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Analysis of the need for biotechnology research on cassava, yam, and plantain[1]

R. Asiedu, S.Y.C. Ng, D. Vuylsteke, R. Terauchi and S.K. Hahn

International Institute of Tropical Agriculture, PMB 5320, Ibadan, Nigeria

Abstract

Cassava, yam, and plantain are staple food crops essential to food security in the humid and subhumid tropics of Africa. However, these crops have long growth cycles, high perishability and slow multiplication rates of propagation materials, and they are subject to a number of biological stresses. These stresses include diseases, insects, mites, nematodes, and weeds. There are gaps in our knowledge, especially in the area of genetics, which influence the pace of breeding. Intensification of research is needed in cytogenetics, molecular genetics, in vitro culture, cryopreservation, and disease diagnosis.

Cassava (*Manihot esculenta* Crantz), yam (*Dioscorea* spp.), and plantain (*Musa* spp.) are major food crops in much of humid and subhumid Africa and a major source of energy for millions of people in these regions. Their adaptation to the food and farming systems and their multiplicity of uses make them indispensable to food security. They are grown mainly by subsistence farmers, but commercialization is increasing in some countries.

The planting materials of the three crops have slow multiplication rates and, like the produce, are bulky to transport. All three crops are highly perishable and thus suffer severe post-harvest losses; also, their long growth cycles expose them to various biotic and abiotic stresses, some of which could be avoided by short-duration crops. The major biological constraints to increased and sustainable production are diseases, insects, mites, nematodes, and weeds. These constraints, in conjunction with the limited use of inputs in traditional cultivation systems, have kept the yield levels of most cultivars far below their potential.

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Constraints to Production and Research

Cassava

African cassava mosaic virus (ACMV) is widespread and is the most important insect-borne disease of cassava in Africa. Caused by a gemini virus (Bock and Woods 1983) and transmitted by the white fly *Bemisia tabaci*, it can cause yield losses of up to 95% in Africa. The International Institute of Tropical Agriculture (IITA) has successfully incorporated resistance to ACMV into high-yielding cultivars. More research is required to identify any existing pathogenic variations and to explain the mechanism(s) of resistance, as well as the influence of environmental factors, such as ambient temperature, on symptom expression.

Cassava bacterial blight disease (CBB) occurs in many countries in South America and Africa, often resulting in complete crop failure in susceptible cultivars. It is caused by *Xanthomonas campestris* pv *manihotis* and leads to leaf spot, complete wilt, and tip dieback. Some clones with resistance to CBB have been developed. Cassava anthracnose and cassava root rot diseases are also widespread and can cause significant reductions in yield.

Cassava mealybug (*Phenacoccus manihoti*) and cassava green mite (*Mononychellus* spp.) are important dry-season pests of cassava. Cassava mealybug (CM) can reduce tuber root yield severely, especially in late-planted crops. Cassava green mite (CGM) can cause losses of over 40% (Nyiira 1975). Both pests are being tackled through host plant resistance breeding and biological control. Cassava varieties with moderate resistance have been developed or identified. Resistance has been shown to be associated mainly with pubescence of young leaves (Hahn et al. 1989; Kanno et al., 1991), but there are indications that antibiosis, preference, and tolerance are also involved. Additional sources of resistance are being sought from new introductions of cassava germplasm and wild *Manihot* species from Latin America. Hybrids produced recently at IITA between cassava and *Manihot tristis* (currently undergoing second-season evaluation) are fairly promising in terms of resistance to CGM. Biological control of the mealybug has been successful.

Cassava contains cyanogenic glucosides which release hydrocyanic acid (HCN) during hydrolysis, following tissue damage. Although varietal differences in levels of total cyanide have been established, no acyanogenic cultivar has been reported. The presumed relationship between HCN content and taste of the tuberous root (sweet or bitter) has led to confusion in the classification of cassava into low or high cyanide types. Research is required to clarify the situation and to positively identify what compounds account for bitterness in cassava. Moreover, the methodologies used for determining total HCN in the crop have produced variable results. Even with the best of techniques, the high variability resulting from environmental influences on field-grown materials renders most of the existing procedures in breeding programmes inefficient. There is an urgent need for more efficient screening procedures for identifying low HCN or acyanogenic clones. It is necessary to clarify the role the cyanogenic glucosides and/or their breakdown products play in the plant. Meanwhile, we can intensify molecular and conventional approaches to modify the genetic capacity of the plant to produce the glucosides. Another approach would be to increase the activity of endogenous degradative enzymes, such as linamarase, in the plant so that the glucosides can be rapidly and completely degraded for the release of the volatile toxin, hydrogen cyanide, during processing (M. Bokanga, IITA, 1990, pers. comm.). Ultimately, the best course may be to develop varieties that are low in cyanogenic glucosides but high in the appropriate degradative enzymes.

High dry-matter content of the tuberous root is desirable, but it seems to be negatively correlated with fresh tuberous root yield (Whyte 1987). Also, high dry-matter content has been associated with susceptibility to post-harvest physiological root deterioration (Jennings and Hershey 1985). Further resarch is needed on the physiological, biochemical, and genetic bases to determine the feasibility of combining high tuberous root yield, high root dry-matter content, and slow post-harvest deterioration in one genotype.

For millions of Africans, the preferred way of using cassava is direct consumption after roasting or boiling the fresh tuberous root (Hahn 1989). In some areas, the boiled root is pounded into a thick paste, often in combination with boiled plantain or cocoyam. Mealiness of the boiled tuberous root, as well as low cyanide content, is essential for these uses. Very little is known, however, about the biochemical/biophysical basis of mealiness. There is variation between cassava clones and there may be interactions between clone, age, and climate in their influence on the trait. It is important to gain a better understanding of this valuable characteristic in order to develop the ability to control it or breed more efficiently for it. Selection is hampered by the poor correspondence between young and adult plant expressions of the characteristic and the influence of climatic changes.

Cassava tuberous roots are low in protein. Attempts to breed for a higher level of protein have included the use of closely related species such *Manihot melanobasis* (Bolhuis 1953). The debate over whether to continue this effort using molecular approaches or to accept the main physiological function of cassava roots as storage organs for starch is unresolved. It has been argued that cassava is normally consumed with a protein source, such as fish or legumes; however, these accompaniments are often in short supply in communities with high dependence on cassava. On the other hand, the possibility of a trade-off between root yield and protein content should be noted. For the time being, it might be a good idea to refine fermentation processes for enhancement of the protein content of cassava products.

As cassava is cultivated under increasingly marginal conditions, its association with mycorrhizae, reported to boost its performance, ought to be investigated further.

Yam

The major yam species grown in Africa are: white yam (*Dioscorea rotundata*), yellow yam (*D. cayenensis*), water yam (*D. alata*), and trifoliate yam (*D. dumetorum*). The general preference for large tubers necessitates large planting setts, large mounds, and staking. The availability of enough good quality planting setts has often been the most serious constraint to production. The minisett technique (Otoo et al. 1985) has gone a long way towards solving the problem in the yam belt of West Africa. Sprouting leads to loss in tuber weight and quality. Some extension of tuber dormancy has been achieved through the application of gibberellic acid (GA3) (Wickham et al. 1984).

Yam mosaic virus and water yam chlorosis cause yield loss through their effects on the shoot; nematodes and yam beetles affect the tubers directly. It is likely that yam beetles would be vulnerable to alpha-amylase inhibitors (present in other foods, such as common bean), as well as to digestive proteinase inhibitors (L.L. Murdock, Purdue University, 1990, pers. comm.). Studies on the impact of such inhibitors on the growth, development, and survival of the insect when it feeds on foods containing them could lay the foundation for introducing insect resistance into yam by genetic engineering. Root-knot nematodes affect marketability and storability of tubers through discoloration and galling of the surface.

Poor flowering and asynchrony of flowering has been a major obstacle to hybridization in yams. Many cultivars do not flower at all. Flowering is erratic in some cultivars which appear to be sensitive to climatic changes in a manner not well understood. Even more frustrating are the situations where flowers of only one sex are available. A lot more work needs to be done to gain an understanding of the physiology of flowering. The role of premature necrosis of the shoot, which prevents the plants from reaching the basal vegetative phase desirable for flowering, needs further clarification.

Plantain

Black sigatoka is the most important disease of plantain at present. This virulent fungal disease, caused by *Mycosphaerella fijiensis*, is threatening plantain and cooking banana production in many African countries. IITA has identified resistant diploid wild bananas and produced resistant hybrids from crosses between susceptible plantains and a resistant wild diploid banana. The relatively rapid decline in plantain productivity in Africa requires further research. Current thinking is that it is influenced by rising mats, attacks by the banana weevil and nematodes, and declining soil fertility. Lodging can lead to serious losses. Poor seed set and viability impose restrictions on genetic improvement through hybridization.

Biotechnology Research

The need to intensify biotechnological research and to initiate new research on cassava, yam, and plantain cannot be overemphasized. In vitro culture is critical for rapid multiplication, virus elimination, germplasm exchange, and germplasm conservation. Somaclonal variation can be useful as an additional source of genetic variability, but it can be a nuisance in germplasm conservation. More research is needed on the causes and frequency of the phenomenon in plantains. Meanwhile, molecular markers could play a useful role in rapid identification of off-types. Embryo culture is a routine procedure used for many crops. In cassava and plantain, it has been very successful using fairly mature seeds, whereby the embryos were helped to germinate more readily, free from seed coat obstructions (Ng, Paper 3.4, pp 135-141 this volume; Vuylsteke, Paper 3.5, pp 143-150 this volume). Similar procedures could be used for yams. It would be even more useful to develop appropriate media that could support very young embryos of the three crops, so that these could be rescued from young fruits in order to accelerate hybridization and prevent losses through premature embryo abortion, especially in interspecific crosses, or damage to the plant.

Protoplast culture needs to be perfected to facilitate genetic transformation. Regeneration remains a bottleneck, depriving these crops of some of the new and exciting avenues for gene transfer. The promising results from somatic embryogenesis could be extended to serve as an additional avenue. Also, promising physical methods, such as using the particle accelerator to transfer DNA into meristems, ought to be pursued vigorously.

It would be useful to extend the successful cryopreservation of plantain to yams and cassava for long-term preservation without loss of totipotency or genetic integrity.

Because they are vegetatively propagated crops, the dangers of disease transmission are rather acute. Modern and efficient methods of diagnosing disease agents, including latent viruses, are critical to the safe exchange of germplasm.

Despite years of research on these crops, their reproductive biology has not been clarified. Many questions remain concerning control of flowering, compatibility of parents, and fruit development. There is poor understanding of the genomes present in the cultigens and their related wild species. The differences in ploidy, which have important influences on cross-compatibility and success of backcrossing, have not been studied systematically. The need for these basic cytogenetic studies can no longer be ignored. They would complement and accelerate studies on molecular genetics towards the ultimate goal of understanding these crops well enough to enable planned and precise genetic manipulation.

Genetic analysis is at an early stage for all three crops. The genetic control of only a few traits is known (Hahn et al. 1989) and few morphological markers have been established. This situation may be explained partly by the long growth cycles of these crops, as well as the complexity of their genetic analyses. These factors, coupled with the complexities of screening for economically important traits, call for the urgent establishment of genetic markers to help breeders make more rapid progress. For instance, linkage of biochemical/molecular markers to agronomic traits and applying them to plants at the seedling or early growth stages would considerably improve the efficiency of selection. The production of haploids through a culture of anthers/microspores or unpollinated ovaries could open up another avenue for simpler genetic analyses. Phylogenetic studies on cassava, yam, and plantain need to be accelerated using the new techniques of molecular biology.

As the cultivation of cassava, yam, and plantain shifts to marginal lands, biotechnology has an increasingly important role to play in the development of these essential crops. They are not just sources of food for the people of Africa; they constitute an integral part of African culture.

Discussion

ENE-OBONG: In cassava, can the problem of determining the nature of inheritance of resistance to mosaic virus be solved without a good knowledge of the plant's chromosomes? Can't the problem be associated with the ploidy nature, as we are dealing with polyploids?

ASIEDU: The problem is not one of determining the inheritance of resistance but of understanding the mechanism by which the resistance is expressed. In terms of inheritance, we already know that it is controlled by recessive genes.

FAUQUET: There is not one resistance to African cassava mosaic virus (ACMV) but several. A viral infection is the result of a series of events, beginning with the virus inoculation and ending with the spread of the items to the next generation. Several genes are controlling these different steps of "resistance" and their expressions can change according to the growth of the plant and the environmental conditions.

ROBERTSON: There is a simple answer as to why a virus may cause symptoms which then disappear. We have found, in both the laboratory and the field, that expression of a symptom can be related to temperature. When tissues are colder (about 25°C) virus symptoms appear, while at 28-30°C they can disappear. In a sense, the plant wins the battle against the virus when it is at its most compatible temperature.

MURDOCK: You mentioned the yam tuber beetle. This insect apparently thrives on a diet rich in carbohydrate (starch) and poor in protein. It is probably vulnerable to alpha-amylase inhibitors present in other foods, such as common bean, as well as to digestive proteinase inhibitors. Studies of the impact of such inhibitors on the growth, development, and survival of the insect when it feeds on diets containing them could lay the foundation for introducing insect resistance into yam by genetic engineering. To initiate such studies, some kind of dietary bioassary would be required. Can this insect be reared in the laboratory, and, in particular, is there a diet available on which it can be reared?

MBANASO: My comment is on increasing the protein in cassava as an area for biotechnology research for cassava improvement. I suggest this area be given a low rating in priority since cassava-based products are usually eaten with protein-enriched sauces.

ASIEDU: We ought to separate the two issues of gene splicing or transformation techinques and techniques such as the application of moleculer markers which assist breeders in doing what they are already doing by increasing their efficiency.

ROBERTSON: In the debate on whether we need a strong breeding programme before we go to genetic manipulation, it is natural that plant breeders fear that when genetic engineers make their "wild" claims they may be seen to be out of a job or, worse, restricted in their funding. Nevertheless, especially where countries have weak breeding programmes, it is possible in some cases (for example, virus resistance, insect resistance, altering protein levels and quality) to take local cultivars and improve them by the "quick fix" of single gene addition. Plant breeders need not fear, they will be needed forever. Yet simple additions can be very useful and do not, in fact, need already established breeding programmes to be in place before the genetic engineers can begin their work.

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