed by *O. tuberosa*, (18  $\pm$  1.0%), and *A. xanthorrhiza* (4  $\pm$  1.0%) (Table 1). Comparison of gelatinization results of the three starches showed that *C. edulis* and *A. xanthorrhiza* had higher  $T_c$  than *O. tuberosa*. The high transition temperature may reflect more stable amorphous regions or a lower degree of chain branching (Biliaderis *et al.*, 1980). This is supported by the degree of polymerisation of *C. edulis* and *A. Xanthorrhiza* which had a lower degree of polymerisation than *O. tuberosa* (Table 3).

**Table 2** Gelatinization parameters of *Arraccacha xanthorrhiza*, *Canna edulis* and *Oxalis tuberosa* enthalpy ( $\Delta H$ ), onset ( $T_o$ ), peak ( $T_m$ ), and final ( $T_c$ ) temperatures of gelatinization

Starch	$\Delta H$ (J g <sup>-1</sup> ) <sup>a</sup>	$T_o$ (°C) <sup>a</sup>	$T_m$ (°C) <sup>a</sup>	$T_c$ (°C) <sup>a</sup>
<i>A. xanthorrhiza</i>	17.6 $\pm$ 0.3	53.8 $\pm$ 0.1	60.1 $\pm$ 0.1	65.9 $\pm$ 0.8
<i>C. edulis</i>	15.7 $\pm$ 0.2	56.8 $\pm$ 0.0	61.2 $\pm$ 0.1	67.7 $\pm$ 0.9
<i>O. tuberosa</i>	14.6 $\pm$ 0.2	50.2 $\pm$ 0.1	55.9 $\pm$ 0.2	63.3 $\pm$ 0.4

<sup>a</sup>Measures made at triplicate

**Table 3** Chain length distribution of amylopectin, degree of polymerisation (DP), of *Arracacha xanthorrhiza*, *Canna edulis*, and *Oxalis tuberosa*

Starch	Average DP <sup>a</sup>
<i>A. xanthorrhiza</i>	17
<i>C. edulis</i>	17
<i>O. tuberosa</i>	18

<sup>a</sup>Measures made at least at duplicate

The present investigation demonstrated that the physicochemical properties were considerably different among the three starches examined. In Europe, there are several projects dealing with genetic modifications to obtain potato varieties with either very high or very low amylopectin contents for industrial purposes. The use of *A. xanthorrhiza* with a natural amylose content of 4% can be an alternative to such starches. It

has also the highest enthalpy among the starches investigated, which is similar to what is known for amylopectin potato starch.

### Acknowledgement

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## Poster

# Enzyme-based dip-stick: An easy-to-use alternative for estimation of cyanogen level in cassava roots

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A protocol to use the enzyme-based dip-sticks with intact or crushed cassava root slices for the determination of cyanogen content was developed. Analyses based on five roots per cassava variety compared well with those obtained by spectrophotometric assay of the composite root extracts. Based on 90 varieties, a correlation ( $r$ ) of 0.88 was obtained. The dip-stick method could also be used for semi-quantitative analysis of cyanogen level by comparing the colour of picrate paper against those prepared with known amounts of hydrocyanic acid (HCN). Analysis carried out for 122 cassava varieties using intact root slices showed good correlation with results obtained from spectrophotometric assay using composite root extracts ( $r = 0.79$ ). Overall, this simple and easy-to-use method should prove useful not only as a field method where a spectrophotometer might not be available, but also capable of handling large numbers of samples.

Keywords: Cassava roots; Cyanogenic potential; Dip-sticks; Cyanogen determination

Several enzyme-based methods are available for quantitative determination of cyanogen level in cassava roots (Cooke, 1978; Essers *et al.*, 1993; Yeoh and Truong, 1993; Brimer, 1994; Yeoh and Tan, 1994; Tatsuma *et al.*, 1996). However, when it comes to handling large numbers of samples, many of these methods are viewed as laborious and time consuming. This could be seen in the preparation of the root samples as well as in the multitude of steps needed for the analysis. Therefore, laboratories having to deal with large quantities of samples prefer the semi-quantitative procedures which unfortunately have some limitations (Sadik *et al.*, 1974; Williams, 1979; CIAT, 1993; Bainbridge *et al.*, 1996). A recently reported enzyme-based dip-stick for the determination of cassava flour (Yeoh and Egan, 1997) may provide a solution to these problems if root tissues can be used directly for analysis.

The objective of this study was to develop a simple and less laborious sample preparation procedure that could be used with the dip-sticks, which could result in a simple and easy-to-use protocol for the determination of cyanogen in cassava roots. It could also be used for semi-quantitative estimation, making it a suitable field method.

## Materials and Methods

### Plant material

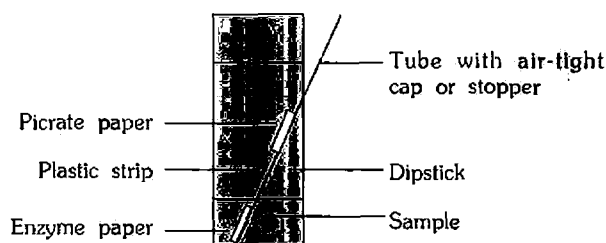
Cassava varieties were collected in 1997 from the germplasm collection maintained at the Centro Internacional de Agricultura Tropical, Cali, Colombia. Freshly harvested roots were used for analysis.

### Preparation of enzyme-based dip-stick

This was prepared as described by Yeoh and Egan (1997). A linamarase-impregnated paper (Whatman 17 Chr chromatography paper, 10 mm × 10 mm, containing 0.15 U) and an alkaline picrate paper (Whatman 3 MM chromatography paper, 10 mm × 20 mm) were glued on the same side of a plastic strip (10 mm × 50 mm) (Figure 1).

### Sample preparation and analysis

Root slices were prepared by punching out a disc from a 10–15 mm section of cassava root with a cork borer (6 mm i.d.), then sliced. To prepare the slices quickly and with consistency, a cutting device comprising 2 blades separated by a 1-mm spacer was used. Each root disc



**Figure 1** Design of an enzyme-based dip-stick and experimental setup

weighed about 0.10–0.01 g. The tissue was weighed directly into a glass test-tube containing 0.5 mL water. If necessary, it was rapidly crushed while submerged in the water with a glass rod. One-third to half a slice was used if it was suspected that the cassava variety was of high cyanogenic potential. An enzyme-based dip-stick was then inserted and the test tube sealed air-tight with a rubber stopper. All the tubes were left at room temperature (26–30°C) overnight (18–20 h).

For quantitative measurement, the picrate paper was soaked in 2.5 mL water to elute the colour and its absorbance read at 510 nm. The cyanogen level was expressed as mg HCN kg<sup>-1</sup> root using linamarin as the reference compound. For semi-quantitative estimation, an eight-point colour chart (for HCN ranging from 0 to 40 mg) was prepared using linamarin. Since the root tissue used weighed about 0.1 g, the cyanogen level could be estimated directly from the chart as mg HCN kg<sup>-1</sup> root on a fresh weight basis.

### Composite root extract

This was prepared using the the proximal, middle, and distal portions of three peeled roots. These were chopped into small pieces and thoroughly mixed. A 60-g portion was sampled and homogenised in 200 mL 0.1M H<sub>3</sub>PO<sub>4</sub> containing 25% (v/v) ethanol (Essers *et al.*, 1993). The homogenate was filtered through Whatman filter GF/A and the filtrate used for analysis. Duplicate analyses were carried out for each sample using an enzyme-based spectrophotometric method (Essers *et al.*, 1993).

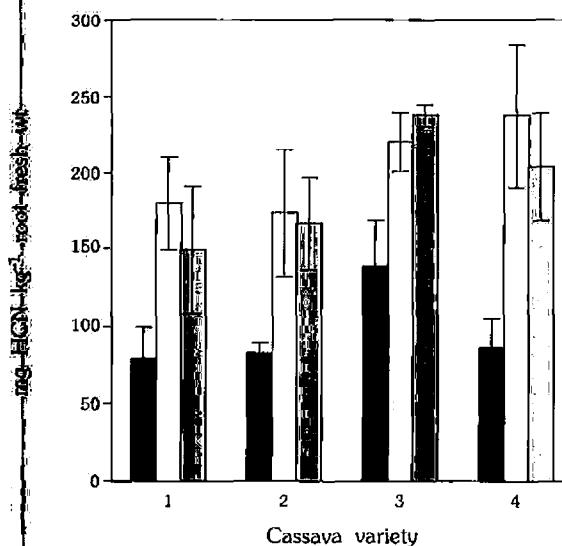
## Results and Discussion

The concept of an enzyme-based dip-stick for determination of the cyanogen level in cassava was first reported by Yeoh *et al.* (1966). This idea was later developed for the determination of cyanogenic potential in cassava flour (Yeoh and Egan, 1997). It was also envisaged that such a method could also provide a simple and less laborious analysis of cyanogen content in cassava roots if it could be used directly with root tissues. For practical purposes, one must be able to prepare the samples rapidly and with some consistency.

In a preliminary study, it was observed that the cyanogen level of some cassava varieties were underestimated when intact root slices were used. To overcome this, it was necessary to promote better contact between the cyanogenic glucoside and the enzyme. To achieve this, the root slice was quickly crushed with a glass rod before the dip-stick was inserted. The whole process took about 15 to 20 s. The results showed that crushed root slices gave values comparable to those of the control (Figure 2). Thus, to avoid under-estimation, it is recommended that the root slices be broken up for analysis.

Variation in linamarin and cyanide content exist within the same root and between roots of the same plant (Cooke, 1978; Cooke *et al.*, 1978). This information is important as proper sampling must be given careful consideration in order to give reliable information on the cyanogenic potential of the variety. Thus, a root slice from the mid-section of the root using five roots for each variety was sampled. These results compared favourably with those obtained using the composite root extracts prepared from three roots per variety. The spectrophotometric method when compared with the dip-stick method, gave a correlation factor (*r*) of 0.88 (data not shown). Thus, using the dip-sticks in conjunction with root slices, it was possible to provide reliable estimates of cyanogenic potential in cassava roots. In the authors' experience, this protocol was less tedious and required less man-power compared to having to prepare composite root extracts for analysis.

Notwithstanding the need to crush the root slices as discussed above, the use of intact root slices for semi-quantitative estimation would further reduce the time and labour required. For



**Figure 2** Effect of crushing root slices for analysis. Cassava variety: 1, MCOL 1684; 2, CM 507-37; 3, MBRA 881; and 4, MBRA 162. (●), Intact root slice; (□), crushed root slice, and (▨) composite root extract

example, there will be a saving of 4 to 5 h for every 1000 samples determined. As the prepared root slices weighed about 0.10–0.01 g, the cyanogenic potential could be read directly from the calibration chart as 0–400 mg HCN kg<sup>-1</sup> root fresh weight. This method was conducted on 122 cassava varieties and the results when compared against those obtained from composite extract determined by spectrophotometric method gave a correlation factor of 0.79 (data not shown). As expected, some varieties that were underestimated were detected. Nonetheless, the semi-quantitative approach appears to be useful as a potential field method where a spectrophotometer may not be available and under situations where time is an important factor. It was observed that a three-man team could easily handle 250 analyses in under 2 h. Based on an 8 h work day, it was possible to process 200 varieties, using 5 roots per variety.

Overall, this study demonstrated that the dip-stick method could be used in conjunction with cassava root slices. Thus, large numbers of samples, typically encountered in plant breeding programmes, germplasm screening, and field trial evaluation, could be readily handled. There was overall savings in costs, labour, and manpower compared to the other enzyme-based methods. Although the thought of preparing thousands of dip-sticks seemed daunting, it was possible to prepare 1000 pieces of dip-sticks a day. Considering that there are other benefits with the enzyme-based dip-sticks (Yeoh *et al.*, 1996; Yeoh and Egan, 1997), this method of cyanogenic potential determination would be a good alternative to adopt.

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## Poster

# Antimutagenic activity of water extracts from sweetpotato

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Antimutagenicity of the water extracts prepared from the storage roots of four varieties of sweetpotato [*Ipomoea batatas* (L.)] with different flesh colour was investigated using the *Salmonella typhimurium* TA 98. The extract from the whole roots of the purple-coloured Ayamurasaki effectively decreased the reverse mutation induced not only by Trp-P-1, Trp-P-2, IQ, B[a]P, and 4-NQO, but also by dimethyl sulphoxide extract of grilled beef. Comparison of the inhibitory activity of the extracts from the normal Ayamurasaki variety and its anthocyanin-deficient mutant suggested that the anthocyanin pigment in the flesh decreases the mutagenic activity of the mutagens as heterocyclic amines. Two constituents of Ayamurasaki anthocyanin pigments, 3-(6,6'-caffeylferulylsophoroside)-5-glucoside of cyanidin and peonidin, effectively inhibited the reverse mutation induced by Trp-P-1, Trp-P-2, and IQ. Furthermore, determination of the inhibitory activity of sectional portions using the Joy White variety with white flesh demonstrated that the inhibitory components are abundant in the cortical portion of the storage root, suggesting the involvement of phenolics in the antimutagenicity of the extract from the outer tissue.

Keywords: Sweetpotato; Storage root; Antimutagenicity; Anthocyanin; Phenolics

Sweetpotato [*Ipomoea batatas* (L.)] represents the sixth most important food crop in the world. Kozai *et al.* (1996) reported that sweetpotato will play an important role in solving the global issues on food, energy, and natural resources and the environment in the 21st century. Sweetpotato will be an important food crop in Asian and African countries where the populations are expected to increase significantly in the future. Several varieties of sweetpotato contain higher contents of various vitamins, minerals, and protein than other vegetables (Woolfe, 1992). Despite its agronomic and nutritional advantages, consumption of sweetpotato has declined in recent decades. To circumvent this problem, interest has been focussed on the development of new uses. Therefore, further understanding of the physiological functions of sweetpotato is considered to be one of the important factors for developing new uses. Several investigators reported the suppression of melanogenesis of mouse melanoma B16 (Shimozono *et al.*, 1996) and antioxidant activity (Hayase and Kato, 1984) by sweetpotato extract as well as the reducing effects of purple-coloured sweetpotato juice against carbon tetrachloride-induced liver injury (Suda *et al.*, 1997). Recently, new varieties of sweetpotato with different flesh colour have been released for new utilization by the Kyushu National Agricultural Experiment Station. Estimation of general nutritive value such as vitamin or mineral contents of these varieties has been investigated, but too little is known about the physiological function.

Recent development of screening methods for environmental carcinogens by determining their mutagenicity has enabled various types of mutagens and carcinogens to be detected and identified in foods (Ames *et al.*, 1975). Some of these substances have been found to be generated during storage, cooking, and digestion of foods (Nagao *et al.*, 1977; Kasai *et al.*, 1979; Yamaizumi *et al.*, 1980). On the other hand, it is now known that various types of inhibitors that act against mutagens and carcinogens exist in food, and they play an important role in reducing the risks of mutagenesis and carcinogenesis (Shinohara *et al.*, 1988). However, antimutagenic activity of sweetpotato varieties has not been investigated.

In the present paper, the effects of the water extracts of the several varieties of sweetpotato-storage roots with different flesh colour on the mutagenicity of some mutagens and anthocyanin pigments crystallized from purple-coloured sweetpotato are investigated.

## Materials and Methods

### Sweetpotato materials

Four varieties of sweetpotato roots with different root flesh colour, Koganesengan, Kyushu 114, Joy White, and Ayamurasaki, as well as its anthocyanin-deficient mutant (yellow flesh) were cultivated under the same conditions in an ex-

perimental field at Miyakonojo (Japan) (Table 1). Harvested roots were cut into two portions and one half was used as the whole root. The remaining one half was separated into the peeled outer layer (about 0.5 cm thick) as the outer tissue and the inner portion as the inner tissue, respectively. The peeled outer layer comprised all of the cortical region, which included the periderm, laticifer, and cambium. All sections in each case were diced, lyophilized, and ground to flour. The flour samples were stocked at  $-20^{\circ}\text{C}$  until use.

### Chemicals and bacteria

Trp-P-1, Trp-P-2, IQ, 4-NQO, and B[a]P were obtained from Wako Pure Chemical Industries Ltd. Chlorogenic acid was the product of Sigma Chemical Co. The S-9 fraction prepared from rat liver pretreated with phenobarbital and 5,6-benzoflavone and cofactors were the products of Oriental Yeast Co., Ltd. Two anthocyanin pigments, 3-(6,6'-caffeylferulylsophoroside)-5-glucoside of cyanidin and peonidin were supplied by Dr M. Yamaguchi of Minami-Kyushu University. Other chemicals used were of special grade. Strain TA 98 of *Salmonella typhimurium* was supplied by the Institute for Fermentation, Osaka, Japan (IFO). The bacterium was cultured in nutrient broth for 16 h at  $37^{\circ}\text{C}$  prior to the mutagenicity assay.

### Preparation of sweetpotato-water extract

The extract was made from the lyophilized flour (1 g) using 20 mL of ice-cold water for 1 h. The suspension was centrifuged at  $18\,000 \times g$

for 20 min and the resultant precipitate was re-extracted under the same conditions. The collected supernatant was lyophilized.

### Assay of antimutagenicity

The mutagenicity assay was done by a modification of the method of Yahagi *et al.* (1977). The antimutagenic activity of the sweetpotato-water extracts of various varieties was evaluated on TA 98 using the five purified mutagens, Trp-P-1, Trp-P-2, IQ, B[a]P, and 4-NQO. Of these compounds, Trp-P-1, Trp-P-2, IQ, and B[a]P require metabolic activation to cause mutation in TA 98, but 4-NQO does not. The S-9 mix contained 50  $\mu\text{mol}$  of sodium phosphate buffer (pH 7.4), 4  $\mu\text{mol}$  of  $\text{MgCl}_2$ , 16.5  $\mu\text{mol}$  of KCl, 2.5  $\mu\text{mol}$  of glucose-6-phosphate, 2  $\mu\text{mol}$  of NADH, 2  $\mu\text{mol}$  of NADPH, and 50  $\mu\text{L}$  of S-9 fraction in a total volume of 0.5 mL. Mutagenicity was tested on TA 98 with or without S-9 mix according to the type of mutagen. For the inhibition test, 0.1 mL of each mutagen, 0.1 mL sweetpotato-water extracts or dimethyl sulphoxide (DMSO)-solubilized anthocyanin, and 0.5 mL S-9 mix or phosphate buffer were simultaneously incubated with 0.1 mL of bacterial suspension at  $37^{\circ}\text{C}$  for 20 min, and then poured on minimal-glucose-agar plates with 2 mL of soft agar.

### Preparation of DMSO extract of grilled beef (DEGB)

For the preparation of DEGB, sliced beef (fillet, purchased from a market) was grilled to well-done, lyophilized, and ground to flour. Five grams of the lyophilized flour was extracted with 10 mL of DMSO for 60 min at room temperature. The solution was sterilized by filtration (Minisart NML 0.45  $\mu\text{m}$ , Sartorius). The DEGB was added to the reaction mixture at a dose of 100  $\mu\text{L}$  plate $^{-1}$  without dilution.

**Table 1** Effects of sweetpotato water extracts on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98<sup>a</sup>

Variety	Flesh colour	Amount of extract (mg plate $^{-1}$ )	His <sup>+</sup> revertants plate $^{-1}$ <sup>b</sup>	Inhibition
Control	—	—	625 $\pm$ 12	—
Ayamurasaki	Purple	1.0	372 $\pm$ 31	41
		5.0	282 $\pm$ 23	55
		10.0	240 $\pm$ 9	62
Koganesengan	Yellow	1.0	628 $\pm$ 27	0
		5.0	632 $\pm$ 22	0
		10.0	593 $\pm$ 16	5
Joy White	White	1.0	630 $\pm$ 47	0
		5.0	627 $\pm$ 48	0
		10.0	598 $\pm$ 18	4
Kyushu 114	Orange	1.0	595 $\pm$ 21	5
		5.0	571 $\pm$ 31	9
		10.0	574 $\pm$ 19	8

<sup>a</sup>Trp-P-1 was added at a dose of 0.075  $\mu\text{g}$  plate $^{-1}$

Mutagenicity was tested with S-9 mix

<sup>b</sup>Each value represents the mean  $\pm$  S.D. of triplicate plates

The values shown have had the spontaneous mutation frequency subtracted

## Results and Discussion

The antimutagenic effect of the water extracts from whole storage root of four varieties with different flesh colour was examined using Trp-P-1 at a dose of 0.075  $\mu\text{g}$  plate $^{-1}$  (Table 1). The Ayamurasaki extract showed a dose-dependent antimutagenicity. The inhibitory activities were 41, 55, and 62% at doses of 1.0, 5.0, and 10.0 mg plate $^{-1}$  of the extract, respectively. The extracts from Koganesengan and Joy White showed the inhibitory activities of about 5% at a dose of 10.0 mg plate $^{-1}$ . The inhibitory activities of the extracts from Kyushu 114 were 5, 9, and 8% at doses of 1.0, 5.0, and 10.0 mg plate $^{-1}$  of the extract, respectively. The antimutagenic effect of the extracts from whole-storage roots of Koganesengan, Joy White, and Kyushu 114 on Trp-P-1 was minor compared with the one from Ayamurasaki. The effects of

the Ayamurasaki water extract on some purified mutagens other than Trp-P-1 were examined in the subsequent experiments.

The antimutagenic activity of the Ayamurasaki water extract was evaluated using the purified mutagens such as Trp-P-2, IQ, B[a]P, and 4-NQO (Table 2). Of these compounds, S-9 mix was added for the assay using Trp-P-2, IQ, and B[a]P to cause mutation in TA 98 but not for 4-NQO. The extract inhibited Trp-P-2-induced mutation by 21 to 76%, IQ by 34 to 77%, B[a]P by 4 to 58%, and 4-NQO by 10 to 53%.

The present data indicate that the Ayamurasaki water extract effectively decreased the reverse mutation induced by all purified mutagens tested. Subsequent studies were conducted to confirm whether the Ayamurasaki water extract inhibits the reverse mutation induced by mutagenic substance in foods. The DEGB was prepared as an example of a mutagenic substance in daily foods according to the report by Yamada and Tomita (1994). As shown in Table 3, the inhibitory activities of the Ayamurasaki water extract were 20, 30, 53, and 64% at doses of 1, 2.5, 5.0, and 10.0 mg plate<sup>-1</sup> of the extract, respectively. The Ayamurasaki extract also showed the dose-dependent antimutagenicity against the reverse mutation induced by DEGB, as well as by Trp-P-1, Trp-P-2, and IQ. Thus, it was confirmed that the Ayamurasaki water extract effectively decreased the reverse mutation induced by a mutagenic substance in foods.

The comparison of the antimutagenicity be-

**Table 2** Effect of Ayamurasaki water extract on the mutagenicity of Trp-P-2, IQ, B[a]P, and 4-NQO against *Salmonella typhimurium* TA 98

Mutagen (µg plate <sup>-1</sup> )	Amount of extract (mg plate <sup>-1</sup> )	His <sup>+</sup> revertants plate <sup>-1,c</sup>	Inhibition (%)
Trp-P-2 (0.02) <sup>a</sup>	—	866±57	—
	1.0	680±33	21
	5.0	267±26	69
	10.0	205±11	76
IQ (0.02) <sup>a</sup>	—	921±22	—
	1.0	607±32	34
	5.0	378±9	59
	10.0	211±27	77
B[a]P (10) <sup>a</sup>	—	252±24	—
	1.0	241±10	4
	5.0	190±19	25
	10.0	107±9	58
4-NQO (0.8) <sup>b</sup>	—	305±9	—
	1.0	275±13	10
	5.0	184±7	40
	10.0	143±16	53

<sup>a</sup>Mutagenicity was tested with S-9 mix

<sup>b</sup>Mutagenicity was tested without S-9 mix

<sup>c</sup>Each value represents the mean ± S.D. of triplicate plates

The values shown have had the spontaneous mutation frequency subtracted

**Table 3** Effect of Ayamurasaki water extract on the mutagenicity of DEGB against *Salmonella typhimurium* TA 98

Test system <sup>a</sup>	Amount of extract (mg plate <sup>-1</sup> )	His <sup>+</sup> revertants plate <sup>-1,b</sup>	Inhibition (%)
DEGB (100µL)	—	229±20	—
	1.0	207±5	20
	2.5	160±23	30
	5.0	108±5	53
	10.0	82±5	64

<sup>a</sup>Mutagenicity was tested with S-9 mix

<sup>b</sup>Each value represents the mean ± S.D. of triplicate plates

The values shown have had the spontaneous mutation frequency subtracted

between the water extracts from the Ayamurasaki normal and its anthocyanin-deficient mutant roots is shown in Table 4. Trp-P-1 was used as a mutagen at a dose of 0.075 µg plate<sup>-1</sup>. The inhibitory activities of the extract from the Ayamurasaki normal root were 37, 54, and 64% at doses of 1.0, 5.0, and 10.0 mg plate<sup>-1</sup> of the extract, respectively. However, the inhibitory activity of the extract from the Ayamurasaki mutant was 5% at a dose of 10 mg plate<sup>-1</sup> of the extract. Thus, the antimutagenicity of the extract from the Ayamurasaki mutant was at the same level as the ones from Koganesengan, Joy White, and Yushu 114 (Table 1). These results suggest that effective inhibition of the reverse mutation by the Ayamurasaki extract may be attributed to the anthocyanin pigments in purple root flesh.

Two crystallized constituents of Ayamurasaki anthocyanin pigments, 3-(6,6'-caffeylferulylsophoroside)-β-glucoside of cyanidin (YGM-3) and peonidin (YGM-6) were applied to confirm the antimutagenic activity of the anthocyanin. Both anthocyanin constituents, which correspond to the third and sixth peaks, respectively, seen under an earlier high performance liquid chromatography

**Table 4** Effect of Ayamurasaki normal or mutant water extract on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98<sup>a</sup>

Test system	Amount of extract (mg plate <sup>-1</sup> )	His <sup>+</sup> revertants plate <sup>-1,b</sup>	Inhibition (%)
Control	—	688±19	—
Normal	1.0	431±22	37
	5.0	316±27	54
	10.0	250±29	64
Mutant	1.0	683±17	1
	5.0	687±12	0
	10.0	657±33	5

<sup>a</sup>Trp-P-1 was added at a dose of 0.075 µg plate<sup>-1</sup>

<sup>b</sup>Mutagenicity was tested with S-9 mix

Each value represents the mean ± S.D. of triplicate plates

The values shown have had the spontaneous mutation frequency subtracted

analysis of the crude anthocyanin extract from the purple-coloured flesh were designated as YGM-3 and YGM-6 (Odake *et al.*, 1992). The effects of YGM-3 and YGM-6 on the reverse mutation induced by Trp-P-1, Trp-P-2, and IQ are shown in Table 5. YGM-3 inhibited Trp-P-1-induced reverse mutation by 51 to 82%, Trp-P-2 by 77 to 95%, and IQ by 42 to 79%. YGM-6 inhibited Trp-P-1-induced reverse mutation by 45 to 70%, Trp-P-2 by 51 to 80%, and IQ by 38 to 70% at the corresponding concentrations of the pigment. Thus, YGM-3 and YGM-6 showed the dose-dependent antimutagenicity against the reverse mutations induced by the mutagenic heterocyclic amines.

As shown in Table 1, the water extracts from whole roots of varieties other than Ayamurasaki hardly decreased the reverse mutation induced by Trp-P-1. Yamada and Tomita (1996) indicated that caffeic acid and chlorogenic acid have inhibitory effects on the mutagenicity of Trp-P-1 and Glu-P-2. Chlorogenic acid is a main component of sweetpotato phenolics (Walter *et al.*, 1979; Shimozono *et al.*, 1996), and these contents are much higher in the outer tissue than the inner (Walter and Schadel, 1981). Those reports suggest that the extract from the outer tissue of sweetpotato roots may effectively decrease the reverse mutation by the mutagen. The effect of the water extracts from each sectional portion of Joy White variety on

the mutagenicity of Trp-P-1 on TA 98 is shown in Table 6. Trp-P-1 was used at a dose of 0.075  $\mu\text{g plate}^{-1}$  and the water extract from the whole root, inner tissue, or outer tissue was added at a dose of 10  $\text{mg plate}^{-1}$ . The antimutagenic activity was not detected in the extract of the whole roots or inner tissue. The extract from the outer tissue showed 24% inhibitory activity. The antimutagenic activity of chlorogenic acid on Trp-P-1 and Trp-P-2 is shown in Table 7. Trp-P-1 and Trp-P-2 were added at doses of 0.075 and 0.02  $\mu\text{g plate}^{-1}$ , and chlorogenic acid at doses of 0.5, 1.0, and 5.0  $\text{mg plate}^{-1}$ . Chlorogenic acid inhibited Trp-P-1 induced mutation by 30 to 59% and Trp-P-2 by 38 to 70%. Those results support the data of Yamada and Tomita (1996) that chlorogenic acid had inhibitory effects on the mutagenicity of Trp-P-1.

The water extract from Ayamurasaki with purple-coloured flesh effectively inhibited the reverse mutation induced not only by the of purified mutagens but also by DEGB (Tables 1, 2, and 3). Furthermore, the anthocyanin-deficient mutant of Ayamurasaki variety suggested that the antimutagenic activity of the Ayamurasaki water extract might be due to the anthocyanin

**Table 5** Effects of YGM-3 and YGM-6 on the mutagenicity of Trp-P-1, Trp-P-2, and IQ against *Salmonella typhimurium* TA 98<sup>a</sup>

Mutagen ( $\mu\text{g plate}^{-1}$ )	Amount of anthocyanin ( $\text{mg plate}^{-1}$ )	His <sup>+</sup> revertants $\text{plate}^{-1,b}$	Inhibition (%)
Trp-P-1 (0.075)	—	693±43	—
	YGM-3 (0.5)	340±27	51
	YGM-3 (1.0)	188±18	73
	YGM-3 (5.0)	125±3	82
	YGM-6 (0.5)	381±17	45
	YGM-6 (1.0)	253±8	63
	YGM-6 (5.0)	208±35	70
Trp-P-2 (0.020)	—	825±9	—
	YGM-3 (0.5)	193±10	77
	YGM-3 (1.0)	127±9	85
	YGM-3 (5.0)	38±7	95
	YGM-6 (0.5)	402±17	51
	YGM-6 (1.0)	297±27	64
	YGM-6 (5.0)	168±10	80
IQ (0.020)	—	884±20	—
	YGM-3 (0.5)	517±21	42
	YGM-3 (1.0)	241±19	73
	YGM-3 (5.0)	182±10	79
	YGM-6 (0.5)	550±16	38
	YGM-6 (1.0)	361±21	59
	YGM-6 (5.0)	269±20	70

<sup>a</sup>Mutagenicity was tested with S-9 mix  
<sup>b</sup>Each value represents the mean  $\pm$  S.D. of triplicate plates  
 The values shown have had the spontaneous mutation frequency subtracted

**Table 6** Effect of water extracts from Joy White on the mutagenicity of Trp-P-1 against *Salmonella typhimurium* TA 98

Mutagen <sup>a</sup> ( $\mu\text{g plate}^{-1}$ )	Portion <sup>b</sup>	His <sup>+</sup> revertants $\text{plate}^{-1,c}$	Inhibition (%)
Trp-P-1 (0.075)	—	693±43	—
	Whole root	682±31	0
	Inner tissue	691±25	0
	Outer tissue	524±42	24

<sup>a</sup>Mutagenicity was tested with S-9 mix  
<sup>b</sup>Water extract was added at a dose of 10  $\text{mg plate}^{-1}$   
<sup>c</sup>Each value represents the mean  $\pm$  S.D. of triplicate plates  
 The values shown have had the spontaneous mutation frequency subtracted

**Table 7** Effect of chlorogenic acid on the mutagenicity of Trp-P-1 and Trp-P-2 against *Salmonella typhimurium* TA 98<sup>a</sup>

Mutagen ( $\mu\text{g plate}^{-1}$ )	ChA ( $\text{mg plate}^{-1}$ )	His <sup>+</sup> revertants $\text{plate}^{-1,b}$	Inhibition (%)
Trp-P-1 (0.075)	—	693±23	—
	0.5	483±25	30
	1.0	324±19	53
	5.0	283±16	59
Trp-P-2 (0.02)	—	825±29	—
	0.5	509±32	38
	1.0	392±23	53
	5.0	246±23	70

<sup>a</sup>Mutagenicity was tested with S-9 mix  
<sup>b</sup>Each value represents the mean  $\pm$  S.D. of triplicate plates  
 The values shown have had the spontaneous mutation frequency subtracted

pigments in the flesh (Table 4). However, the antimutagenic activity of the Ayamurasaki water extract is fully contemplated due to the water-soluble components such as amino acids (Watanabe *et al.*, 1994), vitamins (Osawa *et al.*, 1980; Shimoi *et al.*, 1992), and phenolics other than the anthocyanin pigments. The present data also indicated that the phenolics were concerned in the antimutagenic activity of the sweetpotato water extract (Tables 6 and 7). Therefore, the antimutagenic activity of the Ayamurasaki anthocyanin pigments using YGM-3 and YGM-6 were confirmed. On the other hand, YGM-3 appears to have relatively stronger antimutagenic activity against the mutagens tested than YGM-6 (Table 5). This may reflect the structural difference between the cyanidin and the peonidin. Furthermore, Yamada and Tomita (1996) reported that compounds analogous to caffeic acid or chlorogenic acid effectively decrease the mutagenic activity of the mutagens as heterocyclic amines. Otake *et al.* (1992) and Goda *et al.* (1996) also reported that anthocyanins of the purple-coloured sweetpotato have a caffeoyl or feruloyl group in their chemical structures. Those results suggest that the remarkable inhibition of the reverse mutation by anthocyanin pigments of purple-coloured sweetpotato may be due to the caffeoyl or feruloyl group in their chemical structures. Further work on the relationship between the chemical structure and antimutagenic activity is required to support the release of highly qualitative varieties of purple-coloured sweetpotato

Chlorogenic acid is a main component of sweetpotato phenolic (Walter *et al.*, 1979; Shimozono *et al.*, 1996). Yamada and Tomita (1996) and the present data (Table 7) suggest that chlorogenic acid effectively inhibited the reverse mutation by the heterocyclic amines. Tsuchiya *et al.* (1996) indicated that the superoxide anion scavenging activity of chlorogenic acid was stronger than that of beta-carotene and BHA, and chlorogenic acid acted preventively against paraquat-induced oxidative stress *in vivo*. Shimozono *et al.* (1996) also reported that the suppression of the melanogenesis of mouse melanoma B16 cells by the extract from steamed sweetpotato was due to the phenolics. Walter and Schadel (1981) and the authors' data (data not shown) indicated that the phenolics including chlorogenic acid were abundant in the outer tissue of the sweetpotato. These results suggest that utilization of the whole root including the skin is important for effective appearance of the physiological functions of the sweetpotato. Such utilization is connected with effective use of living resources and results in decrease of the negative environmental impact by the waste from the sweetpotato processing.

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## Poster

# Seeds and seedlings of *Dioscorea opposita* through natural crossing

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*Dioscorea opposita* is the most popular edible yam in Japan, but crossbreeding is seldom conducted because of its high sterility. However, a small number of seeds can be obtained by open pollination in Kanagawa Prefecture, a region with a climate warmer than the major yam-producing areas in Japan. In the northwest corner of a 250-m<sup>2</sup> field of a female variety of yams, 18 m<sup>2</sup> of male varieties were planted. A total of 3726 seeds were harvested from 6468 capsules on female plants of *D. opposita*. The latter had been planted closer to the male cultivars. The stickiness of the pollen grain showed that pollination occurred enyomophilically and not anemophilically. The mean number of fully matured seeds per capsule was less than one, but some well-developed capsules contained six seeds. The harvested seeds were classified into four groups according to endosperm development. The seeds in class 2 (large but immature endosperm) were sown to test germination time at low temperatures. Some seeds enlarged, and some of these sprouted in one to three months. Low temperatures promoted sprouting to some degree. The germination rates of all plots were less than 10%.

Keywords: *Dioscorea opposita*; Natural crossing; Seed; Pollination; Germination

*Dioscorea opposita* was introduced to Japan in ancient times from China and subsequently multiplied only by vegetative propagation, cultivating varieties in the process. Because of seed sterility, it is difficult to obtain hybrid seed, and breeding has been conducted using clonal variations encountered during cultivation. Hybrids could be obtained easily from immature seeds by embryo culture. Seedlings which were bred by this method had broad variations in leaf shape, rhizome shape, and rhizome quality. However, there is a limit to the number of the seedlings to be derived from embryo culture in terms of labour. In practical use, it is necessary to establish a method of obtaining a large number of seedlings in yam breeding without the labour-intensive method. Fortunately, the authors discovered that *D. opposita* produced many fruits and seeds which were due to germinate in 1996. If seeds could be obtained by natural crossing, breeding would be vastly increased. This report examined capsule and seed setting by open pollination, germinating ability, method of cultivation for obtaining seeds, and a method of sowing the seeds.

### Materials and Methods

#### Obtaining seeds through natural crossing

Capsule and seed setting were observed in an experimental field in Kanagawa Prefecture where

the climate is mild compared to the main yam production areas in Japan. Kanagawa Prefecture is situated in the warm band at 35° NW, 139° E longitude. The total area of the yam field is 250 m<sup>2</sup>. The field contained volcanic soil. Male varieties such as 'Sagamiwase', 'Tsukui', 'Tokkuri', and 'Okubo' were planted in the northwest corner 18 m<sup>2</sup> and the female variety 'Yamatoimo' was planted in the remaining space (75 cm × 20 cm) between the plants. The weight of seed tubers was 70 g in the female variety and 150 g in the male varieties. The field was trenched at a depth of 60 cm after a basal dressing of N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O at 25:25:225 kg ha<sup>-1</sup>. Vines were trained to a net 18 cm × 18 cm and 180 cm in height perpendicularly to the centre of two rows. Flower shapes were examined at the beginning of flowering, followed by the number of fruits and seeds in each plot on 17 October. The types and numbers of insects visiting the flowers were captured for investigation in a yellow adhesion sheet, 4 cm wide and 70 cm long. This was done twice (August 5 to 15 and August 18 to 28), first during flowering and again after flowering.

#### Seed size and germination

The seeds obtained were classified into four groups based on the state of the endosperm. The endosperms were then sown in nursery soil in 128-hole plastic trays 3 cm × 3 cm on 20

May 1997, after measuring each seed diameter, endosperm diameter, and individual seed weight. They were incubated at 25°C at night and 30°C during the day in the greenhouse. Germination percentage and days for germination were investigated.

### Seed dormancy

Seeds of group 2 were used for testing low-temperature effects on seed germination. Seeds sown on 27 November 1996, served as the control plot. Subsequent sowings were on 25 December (28 days after the control plot), 27 January 1997 (61 days after the control plot), 28 February (93 days after), and 28 March (121 days after). The seeds were germinated in 128-hole plastic trays 3 cm × 3 cm. They were incubated at 15°C by night and 25°C by day in a greenhouse. The number of the germination days and the germination percentages were tested with and without the cold treatment.

## Results and Discussion

### Obtaining seeds through natural crossing

Many capsules were formed in the female plants on the northwest side close to the male variety, especially at the east and west edge of each line. Seed number per square metre was less than 50 in almost all plants. However, more than 50 seeds m<sup>-2</sup> were found in the plot close to male cultivars, Sagamiwase and Tokkuri (data not shown). As a result, 3726 seeds were obtained from 6488 capsules. The shape of flowers in male and female varieties is shown in Table 1. The height and the width of the flowers were only 1.7 and 2.5 mm in female plants, and 2.4 and 2.4 mm in male plants of cv. Tsukui, respectively. Moreover, petal width was less than 1 mm when opened. Anemophilical pollination was not likely because the pollen grains were sticky. From August 5–15, many small drosophila-like flies gathered among the male or female flowers. However,

**Table 1** Shape of the flower

	Yamatoimo		Tsukui	
	mean (mm)	C.V.	mean (mm)	C.V.
Height of flower	1.7	8.9	2.4	12.5
Width of flower	2.5	6.3	2.4	4.8
Width of petal opening	0.8	36.4	0.4	41.7
Length of ovary	4.7	8.1	—	—
Width of ovary	1.9	7.2	—	—

C.V. is Coefficient of Variation

from August 18–28 when flowering ended, they decreased and aphids increased. Not only the narrow width of the petal, but also the strong cinnamon flavour suggests that the flies may be involved in pollination. These results thus indicate that *D. opposita* bears sufficient fruits and seeds following mixed planting of male with female varieties.

### Seed size and germination

The fruits of *D. opposita* were composed of three compartments, mean height was 11.2 mm, and radius was 16 mm. They appeared to be thicker and smaller than those of *D. japonica* which is a wild species in Japan. As the seed matures, there is a chord consisting of a circle about 10 mm in diameter, a large endosperm, and a wing which surrounds it. Seeds were classified into groups from 1 to 4 based on the degree of endosperm growth:

**Table 2** Germination of classified seeds

Group	No. of seeds sown	No. of seeds germinated	Germination percentage	Number of days for germination	S.E.
1	128	44	34.4	19.8	49.3
2	128	4	3.1	18.8	48.3
3	128	3	2.3	28.3	5.3
4	128	0	—	—	—

S.E. is Standard error

**Table 3** Germination percentage and the number of days

Low temp. treatment	Days treated	Germination percentage (%)	Number of days	S.E.
—	0	8.6	79.3	89.8
	28	6.3	69.5	114.0
	61	3.9	64.6	324.3
	93	3.9	52.0	94.5
+	121	3.1	39.0	0.0
	28	8.6	68.0	42.8
	61	5.5	54.4	116.6
	93	3.1	34.8	87.6
	121	8.6	28.3	38.6
Days treated <sup>a</sup>		n.s.	**	
Low temp. treatment T-test <sup>b</sup>				
	28	—	n.s.	
	61	—	n.s.	
	93	—	*	
	121	—	**	
	Total	n.s.	*	

\*\*<sup>a</sup>, Significant at 1% level by F-test

<sup>b</sup>, significant at 5% level, \*\*, significant at 1% by t-test

n.s. is not significant

S.E. is standard error

(1) normal endosperm: albumen develops sufficiently, mushrooms out from testa; (2) endosperm is immature: albumen size is adequate but lacking in content, and the central part has collapsed; (3) arrested endosperm growth: small and not developed; and (4) endosperm not developed: arrested growth, many wither away.

In the greenhouse environment, 34.4% of group 1 seeds successfully germinated but germination percentage decreased to 3.1% in group 2 and 2.3% in group 3. No seed germination was observed in group 4 (Table 2). When digging up the soil in the tray after the germination test, there were many germinated seeds with elongated radicles in group 2. However, their original germinating ability diminished until the sprouts could no longer survive. In particular, because germination took about the same number of days as general crops, the occurrence of crust on soil surface as well as seed-eating insects, lichen, and fungi, became major obstacles to germination. It took more days in group 3 than group 1 and 2 for seed germination, and the seeds of groups 3 and 4 did not develop roots in the soil. The germination rate of the mature seeds such as those in 1 and 2 was high in some plots, but they did not coincide with the plots setting many fruits and seeds.

### Seed dormancy

The germination test results are shown in Table 3. The germination rate failed to reach 10% in all plots, and no difference was found in the rates. On the other hand, the longer the cold treatment, the fewer the days needed for germination. In the plots receiving cold treatment for 61-121 days, germination took 15-40 days fewer than for the non-treatment plots. Thus, in the plots sown on 27 November, the germination rate reached 8.6% at 79.3 days, whereas in the plot sown after maintaining cold temperature at 5°C from 27 November until 28 March of the following year, 8.6% germinated 28.3 days thereafter. In conclusion, *D. opposita* takes several months as does *D. rotundata* (Sadik, 1977). These seeds have a 2-3 month dormancy period, and immediately after sowing they show dormant characteristics and take a number of days to germinate, in accordance with which there are fewer days until germination. It was clear that cold treatment was effective in reducing the number of days until germination.

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## Poster

# Functional analysis of the cassava vein mosaic virus promoter and its usage for cassava genetic engineering

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Cassava vein mosaic virus (CsVMV) is a plant pararetrovirus infecting cassava plants in Brazil. The promoter which directs the synthesis of the terminally redundant genome length viral ribonucleic acid (RNA), was isolated and used to express heterologous genes in transgenic plants. A deletion analysis of the upstream region of the promoter was carried out in order to study its functional structure. Effects of the deletions were examined in tobacco plants using the *uidA* reporter gene. Additional data were obtained by protoplast transfection experiments and *in vitro* deoxyribonucleic acid (DNA) binding assays. The constitutive pattern of the promoter expression is due to the promoter sequence of organo-specific *cis* elements. The specificity of the virus for cassava plants and the constitutive as well as the molecular function of its promoter, make it a good alternative to the 35S.

Keywords: Cassava mosaic vein virus; Genetic engineering; Promoters; Pararetrovirus; Brazil

Cassava vein mosaic virus (CsVMV) is a double-stranded deoxyribonucleic acid (DNA) virus that infects cassava plants in Brazil. Electron-microscopy studies of infected plants showed isometric viral particles localized in all cell types and accumulated in cytoplasmic inclusion bodies (Kitajima and Costa, 1996). Based on these data, CsVMV was listed as a putative member of the *Caulimovirus* genus. Consensus sequences of pararetroviruses were found in the CsVMV genome suggesting that this virus has a replication mechanism similar to that of the badnaviruses and caulimoviruses. However, the genomic organization of the CsVMV exhibits specific features not found in other pararetroviruses (Calvert *et al.*, 1995; de Kochko *et al.*, 1997). The CsVMV genome is 8159 nucleotides long and is organized in 4 (maybe 5) open reading frames (ORF) (GenBank accession # U59751). Consequently, it was suggested that the CsVMV might be representative of a new genus of plant pararetroviruses.

Different transcriptional promoters used to express foreign genes in transgenic plants have been isolated from plant pararetrovirus genomes. The 35S promoter of the cauliflower mosaic virus (CaMV) directs a constitutive gene expression in both monocotyledonous and dicotyledonous transgenic plants (Odell *et al.*, 1985; Terada *et al.*, 1990; Yang and Christou, 1990). Similarly, the promoter 34S from the

figwort mosaic virus (FMV) is active in all tissues of transgenic plants and is of comparable strength to the 35S promoter (Sanger *et al.*, 1990). Promoters were also isolated from the rice tungro bacilliform virus (RTBV) and the commelina yellow mottle virus (ComYMV). These two promoters isolated from badnaviruses displayed a vascular specific gene expression pattern in transgenic plants (Medberry *et al.*, 1992; Yin and Beachy, 1995). As a pararetrovirus infecting cassava, the CsVMV was a strong candidate to isolate a new promoter to express genes in transgenic cassava plants.

### Isolation and Expression in Transgenic Plants of the CsVMV Promoter

Sequence analysis of the CsVMV genome allowed the identification of a consensus TATA box similar to that present in plant pararetrovirus promoters. Specific primers were used to amplify, by polymerase chain reaction, a 511 nucleotides fragment containing the TATA motif. This fragment was fused to the coding sequence of the *uidA* reporter gene (coding for the  $\beta$ -glucuronidase, GUS; Jefferson *et al.*, 1987) and the resulting chimeric construct was used to study promoter expression. A primer extension experiment showed that this CsVMV

DNA fragment could initiate the transcription 35 nucleotides downstream of the putative TATA box. According to this transcription start site, the CsVMV promoter fragment extends from position -443 to +72. This fragment was able to cause a high level of gene expression in protoplasts isolated from tobacco or cassava cell suspension. These preliminary experiments showed the CsVMV promoter was of similar strength to the enhanced 35S promoter. Genetic transformation of cassava plants was not accomplished when this project was initiated. Consequently, the expression pattern of the CsVMV:*uidA* fusion gene was first analysed in transgenic tobacco and rice plants (Verdaguer *et al.*, 1996). The results suggested that the CsVMV promoter is more active in vascular elements, in cells containing chloroplasts, and in meristematic zones. The CsVMV promoter was active in all organs tested and in different cell types independently of the developmental stage. The expression pattern was similar in transgenic tobacco and rice plants, suggesting that promoter activity is not dependent on transcriptional factors specific to a plant species.

This study shows that the CsVMV promoter is a strong promoter capable of expressing genes in transgenic plants. Because of its constitutive properties, the CsVMV promoter can be used in plant biotechnology as an alternative to the widely used 35S CaMV promoter.

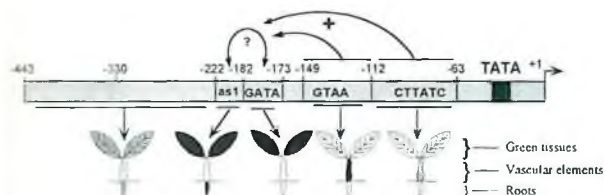
### CsVMV Promoter Expression in Cassava Plants

Micro-bombardment experiments on cassava tissues using the CsVMV:*uidA* fusion gene have been carried out (Verdaguer *et al.*, 1996). These experiments provided the first evidence of promoter activity in cassava plants. Preliminary results of transgenic cassava plants carrying the CsVMV:*uidA* gene showed that the CsVMV promoter is very active in the vascular elements. The GUS staining was also detectable in leaf tissues and was somehow stronger in the younger leaves. Efficiency of the CsVMV promoter to express genes of impact in cassava plants was confirmed by transformation experiments using a plasmid containing the *nptII* gene that confers resistance to the aminoglycoside family of antibiotics. Transgenic cassava plants that carry a CsVMV:*nptII* fusion gene were regenerated after selection on medium containing paramomycin. Also, the CsVMV promoter was used to express the coat protein of the cassava common mosaic virus (CsCMV) in transgenic cassava. Western analysis performed on leaf extracts from regenerated plants showed a high level of accumulation of the CsCMV coat protein.

### Promoter Elements and Deletion Constructs

A deletion analysis of the region upstream of the TATA box of the CsVMV promoter was carried out to identify important *cis*-regulatory elements. A better control of gene expression in transgenic plants could be achieved through a better understanding of the mechanisms of promoter regulation. Likewise, an enhanced version of the 35S CaMV promoter was constructed after the identification of its enhancer region (Kay *et al.*, 1987). It has also been shown (Benfey and Chua, 1990) that the 35S CaMV promoter pattern of expression is controlled by different modules which have different tissue-specific functions. Accordingly, it was possible to alter the promoter's profile of expression by specific deletions or duplication of tissue-specific elements. The CsVMV promoter has very little sequence homology with the 35S CaMV promoter and the 34S FMV promoter. This suggests the presence of different *cis*-elements and therefore the possibility of different regulation mechanisms. The deletion analysis of the CsVMV promoter was thus undertaken to address this question.

A set of deleted promoters were engineered and cloned upstream of the *uidA* gene. The promoter activity of the different deletions was monitored in transgenic tobacco plants using the expression of the reporter gene. Different staining patterns as well as significant and reproducible differences in the staining intensity were detected between promoter constructs. These differences indicated the effect of the deletions on promoter function. The results showed that the constitutive pattern of the CsVMV promoter in transgenic plants is due to the interaction between distinct specific domains. A domain that control promoter expression in the vascular elements was identified between the position -173 to -63. This domain, when associated with the TATA box region is sufficient to direct a high level of gene expression in vascular elements of transgenic tobacco. The region spanning from nucleotides -222 to 173 contains *cis*-elements that control promoter expression in green tissues and in root tips. In this region, the present results suggested that a sequence homologous to the activating sequence 1 (*as1*) previously identified in the 35S CaMV promoter (Lam *et al.*, 1989) is involved in the root tissue expression. Expression in mesophyll cells might be controlled by both the *as1* element and a GATA motif. Also, it was shown that additional sequence elements located between positions -149 and -64 are required for promoter activation in green tissues. Since these latter elements cannot direct gene expression in mesophyll cells by themselves, it is probable that synergistic interactions are involved in the



**Figure 1** Schematic representation of the functional map of the CsVMV promoter. Effects of the different promoter regions on *uidA* gene expression in transgenic plant are represented. The darkened areas on the plant model represent a strong level expression, while grey areas indicate low level expression. Arrows at the top of the figure symbolize the synergistic interactions of the different *cis* elements defined. Motifs identified that might play an important role for CsVMV promoter regulation are also mentioned. Positions relative to the transcription start site are indicated

regulation of promoter activity in green tissues.

The expression pattern as well as the type of molecular organization suggested that the CsVMV promoter is related to caulimovirus promoters. However, specific features such as a *cis*-element not identified in the 35S CaMV promoter or in the 34S FMV promoters, might indicate different regulation mechanisms.

Different promoter constructs that exhibited distinct tissue-specific profiles of expression in transgenic tobacco plants have been obtained by the authors. A promoter construct with lower expression than the full length in leaf mesophyll cells was isolated. Since this construct is active in all cell types, this pattern of expression was designated as 'constitutive weak'. Vascular-specific promoters that displayed variable levels of expression were also developed. A promoter construct which was nearly inactive in the aerial tissues of transgenic plants displayed a strong GUS staining in root tips and vascular tissues of the roots. This result suggests that this construct is preferentially expressed in root tissues.

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## Poster

# Enhanced production, recipe development, and consumption of sweetpotato in Ghana, West Africa

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One of the major forms of malnutrition in developing countries is iron deficiency anemia (IDA). This is partly linked to insufficient consumption of green leafy and deep orange flesh vegetables. Over half of the women in Ghana are involved in agriculture, and their health and economic well-being is vital to the food security of the country. Sweetpotato roots and leaves have been shown to contain high percentages of Fe and other essential minerals. The goal of this study was to increase the consumption of sweetpotato by women small farmers in the Volta Region of Ghana. Households were surveyed according to food habits and major traditional dishes, and recipes were formulated and tested in the laboratory and at a local restaurant. Results showed that the foliar mineral levels for Ca, Fe, and P ranged from 44.0–81.0, 5.2–5.7, and 33.8–47.8 mg 100 g<sup>-1</sup>, respectively. Sweetpotato leaf recipes tested under laboratory conditions showed liking-ratings from 64–68% (moderately to very much), while restaurant-tested recipes showed higher liking-ratings, ranging from 68–76% (very much to extremely).

Keywords: Sweetpotato; Women small farmers; Consumption; Households; Recipes; West Africa

The consumption and utilization of sweetpotato plants in Ghana fall behind other traditional root crops such as cassava, yam, and cocoyam. Small-scale farmers, particularly women farmers, are unaware of nutritional benefits of the roots and leaves of the sweetpotato plant (Wolfe, 1992). Presently sweetpotato roots are the only portion of the plant consumed by Ghanaians on a limited basis. However, studies have shown that sweetpotato leaves contain 70% more Fe than cassava leaves (Tsou and Hong, 1992). Sweetpotato has a shorter growing period than cassava, and it can be grown during off-season periods when traditional green leafy vegetables are unavailable. In addition, the leaves are high in Ca, Zn (Pace *et al.*, 1985), and fibre and, to a lesser extent, protein (Villareal *et al.*, 1979). Incorporation of sweetpotato leaves into the daily menu plan of the Ghanaian diet through the development of culturally relevant recipes can, therefore, enhance the utilization of the sweetpotato plant and simultaneously increase the production of this crop. This will provide not only increased income for the rural farmer but also essential

nutrients in the diet. This project was initiated to help women farmers increase production and enhance consumption of the sweetpotato plant, specifically the leaves.

## Research Design and Methodology

### Selection of cultivars, site, and foliar mineral analysis

Two local cultivars, Agbeyeye and Damadami, grown in the Volta Region by small-scale farmers and three released by the University of Ghana, were identified based on local preferences and nutritional content. The village of Adnokope in the Akatsi district, the traditional sweetpotato-producing area, in the Lower Volta-Region was selected as the site to grow cultivars for foliar mineral analysis and recipe development. Foliar mineral analysis was conducted on foliage removed at bi-weekly intervals during the crop growth period of sweetpotato plants. Foliage removed was 15 cm in length and was taken from all terminal points of the plant.



## Identification of traditional sweetpotato dishes

To enhance the acceptability and adoption of other sweetpotato dishes into the Ghanaian diet, a rapid rural appraisal (RRA) was conducted to identify traditional dishes that utilize sweetpotato as well as dishes that have the potential to incorporate sweetpotato leaves and roots.

## Results and Discussion

### Foliar analysis

Results indicated that sweetpotato leaves contain high levels of Fe, Ca, and P (Table 1).

### Traditional utilization of sweetpotato roots

Baseline surveys conducted in purposely-selected sweetpotato-growing and marketing areas in Ghana revealed that most sweetpotato roots are prepared at the household level for home consumption or as a small-scale food-selling enterprise and consumed in the following forms.

#### *Boiled (ampesi)*

The sweetpotato roots are peeled or left unpeeled when small, and washed and boiled whole in water with salt until soft. The boiled roots are strained and consumed as a snack or as an accompaniment to stews or soups for meals.

#### *Fried (koliko)*

Peeled sweetpotato roots are sliced into chunks, washed, and steeped in salt water and deep-fat fried. This form is commonly sold as snack foods.

#### *Roasted (anagote meme)*

Unpeeled medium sweetpotato roots are washed and roasted in open charcoal fire until cooked. The skin is then removed and the flesh consumed.

#### *Sweetpotato gruel (Mpotompoto)*

Peeled and sliced sweetpotato roots are covered

with water and boiled until cooked. Some of the pieces are mashed to a thick gruel or porridge and seasoned to taste. This dish is sometimes used as one of the weaning foods for infants.

### Traditional utilization of sweetpotato leaves

Traditionally, sweetpotato leaves are not used in the usual Ghanaian staple food preparations even though there are no prejudices against its use as a leafy vegetable in the diet. The foliage is normally discarded or fed to goats and sheep after harvest. Reasons attributed to this include normal cultural practice and the lack of adequate knowledge of cooking or preparation methods and acceptable recipes.

### Recipe development

Recipe development, using sweetpotato leaves or roots, was based on available data on the food habits of Ghanaians and specifically, on the major staple foods of small-scale women farmers in the Akatsi district of the Lower Volta Region. Most recipes were developed to substitute sweetpotato leaves or roots for a major ingredient in traditional Ghanaian staple dishes. Additionally, recipes based on non-traditional foods were developed to target small-scale women food sellers.

### Lab-tested Ghanaian dishes

#### *Staple leafy vegetable dishes*

*Leafy vegetable stew*—Kontomire leaves, bokor-bokor, gboma, or aleefu are shredded and stewed with meat or fish, palm oil, onions, tomatoes, seasonings, and spices (rating: 8.4, like very much).

*Palava sauce*—Prepared as above but with ground melon seeds instead of meat or fish (rating: 8.3, like very much).

*Leaf and bean stew*—Prepared as above with cooked beans instead of fish, meat, or melon seeds (rating: 8.2, like very much).

#### *Sweetpotato leaf dishes*

*Sweetpotato leaf stew*—Tender sweetpotato leaves are shredded stewed with meat or fish, palm oil, onions, tomatoes, ginger, local seasonings, and spices (rating: 6.9, like moderately).

*Sweetpotato leaf palava sauce*—Prepared as above but with ground melon seeds instead of meat or fish (rating: 7.5, like moderately).

*Sweetpotato leaf bean stew*—Prepared as above with cooked beans instead of fish, meat, or melon seeds (rating: 6.6, like moderately).

**Table 1** Foliar mineral analysis of five varieties of sweetpotato

Genotypes	Protein (%)	Ca	Fe	P
		100 g <sup>-1</sup> mg		
ITS2	5.3	81.0	5.2	42.5
91/198	5.6	75.0	5.5	33.8
82/123	5.6	84.0	5.3	45.2
Local Red (Agbeyeye)	4.4	63.0	5.6	44.9
Local Cream (Damadami)	4.5	44.0	5.7	47.8

## Lab-tested non-traditional sweetpotato dishes

### Without sweetpotato

**Cornish pastries**—Fat is rubbed into sifted flour until the mixture resembles fine breadcrumbs. A little cold water is added to form a dough. The dough is kneaded slightly, rolled out, and cut into round shapes. Filling is placed in the centre of the pastry, the pastry is folded over and sealed with a fork, then baked (rating 8.1, like very much).

**Yam fish cake**—Peeled yam is cooked, mashed, and seasoned. Flaked fish and egg yolk are added to the mashed yam and mixed well. The mixture is formed into balls, dipped in beaten egg white, rolled in breadcrumbs, and fried until golden brown (Rating: 7.6, like very much).

**Muffins**—Flour, baking powder, salt, and sugar, are all sifted into a mixing bowl. Beaten egg is mixed with milk and oil and poured into the mixing bowl and stirred until all dry ingredients are moistened and stirred into a batter. The batter is then spooned into muffin tins and baked (rating: 7.8, like very much).

### With sweetpotato

**Sweetpotato cornish pastries**—Fat is rubbed into sifted flour until the mixture resembles fine breadcrumbs. Cooked, mashed sweetpotato is added and mixed well. A little cold water is added to form a dough. The dough is kneaded slightly, rolled out, and cut into round shapes. Filling is placed in the centre of the pastry, the pastry is folded over and sealed with a fork, then baked (rating 7.2, like moderately).

**Sweetpotato fish cake**—Peeled sweetpotato is cooked, mashed, and seasoned. Flaked fish and egg yolk are added to the mashed sweetpotato and mixed well. The mixture is formed into balls, dipped in beaten egg white, rolled in breadcrumbs, and fried until golden brown (rating: 7.7, like very much).

**Sweetpotato muffins**—Flour, baking powder, salt, and sugar are sifted into a mixing bowl. Beaten egg is mixed with milk and oil and the mixture is added to cooked, mashed sweetpotato and creamed well. The mixture is poured into the mixing bowl and stirred until all dry ingredients are moistened and stirred into a batter. The batter is then spooned into muffin tins and baked (rating: 7.1, like moderately).

## Restaurant-tested sweetpotato dishes

### Traditional

**Sweetpotato fries**—Peeled sweetpotato is sliced into pieces, cooked in brine, drained, cooled,

dipped in egg white, coated with seasoned bread crumbs, and deep-fat fried until golden brown (rating: 68%, like very much to like extremely).

**Sweetpotato meatballs**—Shredded potato greens are mixed well with ground beef, chopped onions, garlic and ginger, and salt and pepper. Bind the mixture with egg yolk, shape into balls, coat with flour, and brush with beaten egg white. Fry until cooked (rating: 68% like very much to like extremely).

### Non-traditional

**Sweetpotato stew**—Shredded tender sweetpotato leaves are cooked with meat or fish, palm oil, onions, tomatoes, fresh grated ginger, local seasonings, and spices (rating: 64% like very much to like extremely).

**Sweetpotato potpie**—Brown ground meat, chopped onions, garlic and fresh ginger, tomato puree, seasoning, cooked for 10 minutes. Cook sweetpotato mashed with margarine and warm milk. Grease pie dish and line with half mixture. Fill to the rim with the meat mixture and cover with rest of the sweetpotato mixture, and brush with beaten egg white and baked (rating: 76% like very much to like extremely).

## Conclusion

Sweetpotato leaves can be used as acceptable substitutes for traditional leafy vegetables in Ghanaian dishes and may be used to provide Fe, Ca, and P nutrients to the diet. A wide range of uses of sweetpotato roots for both traditional and non-traditional Ghanaian foods exists. The adoption of the recipes developed, would enhance the potential of sweetpotato widespread utilization for improved economic and nutritional benefits.

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## Poster

# Characterization and use of lactic acid bacteria in traditional *gari* production

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Three varieties of cassava, TMS 30001, Fernando Pó, and Precoce de Angola were subjected to a spontaneous fermentation under pressure as occurs in traditional preparation of *gari*. The fermentation was carried out at 30°C for two days with regular measurement of pH. After two days, dominating lactic acid bacteria were isolated (total of 30 isolates), and characterized by testing their ability to ferment 49 different carbohydrates (API 50CH) together with the API patterns from 240 different lactic acid bacteria. Data were processed by Jaccard ( $S_j$ ) coefficient and unweighted pair of group algorithm with arithmetic averages. At the  $S_j$ -similarity level of 75%, members of *Leuconostoc mesenteroides*, *L. paramesenteroides*, *L. pseudomesenteroides*, *Lactobacillus plantarum*, and *Lactococcus lactis* subsp. *lactis* were found together with unidentified stragglers. Selected isolates were evaluated as starter cultures for production of *gari*. A good performance of the inoculates was characterized by a decrease in pH from 6.5 to about 4.0 in 24 h giving good organoleptic qualities. The results showed that by using selected starter cultures, the fermentation time can be significantly reduced, thus ensuring a more stable product quality for potentially industrial production.

Keywords: Cassava; *Gari*; Starter culture; Lactic acid bacteria

In Mozambique, *gari* (locally called *rali*) is made by peeling and grating fresh cassava roots. The resulting mash is packed in sacks and pressed using heavy objects to reduce the high moisture content, before it is roasted in ceramic pans to yield the final product.

During the de-watering process which takes 3–5 days, a spontaneous fermentation occurs. This is a desirable process since the development of a lactic acid bacterial flora contributes to the sensory qualities of the final product (Ngaba and Lee, 1979; Dougan *et al.*, 1983). The fermentation flora in *gari* can also reduce the cyanogenic compound, linamarin, existing in cassava roots by the exogenous production of linamarase (Giraud *et al.*, 1992).

The spontaneous fermentation flora involved in *gari* production is reported to belong to *Leuconostoc*, *Lactobacillus*, *Alcaligenes*, and *Corynebacterium* (Okafor, 1977), *Candida* spp. (Collard and Levi, 1959; Okafor, 1977; Ejiolor and Okafor, 1981). The cassava fermentation, meanwhile, follows a typical spontaneous lactic acid bacterial fermentation in which a variety of micro-organisms are present at the beginning, but as the fermentation progresses, the other species are gradually overgrown by species of lactic acid bacteria (Ngaba and Lee, 1979).

In the present study, some predominant lactic acid bacteria occurring in spontaneously processed *gari* were isolated and characterized.

Some of these bacteria were tested as single-strain starters for *gari* production with the intention of shortening the time of fermentation.

## Materials and Methods

Three 12-months old varieties of cassava (*Manihot esculenta* Crantz), TMS 30001 (TMS), Fernando Pó (F. Po), and Precoce de Angola (P. Ang.) were obtained from Umbeluzi Agronomic Station in Maputo (Mozambique). Their roots were mashed using a kitchen grater to simulate the traditional procedure, but in a controlled process. One hundred grams of mash from each cassava variety were wrapped in a sterile cloth with a large pore size, put under pressure (using sterile 0.5-kg metal weights), and left to be de-watered at 30°C, while fermenting. Fermentation was interrupted after two days. Duplicate batches were made and pH was measured regularly.

## Isolation of lactic acid bacteria

After fermentation, 10-g samples were taken from all batches, mixed with 90 mL of sterile 0.9% NaCl solution and treated in a Stomacher apparatus for 2 min at normal speed. Thereafter, the samples were properly diluted and inoculated on MRS agar (Oxoid) at pH 5.5 and on Rogosa agar (Difco), and incubated anaerobi-

cally (BBL Gas Anaerobic System, Becton Dickinson, U.S.A.) at 30°C for three days. Isolates (2-6 from each batch) were randomly selected from the countable MRS agar plates, purified on MRS agar, and stored as dense cultures in freezing buffer (Ahrné *et al.*, 1989) at -80°C. A total of 30 isolates were stored.

### Phenotypic characterization of isolates

The ability to ferment 49 different carbohydrates was tested by the API 50CH test kit (API systems, S.A., Montalieu Vercieu, France) according to the manufacturers' instructions. Inocula were prepared in MRS broth. Before inoculation in API medium, the cultures were washed once in 0.9% NaCl solution. Tests were read after incubation at 30°C for 2 days and 7 days. Results were rated from 0 (violet) to 5 (yellow), and 3-5 were scored positive.

### Numerical analysis

The data matrix which consisted of 49 characters and 30 isolates were compared to a larger data set from 240 different lactic acid bacteria, including the 68 type strains of *Lactobacillus* and a number of type strains of *Lactococcus* and *Leuconostoc*. Data were examined with the Jaccard coefficient,  $S_j$  (Sneath, 1978) and clustering achieved with the unweighted pair group method using arithmetic averages, UPGMA (Romersburg, 1984). The BioNumerics program was used (Applied Maths, Kortrijk, Belgium).

### Cassava fermentation with added single-strain starters

Cassava was processed as described above from the cassava variety TMS, but was not incubated under pressure. This was done to mimic an industrial production situation where grated cassava mash is left for fermentation in a large container before pressing and frying. The cassava mash was thoroughly mixed before it was divided into smaller portions (batches). Each batch of 100 g was inoculated with  $2 \times 10^7$

colony-forming units (CFU)  $g^{-1}$  of a starter culture. Single starters were used in this study. One single starter (*L. plantarum* 97) previously isolated from Nigerian ogi, and able to ferment starch, was also included. Fermentation was performed at 30°C with continuous pH-measuring. The pH changed from 5.9 before fermentation to 4.0 and 5.0 after 24 h depending on starter cultures. After fermentation, total counts of lactic acid bacteria were checked, as well as the occurrence of the inoculated strains. The rest was frozen and stored until sensory evaluation was made.

### Sensory evaluation

After pressing and frying the fermented mashes with the selected single-strain starter, sensory characteristics of the resulting *garí* were evaluated by people who were accustomed to the product. General appearance, colour, smell, and taste were evaluated.

## Results and Discussion

### Spontaneous cassava fermentation

The three cassava varieties let to ferment spontaneously showed the expected pH decrease (Table 1). The variations in final pH could partly be explained by differences of the cassava varieties or in single cassava roots in terms of nutritional content (specially the content of easily fermentable sugars) and partly by differences in the bacterial flora present on single cassava roots. Nevertheless, the number of lactic acid bacteria was high in all batches at the end of fermentation (Table 1).

### Numerical analysis of the lactic acid bacterial flora

The fermentation patterns (as obtained on API 50CH) of the dominating lactic acid bacterial flora in the fermented cassava were numerically

**Table 1** pH changes and numbers of colony-forming units (CFU)  $g^{-1}$  obtained on Rogosa agar and MRS agar after spontaneous fermentation of three varieties of cassava

Cassava variety and batch no.	pH			$\log_{10}$ CFU $g^{-1}$ obtained on	
	before	after 24 h	after 48 h	MRS agar	Rogosa agar
F. Po 1					
F. Po 2	6.6	6.2	4.8	9.3	8.8
	6.6	6.2	5.0	9.3	9.3
TMS 1	6.4	6.2	5.4	7.6	8.6
TMS 2	6.4	6.0	4.5	7.6	8.7
P. Angola 1	6.4	6.1	4.6	9.3	9.5
P. Angola 2	6.4	6.3	5.3	9.7	8.9

compared with the fermentation patterns of 240 different lactic acid bacteria. At the  $S_D$ -similarity level of 75%, a number of different clusters were formed, most of them representing only one species of *Lactobacillus*, *Lactococcus*, or *Leuconostoc*. Among the cassava isolates, members of the following species were found: *L. paramesenteroides* (five isolates, representing at least three different strains), *L. pseudomesenteroides* (seven isolates, representing at least four different strains), *L. mesenteroides* (five isolates, representing at least four different strains), *L. plantarum* (two isolates, probably representing two different strains), *L. acidophilus*, *L. crispatus*, *L. gasseri*, and *L. jensenii* (one isolate), and *L. lactis* subsp. *lactis* (two isolates, representing two different strains). Eight strains appeared as stragglers, i.e., they did not form a cluster with any other strain at the  $S_D$ -similarity level of 75%. None of the isolates had any pronounced ability to ferment starch, the major carbohydrate compound in cassava.

The presence of a combined fermentation flora consisting of *Lactobacillus* spp., *Lactococcus* spp., or *Leuconostoc* spp. is in accordance with previous findings (Okafor; 1977; Ngaba and Lee, 1979; Ejiofor and Okafor, 1981).

#### Cassava fermentation with selected single-strain starters

The results of the addition of selected single strain starters indicate that the fermentation time can be significantly reduced, thus ensuring a more stable product quality for a potential industrial production. In terms of lowering the pH, the single-starters performing the best were *L. plantarum* FPO 1:1, *L. plantarum* 97, and *L. mesenteroides* FPO 2:3. Two of these were isolated from the spontaneous cassava fermenta-

tion. A starch-fermenting strain of *L. plantarum* has previously been isolated from cassava and characterized (Giraud *et al.*, 1994). Since the concentration of easily fermentable sugars may be a limiting factor for the fermentation process, such a strain may be favourable.

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## Abstracts of Other Papers Presented

### **Production of yellow yams (*Dioscorea cayenensis*) on small farmers' holdings in Jamaica using the minisett technology**

Thomas Burton, *Rural Agricultural Development Authority, Ministry of Agriculture and Mining, Jamaica*

Yams have been established in Jamaica to be a very high priority crop for both local and international consumption. There is need, therefore, to produce a yam which has uniform size and shape for ease of handling and packaging, and with a greater shelf-life. The minisett technique was introduced to produce such yams. On-farm trials and demonstrations were conducted to expose farmers to the technique. After one year, the farmers who had harvested their crop across seven parishes were interviewed. Of these, 77 were in favour of the technique, while 9 were not. It appeared that those who had adopted the technique had been influenced by the higher yields obtained from their plots.

### **Growth and tuber yields of sweetpotato in stress environments**

A. Oswald<sup>1</sup>, *Institute for Plant Production in the Tropics and Sub-tropics (380), University of Hohenheim, 70593 Stuttgart, Germany*

During 1990 and 1991, three series of field experiments were conducted at two sites of the Centro Internacional de la Papa (CIP) in Peru, to test various sweetpotato cultivars for their tolerance to light-limited growth conditions and their response to interspecific competition stress. No shade-tolerant cultivar was identified. In increasing shade, the

plant shoot became the stronger sink and tuber yields were reduced mainly by an altered assimilate partitioning within the plant and, to a lower extent, by a reduced assimilate production. Stress affected tuber numbers more than tuber mean weight. Shading during the tuber bulking phase of sweetpotato reduced tuber yield more severely than shading imposed at the beginning of the growth period. Interspecific competition in intercropping systems with maize caused a linear decline in tuber yield with increasing stress intensity. Yield losses were principally affected by an overall reduction in assimilate production. Therefore, criteria for selection for stress-adapted cultivars could be biomass production or tuber numbers per plant. Screening under shade should also produce cultivars which perform well in mixed cropping systems.

### **Minisett yam propagation in Jamaica**

George F. Wilson, *JAS, Jamaica Ltd, 27 Shenstone Drive, Kingston 6, Jamaica*

Although yellow yam (*Dioscorea cayenensis*) is the most important root crop in Jamaica, acreage in production has not improved over the years even when there were significant increases in demand. This steady state has been blamed on the very slow multiplication rate associated with traditional methods of yam propagation. Size and shape of tuber are also among the disadvantages associated with planting material presently used. Since 1984, the Ministry of Agriculture and Mining, with the assistance of the International Institute of Tropical Agriculture (IITA), The University of West Indies (UWI) and the Inter-American Institute for Cooperation in Agriculture (IICA) has been attempting to introduce the yam minisett technique for rapid yam multiplication to local yam growers (mainly small farmers).

Unfortunately, in spite of many demonstrations, the technique has not been widely used. To some, the failure is due to poor and uneven sprouting which deprives the farmers of uniform planting material for establishing economic plots. To others, the failure is due to a modification which used minisett material for production of ware yams, instead of producing for seed yam as is done in Africa. Still there are some who believe that using minisett material for production of smaller ware yams is not justified economically, as there is not a strong enough preference for smaller ware yams in the market. This study investigates the advantages and disadvantages of the minisett technique in yellow yam production and suggests approaches that should make the technique effective. It also looks at present attempts to modify the technique by using larger sett pieces.

### **Starch derivatives with food applications from tropical tuber crops**

S.N. Moorthy, *Central Tuber Crops Research Institute, Trivandrum, 695 017, India*

Tropical tuber starches possess a wide variability in physiochemical and functional properties useful in food applications which are not observed in cereal starches, e.g., the high viscosity, clarity, and soil stability of cassava starch; viscosity stability of aroid starches; and clarity and gel strength of yam starches and *Canna edulis* starch. However, the desirable properties are often accompanied by some negative characteristics which can be modified by physical and chemical treatments. The chemical modifications include cross-linking with bifunctional reagents and derivatisations like etherification and esterification with acids, anhydrides, and related chemicals. The Central Tuber Crops Research Institute has been able to produce modified starches from all tuber starches with improved properties.

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**Feed from root and tuber crops for ruminants: Forage yield and nutritive value of sweetpotato cultivars**

A. Larbi and J.W. Smith, *International Livestock Research Institute (ILRI), Humid/Subhumid Zone Programme, Nigeria*

H.N. Nwokocha, *National Root Crops Research Institute (NRCRI), PMB 7006, Umuahia, Nigeria*

R.O. Balogun, *National Animal Production Research Institute, PMB 1096, Zaria, Nigeria*

Sweetpotato [*Ipomoea batatas* (L.)] tops and roots unsuitable for human consumption are used in the fresh or dried form for livestock feeding in smallholder crop-livestock farming systems in Asia, sub-Saharan Africa, and South America. However, selection for dual-purpose cultivars for forage and root production has not received much research attention. Forage (plant tops) and root yields of 20 sweetpotato cultivars (C) were determined at 8, 12, 16, or 20 weeks after planting (WAP) at Umudike (humid forest) and Otobi (southern Guinea savanna) in south-eastern Nigeria. Crude protein and dry matter loss after 48 h of incubation in rumen-fistulated N'dama steers were determined for plant tops harvested at 12 and 20 weeks. The WAP  $\times$  site and WAP  $\times$  C interactions were significant for forage and root yields. Forage yield was higher at Umudike than Otobi (19.2 vs 13.1 t ha<sup>-1</sup>). In contrast, marketable root yield at Otobi (9.8 t ha<sup>-1</sup>) was higher than Umudike (6.6 t ha<sup>-1</sup>). At Otobi, cultivar TIS-8164 gave highest forage (22.8 t ha<sup>-1</sup>) and marketable root (17.1 t ha<sup>-1</sup>) yields. At Umudike, forage yield was highest in cultivar TIS-8470 (30.9 t ha<sup>-1</sup>) whilst marketable root yield was highest in cultivars TIS-8164 and TIS-86/0306 (12.6 t ha<sup>-1</sup>). Overall, forage yield peaked between 12 to 16 WAP while marketable root yield increased linearly from 8 to 20 WAP. Crude

protein (mean: 106 + 6.7 g kg<sup>-1</sup> at 12 WAP, and 89 + 7.3 g kg<sup>-1</sup> at 20 WAP) and dry matter varied among cultivars. Promising cultivars for forage and root production in smallholder crop-livestock systems include TIS8470 and TIS-8504.OP.162.

**Cassava tuberization under mid-altitude and lowland savannah agro-ecological zones of Nigeria**

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I.J. Ekanayake, *TRIP, International Institute of Tropical Agriculture (IITA), P.M.B. 5320, Ibadan, Nigeria*

Twelve cassava genotypes were grown at two field sites (Jos: 18  $\pm$  5°C, Ibadan: 27  $\pm$  6°C) from 1994-96 to examine the onset of tuberization (TITO), tuberous root number (TRN), and tuberous root dry weight (TRDW). Harvesting was done at 3, 6, 9, and 12 months after planting (MAP). Significant differences ( $P < 0.05$ ) in TRN and TRDW were observed among the four environments [Ibadan (1994 and 1995) and Jos (1994 and 1995)] throughout the growing period. Genotypic differences ( $P < 0.05$ ) were observed among the genotypes either across locations or within locations for TITO, TRN, and TRDW. Early initiation of storage organs was observed in cultivars TMS 4(2)1425, TME1, and TMS 30572 across both locations. At Ibadan, TMS 30572, TMS 4(2)1425, and TME1, and at Jos, TMS 30572 and TME1 were genotypes to show the highest number of tubers at 3MAP. Genotypes TMS 4(2)1425, TMS 30572, and TME1 had more TRN while TMS 50395, TMS 30572, TMS 4(2)1425, and TMS 91934 had greater TRDW at Ibadan. At Jos, TMS 30572, Danwaru, and TME1 had more TRN than other genotypes whereas TMS 30572 and TME1 were better for TRDW. The results showed that low tempera-

tures can induce a delay in onset of the tuberization in cassava which results in an economic yield reduction. Some of the genotypes identified for mid-altitudes may be useful for further breeding programmes targeted for higher altitudes.

**Etiologie d'une pourriture des tiges et des racines du manioc (*Manihot esculenta* Crantz) dans les zones akposso et adele au Togo**

Tchabana Bèrè, *Laboratoire de Phytopathologie, BP 2318, Lomé, Togo*

Sur le Plateau de Danyi, au Togo, une pourriture des boutures, de la base des rejets et des racines de manioc dans les trois mois qui suivent la plantation provoque des pertes importantes (jusqu'à 30% de mortalité). L'étude étiologique de la maladie a permis de mettre en évidence le rôle d'un agent pathogène principal, *Lasiodiplodia theobromae* Pat. ainsi que l'intervention de parasites secondaires (*Fusarium* sp., *Diaporthe manihotis*, *Enterobacter cloacae*). L'agent pathogène peut contaminer le matériel végétal avant plantation, la conservation trop longue des boutures après récolte intensifie cette contamination. La présence d'un inoculum d'origine tellurique est suspectée. Pour faire face à cette incidence, des conseils sont donnés sur l'orientation de la lutte contre le parasite.

**Estimating cyanogen content in cassava: New approach based on existing ideas**

Hock-Hin Yeoh, *School of Biological Sciences, National University of Singapore, Kent Ridge, Singapore 119260*

The dip-stick technique commonly and conveniently used for clinical diagnostics was borrowed in developing a dip-stick for detecting linamarin/cyanogenic potential. It was based on the hydrolysis of linamarin by pH-buffered linamarase-impregnated paper

and detection of liberated HCN with alkaline picrate paper. The analysis must be carried out in a closed system due to the nature of the reaction. It could detect from as low as 0.5 to 40 µg hydrocyanic acid (HCN). The dipstick could be used for semi-quantitative or even quantitative measurement of cyanogenic potential in cassava and cassava products (e.g., flour). It was easy to prepare and had a long shelf-life even at room temperature. It would be potentially useful as a field method and could readily carry out large numbers of analyses.

**Protein enrichment of cassava flour by solid state fermentation**

Remigio Zvauya and Modern Muzondo, *Department of Biochemistry, University of Zimbabwe, M.P. 167, Mount Pleasant, Harare, Zimbabwe*

The protein enrichment of cassava flour was achieved during solid state fermentation using the fungus *Aspergillus oryzae*. The protein content increased from 1 to 19% after 50 h fermentation. The reducing sugars, ash, lipid content, and dietary fibre increased while the starch content decreased from 80 to 49 g 100 g<sup>-1</sup> substrate. The amylolytic enzymes were active in the stationary phase. The nucleic acid content of the product increased from 0.04 to 0.48 g 100 g<sup>-1</sup> substrate on dry weight basis. The total cyanogen levels decreased from 158 to 54.2 mg kg<sup>-1</sup> dry weight as a result of the process. The cyanogenic glucoside decreased by 88% during the fermentation process while the acetone cyanohydrin was retained in the cassava. The pre-fermentation process which involved crushing, sun-drying, and milling of the cassava flour reduced the total cyanogen levels by 40%. The whole process resulted in considerable reduction in the cyanogenic content of the product and an improvement in the nutritional quality of the fermented product.

**Feed from root and tuber crops for ruminants: Variation in rumen degradation of whole plant tops among sweetpotato cultivars**

Hideo A. Larbi, J.W. Smith and I. Etela, *International Livestock Research Institute (ILRI), Humid/Subhumid Zone Programme, PMB 5320, Ibadan, Nigeria*

H.N. Nwokocho, *National Root Crops Research Institute (NRCRI), PMB 7006, Umuahia, Nigeria*

N. Anyawu, *International Livestock Research Institute (ILRI), Humid/Subhumid Zone Programme, PMB 5320, Ibadan, Nigeria*

Rumen degradation characteristics are important predictors of voluntary intake and animal growth rate of a forage. However, information on variation in rumen degradation characteristics of forage among sweetpotato cultivars which could be useful in the selection of cultivars for root and forage production is limited. Forage (plant tops) of 20 sweetpotato cultivars was harvested at 12 and 20 weeks after planting (WAP) at Umudike in the humid forest zone of south-eastern Nigeria to determine variation in rumen degradation characteristics. Samples were oven-dried at 60°C and ground through 2.5 mm screen. About 5 g were weighed into 9 cm x 18 cm nylon bags of pore size 41 µm, and incubated in duplicate in rumen-fistulated N'dama steers for 6, 12, 24, 48, 72, and 96 h. Degradation constants were estimated by fitting data to the non-linear model:  $P = a + b(1 - e^{-ct})$  where  $a$  = zero time intercept,  $b$  = insoluble but fermentable fraction in time  $t$ ,  $c$  = degradation rate constant of the  $b$  fraction,  $P$  = extent of degradation ( $a + b$ ), and  $ED$  = effective degradability at rumen outflow rate of 0.03 ( $ED = a + bc(c + 0.03)$ ). Variations in crude protein (ranges: 12 weeks: 43–153; 20 weeks: 68–131 g kg<sup>-1</sup>) and degradation constants among

cultivars was significant ( $P < 0.001$ ). Results indicate cultivars could be grouped into high, medium, and low quality groups based on effective degradation.

**Photosynthetic photochemical response of cassava (*Manihot esculenta* Crantz) to water stress and mycorrhizal inoculation**

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Studies were conducted to observe the influence of vesicular-arbuscular mycorrhizal (VAM) fungi on photochemical photosynthetic efficiency of cassava to irrigation regimes. A drum experiment was conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, during the 1995–96 growing season. Two cassava clones [TMS 4(2)1425 (improved clone) and TME1 (landrace)] were planted each in 75 kg of sterilised soil. The bags were spaced 1 m x 1 m apart. Three treatments of VAM fungi applications were used (*Glomus clarum* and *G. mosseae*) to inoculate two sets separately, while the third set served as the control with no inoculation. The plants were watered on alternate days. At two months after planting, one half of the plants in each treatment was subjected to water stress. All treatments were replicated three times. Results obtained from a portable chlorophyll fluorescence probe indicated that inoculation of VAM fungi enhanced photosynthetic photochemical efficiency of light reac-



tions of the photosystem 11 (PS11) in intact leaf tissue. Water stress significantly ( $P < 0.05$ ) reduced the photochemical efficiency. Leaf chlorophyll contents of the leaves were also increased by VAM inoculation. Photochemical efficiency is a potentially useful criterion in selecting for drought tolerance and nutrient use efficiency associated with photosynthesis in cassava.

#### Determination of cyanogens in fresh and processed cassava using improved solid-state picrate method

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Leon Brimer, *Department of Pharmacology and Pathobiology, Royal Veterinary and Agricultural University, 13 Bulowsveg, DK-1870, Frederiksberg C, Copenhagen, Denmark*

The total cyanogens for fresh and processed cassava were determined using the improved solid-state picrate method. The total cyanogen contents were not significantly different ( $P > 0.05$ ) from those obtained by Cooke's standard enzymic method. For instance, fresh roots and dry cassava gave  $52.6 \pm 3.6$  and  $52.1 \pm 2.0$  and  $109.4 \pm 9.0$  and  $102.8 \pm 9.3$  mg HCN equivalent (eq.)  $\text{kg}^{-1}$  fresh weight by the solid-state and Cooke's methods, respectively. Similar results were obtained for a commercial product. Tapico crisps gave  $141.1 \pm$

$2.9$  and  $143.3 \pm 1.5$  mg HCN eq.  $\text{kg}^{-1}$  fresh weight, respectively. Cyanogen contents as low as 10 mg HCN eq.  $\text{kg}^{-1}$  are detected by the improved solid state assay. Replacing the pH 6.00 buffer with pH 7.00 or 8.00 and without enzyme provided the non-glucosidic cyanogens which were not significantly different from those obtained by the Cooke's assay ( $P > 0.05$ ). For example, fresh roots gave  $25.7 \pm 1.7$  and  $25.1 \pm 4.3$  mg HCN eq.  $\text{kg}^{-1}$  while the partially dried grated cassava gave  $288.1 \pm 2.1$  and  $276.0 \pm 18.1$  mg HCN eq.  $\text{kg}^{-1}$  fresh weight by the Cooke's and solid state assays, respectively. The advantages of the solid state technique over the traditional Cooke's enzymic assay are discussed.

#### Growth of callus in yam explants influenced by 2,4-D and kinetin concentrations and visible light

Angela T. Alleyne, Leonard O'Garro and Ashton J. Delauney, *Department of Biological and Chemical Sciences, Faculty of Science and Technology, Cave Hill Campus, The University of the West Indies, Bridgetown, Barbados*

Callus formation in stem, leaf, and petiole explants of yam (*Dioscorea alata*) was affected by phenolic-induced browning *in vitro* and combinations of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin. Generally petiole explants formed callus faster and more abundantly than leaf and

stem explants on Murashige and Scoog (MS) media amended with 4 mg  $\text{L}^{-1}$  2,4-D and 0.05 mg  $\text{L}^{-1}$  KI. Yam explants when treated with ascorbic acid, polyvinylpyrrolidone, sterile molten wax, and various light wavelengths in attempts to reduce browning, were affected to varying degrees. Only yellow light and orange light to a lesser extent, were effective in reducing extensive browning of these explants.

#### Drying characteristics and the quality of fine cutting sweetpotato

Hideo Fukazawa, Kenichi Yukushido and Hisashi Hosokawa, *Department of Upland Farming, Kyushu National Agricultural Experiment Station, Miyakonojo, Miyazaki, Japan*

For high quality drying roots of sweetpotato, the drying characteristics for cutting form, suitable drying temperature, and air blow mass were researched. Cutting form before drying process, compared with julienne strip dice cut, had good shape and cutting face. Sweetpotato cultivar 'Ayamurasaki' had high anthocyanin content, purple pigmentation, vitamins, minerals, and fibres. One effect of anthocyanin decrease and drying characteristics by drying stress, was that drying time was about 2 h on the air-blow mass 0.015 m  $\text{kg}^{-1}$  (HO) s and air temperature was 60. Decrease of anthocyanin between drying, for the first 1530 min decreased by 20 compared with raw roots. Total decrease of anthocyanin was about 2030 before drying.

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