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AN OVERVIEW OF IITA'S BIOTECHNOLOGY ACTIVITIES FOR CROP IMPROVEMENT*

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1. INTRODUCTION

Biotechnology brings new tools, new ideas, and new approaches to agricultural research and it holds promise to help fulfil the increasing need for food production in Africa. A partnership between on-going agricultural research and biotechnology is called for and IITA and other international agricultural research centers are establishing that partnership in certain fields. Several biotechnology research possibilities relate directly to the needs of developing countries, including modifying plants to increase resistance to pests and diseases and to tolerate unfavorable growing conditions, fixing nitrogen more efficiently, providing more protein, preserving germplasm collections, and developing, and multiplying disease-free materials for exchange across national boundaries. Biotechnology can also improve efficiency and facilitate pathogen diagnosis.

Some of the crop improvement objectives for the improvement of IITA commodities cannot be easily realized by means of conventional breeding. Among these are intractable

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host-plant resistance breeding problems to some diseases and insect pests. New approaches are needed which require the transfer of resistance genes from wild species or even unrelated organisms to improved cultivars. Such intractable problems exist with maize, cowpeas, plantains, cassava and yams. New biotechnological approaches to crop improvement are likely to facilitate more rapid advances in the improvement of plantain/cooking bananas and yams where seed is difficult to obtain. Scientists at IITA are exploring new possibilities offered by biotechnology in order to apply nonconventional technologies in their breeding work, but they have already been applying biotechnology through IITA's tissue culture laboratory in germplasm conservation and distribution of disease-free *in-vitro* materials.

To consolidate the several on-going but somewhat isolated biotechnological efforts at IITA, a Biotechnology Research Unit has been formed with financial support from the Government of Italy. The new facility focuses on applied biotechnology research to serve all the crop improvement programs at IITA.

2. PRODUCTION CONSTRAINTS AND CONTROL STRATEGIES

Cassava, yam, and plantain/banana being vegetatively propagated, there are inherent problems with regards to the propagation, conservation and exchange of germplasm. Next to this impediment, other constraints such as the biotic stresses listed below, cannot easily be dealt with by conventional techniques.

2.1. Cassava

The major diseases and pests of cassava are African mosaic virus (ACMV), cassava bacterial blight (CBB), cassava green spider mite (CGM), cassava mealybug (CM) and nematodes (Hahn et al., 1989; Asiedu et al., 1992a). Yield losses due to ACMV infection range from 20 up to 95%. Tuber losses caused by CM and CGM have been estimated at up to 75%. Another problem for the utilization of cassava is that it contains the cyanogenic glucosides linamarin and lotaustralin which upon hydrolysis release hydrocyanic acid (Bokanga, 1992).

Through its breeding efforts, IITA has developed high yielding cassava clones that are resistant to ACMV and CBB, tolerant to CM and CGM, and with low cyanide content (Hahn et al., 1989). However, shy flowering in some cassava clones, particularly those that have desirable characteristics, has limited the choice of parents for genetic recombinations.

2.2. Yam

Disease and pest problems in yams (*D. rotundata* and *D. alata*) are yam mosaic virus, water yam chlorosis, yam storage rot, yam anthracnose, yam tuber beetles and nematodes (Asiedu et al. 1992a).

Shy flowering and the non-synchronization of male and female flowers are bottlenecks in yam improvement (Asiedu et al., 1992b). Thus, the choice of parents for hybridization and recombination of desirable traits is limited. A cytogenetic study on the ploidy levels, tissue culture and genetic engineering will assist in introducing desirable genes into selected plant materials.

2.3. Plantain/banana

African banana and plantain production has recently been affected by the appearance and spread of the virulent black Sigatoka leaf spot disease, which can cause yield losses up to 50%. The black Sigatoka disease is emerging as a Pan-African epidemic and due to the apparent lack of resistance in the African *Musa* genepool, black Sigatoka has become a major threat to food security in the plantain- and banana-growing regions of Africa (Swennen et al., 1992).

The banana bunchy-top virus (BBTV) is confined to relatively small areas in Gabon, Zaire, Rwanda and Burundi(Vuylsteke et al. 1989). Although it causes serious damage in Asia and the Pacific, for reasons unknown, it is not spreading rapidly and has not caused much damage.

The IITA addresses the problem of black Sigatoka through a short-term and a long-term strategy, i.e. distribution of resistant starchy alternatives and plantain/banana breeding respectively. Breeding for resistance to black Sigatoka is fraught with many obstacles specific to the biology of the preferred parthenocarpic *Musa* cultivars. Low clonal multiplication rates, lack of genetic variability, and barriers to sexual hybridization impede genetic improvement. Bananas are indeed one of the few tropical crops that have not yet been bred successfully. Hence the potential of novel, biotechnological approaches to plant improvement may be considerable, although several sophisticated methods still need to be further developed before practical benefits accrue (Vuylsteke and Swennen, 1992).

IITA, in collaboration with INIBAP, has researched and implemented existing *in vitro* culture technologies in order

to increase the efficiency of conventional multiplication and breeding procedures, but several bottlenecks remain to be solved before more advanced techniques can be applied to the improvement of *Musa*.

2.4. Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp) is an important food legume crop for the semiarid regions of Africa. It is cultivated as a mixed crop, mostly with sorghum and millet, and forms an important part of the diet of a large part of the African population.

Insect pests, diseases, plant parasitic weeds, drought, and heat are major constraints in cowpea production.

The advanced cowpea lines TVx 3236 and TVx 82D-716, which have high yield potential, also have multiple resistance to anthracnose, bacterial blight, brown blotch, web blight, and scab. It appears that resistance to fungal and bacterial diseases has been well established and that adequate sources of resistance are available within the cowpea germplasm. However, some fungal diseases are known to form new strains. Restriction fragment length polymorphism (RFLP) and/or PCR technologies are potentially useful for determining differences among strains of *Colletotrichum*, for example. Use of RFLPs or PCR technologies on the bacterial DNA could also provide a definitive answer to another unresolved question in phytopathological research on cowpea: whether the *Xanthomonas campestris* pathovars that cause distinct symptoms on cowpea, i.e. bacterial blight, bacterial pustule, and bacterial canker, are distinct species or strains (Singh et al., 1992).

Several improved cowpea lines with resistance to as many as five viruses have been developed.

The control of virus diseases is important for increasing food production in the tropics. In order to devise effective control methods, an understanding of the nature and distribution of virus strains is essential. Unfortunately, resistance is sometimes strain-specific, requiring several gene sources for the development of a suitable cultivar. Awareness of the existence of strains and knowledge of their distribution will facilitate development of breeding and screening strategies.

Most cowpea accessions have been screened for resistance to the major pests. In the case of the legume pod borer (*Maruca testulalis*) and a pod-sucking bug (*Clavigralla tomentosicollis*), it has been difficult to identify useful sources of resistance.

The legume pod borer infests stems, floral buds, flowers, and green pods. The greatest damage is caused to flowers, while the most obvious damage appears on the pods.

Pod-sucking bugs constitute a complex of up to nine different species, with *Clavigralla tomentosicollis* as the most dominant and damaging in most parts of Africa.

Cowpea bruchid is a serious pest of cowpea in storage. Complete infestation often occurs within six months of storage at the farm level. Resistance to cowpea bruchid has been extensively studied at IITA. A moderate level of resistance has been identified in TVu 2027 (Singh 1977). After three months' storage, TVu 2027 showed about 20% infestation while other cowpea varieties showed 66-96%

infestation. Similarly, during six months' storage, TVu 2027 showed about 71% infestation while the other varieties were already completely destroyed, indicating the need for better protection for a longer period. This resistance has been incorporated into most advanced breeding lines.

2.5. Parasitic weeds

Striga gesnerioides and *Alectra vogelii* are two major parasitic weeds infesting cowpea. Striga is more common in western Africa and severe losses in yield have often been reported (Aggarwal and Ouedraogo 1989). *Alectra vogelii* has also been reported from western and southern Africa; it causes damage similar to that of striga, with infested cowpea plants suffering serious yield losses.

B301, a cowpea line from Botswana, has shown resistance to a number of striga populations in laboratory tests. The presence of various physiological strains of striga creates an enormous task in breeding for resistance. The use of isozymes and RFLPs is helping with strain identification. RFLPs could also be used to develop an understanding of population genetics in striga and to follow the genetic basis of epidemics.

Drought and heat are major constraints in cowpea production. Most screening methodologies are field based. However, methods for identification of genes for resistance to drought and heat, using RFLPs, have a potential in plant breeding.

2.6. Cowpea Germplasm and Seed health

There are about 15,000 accessions of cowpea in the germplasm unit at IITA, although some may be duplicates. Because RFLP analysis can characterize the genotype of individuals with high precision, it may be an effective tool for classifying this material.

Another important role for biotechnology is in seed health management. Several fungal and bacterial pathogens

are known to be seed-borne. IITA, as well as other institutes in the CGIAR, has as part of its responsibility the movement of both breeders' germplasm and germplasm bank material. There are two ways that biotechnology might help in this respect. Detection of various kinds of pathogens within a seed lot might be faster and more accurate with biotechnological methods.

An RFLP map of the cowpea genome is expected to help identify specific markers for characters relating to pest resistance and to ultimately aid in the selection of desirable genotypes. Some of the constraints described could be possibly overcome with the help of molecular techniques, including RFLPs and DNA fingerprinting.

3. ON-GOING PROJECTS

3.1. Root and Tuber Crops

3.1.1. Meristem culture and disease elimination

IITA has developed and adopted meristem culture media for cassava, yams, sweet potatoes, and cocoyams. Scientists found the combination of heat treatment of the mother plant followed by meristem culture to be effective in eliminating African cassava mosaic virus (ACMV) from cassava and yam mosaic virus (YMV) from white yam (Ng et al. 1990, 1992). For white yam, where the plants are less tolerant to thermotherapy, chemotherapy in *in vitro* cultures using virazole (a chemical known to reduce viruses) is being explored. Meristem culture followed by virus indexing was effective in eliminating sweet potato virus disease complex. About 100 clones of cassava and its related *Manihot* sp., 500

clones of sweet potatoes, 1,500 clones of yams, and 100 clones of cocoyams have been regenerated from meristem culture.

Plantlets of selected improved clones produced from meristem culture were virus indexed (Thottappilly and Rossel, 1988). Methods for indexing ACMV and YMV are sap inoculation to a sensitive test plant *Nicotiana benthamiana* and Enzyme-linked Immunosorbent Assay (ELISA). Approach graft to *Ipomoea setosa*, complementary grafting to pre-infected clones, ELISA, and Immunosorbent Electron Microscopy (ISEM) were used for sweet potato virus complex detection.

3.1.2. Rapid multiplication and international distribution

Media for rapid multiplication and germplasm preservation of cassava, yams, sweet potatoes, and cocoyams have been developed. A multiplication rate of five-fold can be obtained within four to five weeks using single node cuttings. For yam, *in vitro* microtubers and aerial microtubers were also obtained by increasing the sucrose concentration in the culture media. They can be stored for at least three months, sprouted, and planted directly in soil. Virus-free plantlets in sterile containers with culture media are used for international distribution.

3.1.3. Germplasm conservation

A germplasm conservation method based on reduced growth *in vitro* has been used to maintain germplasm collections of root crops at IITA: 1,000 clones of sweet potatoes, 1,500 clones of yams, 200 clones of cassava and related sp., and 50 clones of cocoyams. Under lower incubation temperature,

cassava, yam and cocoyam germplasm can be kept for more than one year when cultured on a normal culture medium. With the addition of 3% mannitol in the culture media and lowering the incubation temperature, sweet potato germplasm can be stored for one to two years.

3.1.4. Biochemical studies on cyanogenesis in cassava

The presence in cassava of cyanogenic glucosides which upon hydrolysis may release hydrocyanic acid (HCN) is a reason for concern for cassava consumers. The biosynthesis of these glucosides, their translocation and accumulation in specific tissues of cassava, particularly in edible root tissues are being studied in collaboration with the Royal Veterinary and Agricultural University of Denmark.

The objective of the study is to provide an understanding of molecular machanisms for varietal differences in the levels of cyanogenic glucosides in cassava which will lead to the development of new strategies for selecting for low-cyanide clones.

The endogenous enzyme linamarase which hydrolyzes cyanogenic glucosides plays an important role in the detoxification of cassava during processing. The wide varietal differences in the levels of linamarase activity observed by IITA scientists in cassava tissues make it possible to improve the crop for this characteristic by conventional breeding methodologies. Molecular biology techniques are needed to establish the biochemical basis of these differences and to develop appropriate screening tools (DNA probes, RFLP markers).

3.2. Plantains and cooking bananas

A plantain/banana tissue culture laboratory was established at the Onne High Rainfall Substation of the IITA in 1983. The tissue culture research and activities performed at that laboratory are fully integrated into the conventional plantain breeding program. A breeding strategy targeting the creation of resistant plantain hybrids was developed at IITA and achieved success within 3 years. The role of tissue culture research and applications in this feat has been considerable. An *in vitro* micropropagation technique for plantains and cooking bananas, developed in the 1980s, is routinely applied to obtain large numbers of plants. Increases in the number of propagules range from 10-30 every two months compared with 6-12 months using conventional field multiplication techniques. Shoot tip culture has been applied successfully in the propagation of over 400 *Musa* germplasm accessions. These materials are also suitable for distribution to national programs through plant quarantine systems because they are considerably lighter and less bulky than conventional propagules, amenable to rapid multiplication if required, and, most importantly, free of non-obscure pathogens.

Embryo culture techniques are applied to enhance seed germination rates. These are further refined by evaluating changes to the culture medium and by the culture of immature embryos. The aim is to improve on the 4-12% germination obtained at present *in vitro* as compared to the 1-2% germination in soil (Vuylsteke and Swennen, 1992).

The occurrence of somaclonal variation (genetic variation among plants regenerated from tissue culture) among *in vitro* propagated plantains has been studied extensively (Vuylsteke et al, 1990). Somaclonal variation is a potential hindrance to the *in vitro* propagation and conservation of germplasm, but has also been highlighted as a potential benefit in terms of creating novel variability for crop improvement such as plant height and disease resistance.

Plant regeneration by somatic embryogenesis in cell suspension cultures has been achieved in *Musa*, but only in wild species (from zygotic embryo explants) and in a few clones of AA and AAA dessert bananas and ABB cooking bananas (Banerjee *et al.*, 1987; Cronauer-Mitra and Krikorian, 1988; Escalant and Teisson, 1988; Novak *et al.*, 1989). No success has yet been reported in any of the African bananas and plantains.

4. NEW INITIATIVES

4.1. Cassava

Wild species of cassava have desirable characteristics for disease and insect resistance and low cyanide levels. Among cultivated species, shy-flowering especially in land-races limits the choice of parents for breeding. Cytogenetic studies of cassava and its wild relatives and embryo/ovule culture will be important new initiatives in cassava breeding (Hahn et al, 1990). Both cultivated cassava and its related wild species all have chromosome number of 2n = 36. IITA scientists have identified six spontaneous sexual tetraploids (2n = 4x = 72) and four spontaneous sexual triploids (2n = 3x = 54) from the diploid interspecific crosses. Some

triploids gave over 200 per cent yield increase over normal diploid cassava. Occurrence of natural tetraploids provides new opportunities for polyploidy breeding and genetic studies, making use of different related *Manihot* species. With the recent advances in biotechnology, it would be possible to make use of the tetraploids for anther culture.

By using electrophoretic studies, it is now possible to identify duplicates and differentiate and/or to classify *in vitro* regeneration lines to their characteristics which can be applied for isolation of variants. This may enhance breeding activities (Table 1).

Embryo culture has been used to culture isolated embyos from some *Manihot* species and cassava to enhance the germination rate of the seeds. Media for immature embryo culture of cassava has also been developed.

Somatic embryos matured to cotyledon stage were obtained from young leaves of several IITA cassava clones. Plantlets were obtained in some clones.

4.2. Yams

Many yams are not flowering under natural conditions, and they are polyploids. Therefore, biotechnological and cytogenetic research has a high priority in yam improvement. Future progress will depend in part upon a much better understanding of the nature of polyploidy and flowering in the genus *Dioscorea*. Micropropagation, or the regeneration of plants from callus tissue, is a possible source of variation in the cultivars which are not flowering.

Media and procedures for embryo culture of yams were developed. Microtubers of white yam are produced using inductive media and used for internatioal distribution.

The origin and phylogeny of the Guinea yam as revealed by RFLP analysis of chloroplast and nuclear ribosomal DNA was studied at IITA (Terauchi et al, 1992).

4.3. Plantains/Cooking Bananas

Tissue culture technologies offer solutions to circumvent problems which handicap certain stages in the genetic improvement of plantains and bananas.

The intractable fertilization barriers that hamper the genetic improvement of plantain and banana could be readily surmounted by advanced biotechnological methods, such as recombinant DNA technology and somatic hybridization. However, to benefit from the full potential of such technology, the basic problem of controllable regeneration of plants from single cells or protoplasts must be solved. Several investigators have done research in this direction on *Musa*, mainly aiming at the production of embryogenically competent tissue and the achievement of somatic embryogenesis. In collaboration with the Catholic University of Leuven, Belgium, IITA is conducting research into the regeneration of *Musa* plants through somatic embryogenesis. Embryogenic cell suspension cultures of the widespread cooking banana clone 'Bluggoe' (*Musa* spp., ABB group) were established by culturing meristematic «scalps», taken from proliferating shoot-tip cultures. Plant regeneration proceeded through the developmental pathway of somatic embryogeny, which at all stages showed conspicuous morphological and histological resemblance with zygotic embryogenesis in a wild *Musa* species (Dhed'a et al 1991). Somatic embryos were produced directly from cells in suspension and not via callus. Germinated banana somatic embryos were successfully established in soil.

The relative simplicity of this cell culture protocol may enhance the feasibility of integrating biotechnological approaches in conventional schemes of banana and plantain improvement.

4.3.1. Germplasm exchange and micropropagation

The introduction of valuable genetic resources for the breeding program and the rapid multiplication of selected genotypes is readily achieved using the well established shoot-tip culture technique. Over 300 new *Musa* accessions were introduced, thereby quadrupling the number of accessions held in the IITA collection. These genetic resources were introduced in a joint effort with the Nigerian Plant Quarantine Service and the International Network for the Improvement of Banana and Plantain (INIBAP). At least 33 of these introductions have shown resistance to black Sigatoka. Among these, several AA diploids are useful sources of black Sigatoka resistance in IITA's plantain breeding program. Five black Sigatoka resistant ABB cooking banana cultivars are being rapidly multiplied in vitro and distributed at a rate of 5000 plants annually to Nigerian national programs and farmers as an alternative to the susceptible plantains.

Micropropagation techniques have also been pivotal in the rapid deployment of IITA's plantain breeding program by supplying large numbers of plants of seed-fertile plantain cultivars for use as female parents in breeding schemes. Micropropagation could in the short term play an increasingly important role in the rapid multiplication and distribution of resistant plantain hybrids.

4.3.2. Somaclonal variation

With the increasing use of in vitro culture for plant production, the occurrence of somaclonal variation, that is increased genetic variation among plants regenerated from tissue culture, has been found to be ubiquitous(Scowcroft, 1984). Because in vitro propagation is a frequently used technique for handling *Musa* germplasm in IITA's plantain improvement program, it was necessary to determine the nature and extent of somaclonal variation among a wide range of plantain cultivars.

Factors influencing the incidence of somaclonal variation are investigated in order to identify guidelines for control of in vitro instability.

4.3.3. Embryo culture

In addition to the generally low seed set, hybrid plantain production is further complicated by low seed germination rates. In soil, plantain seeds germinate at a rate of only about 1%.

An improved embryo culture protocol has been developed, whereby germination rates now range from 10-25% (if calculated on the basis of number of embryos cultured). On average, about 700 plantain seeds are handled in vitro each month, resulting in the production of one plantain hybrid per working day.

Techniques of immature embryo rescue are currently under investigation.

4.4. Cowpeas

Among the most destructive insect pests of cowpeas are the *Maruca* pod borer and three pod-sucking bugs. After screening over 8,000 cowpea accessions, no resistance to

these pests has been identified in the cultivated *Vigna* but very good sources have been found in wild species, particularly *V. vexillata*. However, it has been impossible to obtain progeny after interspecific crosses. Research is underway by both the scientists of IITA and collaborators in institutes in Italy (The Universita Degli Studi Di Napoli and the Universita Degli Studi della Tuscia), at Purdue University, and at University of California at Davis to explore the possibility of transferring the resistant gene(s) from the wild species *V. vexillata* to cultivated cowpeas. These studies included wide crosses, cytogenetics, embryo culture, and regeneration system. Other crosses between cowpeas and several wild *Vigna* species *(V. ambacensis, V. frutescens, V. gracilis, V. luteola, V. membranace, V. nervosa, V. oblongifolia, V. racemosa,* and *V. reticulata)* have not been successful so far.

In collaboration with the University of Ibadan (Nigeria), it has been possible to rescue hybrid embryos after wide crosses between cowpeas and *V. pubescens*. In another collaborative project with the University of Napoli, Italy, protoplast isolation and multiplication techniques have been developed, somatic fusions have been made, and tolerance of cowpeas to abiotic stresses (e.g. aluminium toxicity and drought) is being studied on protoplasts and free cells.

Initial results on a plant regeneration system have been encouraging. IITA collaborators in Italy and the United States were able to generate roots from calli derived from several explants including leaf discs. They were also able

to obtain fast dividing protoplasts which have the capacity to fuse with *Nicotiana* protoplast. Research in this and similar areas is continuing with the prospect of more success in helping to solve the problems caused by insect pests of cowpeas.

Under an agreement with Purdue University, entomological studies are underway to identify bruchid-resistant *Vigna* species and subspecies and make interspecific hybrids, including attempts at embryo rescue of promising crosses. Also, this research involves isolation and characterization of gene products whose genes have potential to confer resistance to insects, focussing mainly on *Maruca*. Candidate genes will include those coding for *Bacillus thuringiensis* protoxins, digestive proteinase inhibitors, and lectins.

4.5. Rice

Some African rice accessions (*Oryza glaberrima, O. longistaminata,* and *O. barthii)* have characteristics important for rice improvement in Africa, particularly immunity to the rice yellow mottle virus and resistance to the stem borer *Diopsis* both stressers being specific for Africa. Transferring the desirable traits from African species to the high-yielding *O. sativa* background has not been satisfactory due to insufficient recombination. Approaches through biotechnology will be investigated in collaboration with advanced laboratories.

4.6. Maize

Modern biotechnology is being applied vigorously to maize in both public and private laboratories in the USA. The interest lies especially in mapping of resistance genes

to accelerate selection, in transforming maize with the BT gene for resistance to *Lepidopterous* borers, and in transformation with herbicide resistance genes. All of this research is potentially useful to IITA's maize research goals and linkage projects are being developed. An especially African target will be to utilize biotechnology to solve the *Striga* problem on maize in Africa and several approaches are being examined. Linkage projects are being developed with advanced laboratories in the USA and Europe already working with molecular aspects of maize streak virus. The viral coat protein gene transfer approach can be explored for all important African viruses in maize. Research on genetically engineered endophytes (inocuous bacteria that live in maize xylem) is a major activity in the private sector and this approach to delivery of resistance compounds to maize should be considered through joint project development.

Soil-borne fungal pathogens which cause stalk rots, ear rots, and produce aflatoxins have received scant research in the tropics. Their study is difficult because detection methods are inadequate. The development of modern diagnostic/ detection methods through biotechnology should accelerate research in pathogen ecology and disease epidemiology. The potential for diagnostics for study of *Striga* biology is to be developed.

In collaboration with the University of Califonia, Davis, USA, IITA has initiated a new project to study "variability in tropical maize downy mildew fungi using molecular markers".

As soon as transformation of maize is possible, there will be much to explore for maize improvement in the tropics of Africa. IITA plans to keep abreast of the rapidly developing aspects of maize biotechnology in the advanced private and public sector laboratories and it will vigorously pursue potential options.

4.7. Diagnostic Research

IITA's Virology Unit will be closely linked with the Biotechnology Unit and will benefit from recombinant DNA techniques. An example is the control of virus diseases by introduction of the viral coat protein gene to plants where resistance is not available. Other developments in biotechnology permit the production of monoclonal antibodies and cDNA probes for the detection of viruses and their strains(Thottappilly and Rossel, 1988). Proper virus diagnosis using monoclonal antibodies can greatly help in the reduction or control of outbreaks of virus diseases. This technology provides a means of meeting the goals of plant disease prevention and control through effective diagnosis.

A laboratory of the U.S. Department of Agriculture (USDA) at Beltsville, Maryland is collaborating with IITA in the production of monoclonal antibodies and cDNA probes for the detection of viruses affecting root and tuber crops. Recently, various strains of viruses affecting IITA's mandated crops were identified. However, their distribution in other countries is not known. This information is crucial for plant breeders.

To help the national programs with the identification of the viruses and strains in their countries, a project proposal was submitted to IDRC (Canada) and approved. The main objective of this project is to produce monoclonal antibodies to detect viruses and their strains in food crops in various African countries. In collaboration with Canada Agriculture, Vancouver, a special workshop was held at IITA in April 1991 and 12 African scientists attended. Because

proper identification of viruses is more difficult than for other plant pathogens, many national programs do not have equipment and other facilities to carry out proper virus identification work. Quarantine regulations prohibit them from sending infected plants or plant parts across their borders to IITA, but once monoclonal antibodies are available, the viruses and their strains can be identified within each country. Then, IITA and national breeders can adopt strategies to incorporate virus resistance in plants.

4.8. Germplasm

Germplasm resources are vital to any crop improvement effort and many opportunities exist for identifying novel genetic traits in wild species and landraces. Tissue culture techniques offer solutions to the problem of root crops germplasm conservation and germplasm exchange. Tissue culture allows the transfer of plant materials in a disease-free condition, especially in the case of vegetatively propagated root and tuber crops and plantains/bananas for use in improvement programs worldwide. Cryopreservation will allow the long-term conservation of germplasm particularly for asexual species difficult to maintain by seed. Cryopreservation of virus-indexed genotypes will also help preserve rare genotypes, control genetic erosion, and increase the genetic variability available to breeders through international exchange of healthy materials. Recently, embryogenic cell suspensions of a cooking banana were cryopreserved and plants successfully regenerated therefrom (Panis et al. 1990) at the Catholic University of

Leuven in Belgium. Regenerated plants are screened for clonal uniformity at IITA. It is envisaged to transfer this technology to IITA in the near future.

5. LINKAGE WITH NATIONAL PROGRAMS

IITA's collaborative research with advanced laboratories gives the Institute the benefit of direct access to the latest biotechnology developments. In turn, it will be able to be more effective in training national scientists and MSc. and PhD. students. The new Biotechnology Unit forms a link between laboratories in the advanced countries and the national agricultural institutes in Africa. This unit will develop strategies to increase the efficiency of breeding programs by finding solutions to problems which cannot be solved by conventional methods.

To strengthen and to broaden collaboration with the national programs and to identify biotechnologically researchable areas, a meeting of African scientists working on cassava, yams and plantain/cooking bananas was held at IITA from August 8-9, 1988; 25 participants represented 14 institutes in 7 African countries. An agreement was reached on a set of recommendations which would guide IITA in developing research applications of biotechnology. A similar biotechnology meeting was held in February 9-10, 1989 at IITA to establish priorities in cowpea biotechnology research. A conference entitled "Biotechnology: enhancing research on tropical crops" was held at IITA from 26-30 November, 1990 and over 130 participants attended the conference. An African Plant Biotechnology Network (APBNET) was initiated with IITA as its secretariat. Also, this conference

identified the constraints in various crops which could be handled by biotechnology.

6. Further needs for the development of biotechnology in the improvement of IITA's mandated crops

An appraisal of the current status of biotechnology in tropical crop improvement makes it clear that only the "lower" technology of plant tissue culture has made some impact yet. The application of the more advanced techniques, listed below, could have great potential in solving specific intractabilities that hamper optimal use of the existing *Musa*, cowpea, maize, yam and cassava germplasm. The further development of these methods and their adaptation to IITA's crops of concern, which often are recalcitrant with regards to both conventional and non-conventional improvement, will require a serious collaborative research effort. For certain research items a *de facto* collaboration has already been established between IITA and other institutes. It is also not necessary for IITA to be engaged in each subject since other institutes involved with research in these crops may have a greater comparative advantage than IITA to carry out particular research subjects. It is envisaged that relevant developments in other parts of the world will also benefit IITA through participation in networking activities.

6.1. Recombinant DNA technology

Transformation of cassava, yam, maize, cowpea and plantain by genetic engineering techniques is worth considering as a potential route for the production of pest and disease-resistant materials.

In the case of cassava, the transfer of a storage protein to fortify the tubers with more and better protein seems to be a researchable issue. A transformation system for the concerned crops still needs to be established. In view of recent progress with *Agrobacterium*-mediated gene transfer to monocots, this is a course meriting due attention. Yam, a monocot, has recently been shown to be amenable to infection by *Agrobacterium* (Schafer *et al.*, 1987).

For *Musa*, no cloned genes are presently available which may directly confer host resistance to fungal disease for any host-pathogen system (particularly for black Sigatoka and *Fusarium* wilt). There are however a number of genes available associated with expression of resistance which could be used experimentally in attempts to confer fungal resistance (Murfett and Clarke, 1987).

INIBAP and IBPGR have recently stressed that an elegant test case would be to control BBTV by transformation of *Musa* with the viral coat protein. Expertise for this approach is available in Australia (Queensland University of Technology). Furthermore, plant regeneration *in vitro* from transgenic *Musa* cells seems a genuine possibility in the near future. Much research has already been performed in Europe on somatic embryogenesis in cell suspensions and callus (Catholic University of Leuven, Belgium; University of Paris-Sud and IRFA/CIRAD, France; FAO/IAEA laboratories, Austria), although more research is warranted in order to achieve controllable plant regeneration in a wide range of important cultivars.

6.2. Haploidy

In view of the high degree of heterozygozity in cassava and *Musa*, the production of homozygous breeding lines through haploidy would be of use in genetic improvement programmes and would be a tool in the study of the genetics of disease resistance.

6.3. *In vitro* selection for useful mutants

Screening in the field for useful somaclonal variants (e.g., with disease resistance) is not feasible. However, screening at the cellular level and with a selection pressure applied *in vitro* is a more practical approach. In the case of *Musa*, research into cell culture has progressed significantly in the past two years and pathologists are well on their way to purify extracts of the fungus *Mycosphaerella* that could be tested as a screening agent.

6.4. Cryopreservation

Cryopreservation for the long term storage of genetic resources of tropical crops is considered priority research by IBPGR. Such research has been/is supported by the latter at K.U. Leuven (Belgium) and CATIE (Costa Rica) for *Musa* and is progressing at CIAT, Colombia for cassava.

A feasible cryopreservation methodology for the crops of concern to IITA would benefit IITA and national research institutes involved in germplasm conservation.

6.5. Molecular methods to detect genetic diversity

The use of molecular techniques for detecting genetic diversity has raised considerable interest because of the sensitivity of these techniques.

Restriction fragment length polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPDs) and, to a lesser

extent, isozymes have shown their appropriateness as genetic markers for plant systematic studies and for clonal identification.

IITA has contacted the USDA/ARS laboratory at the Regional Plant Introduction Station, Griffin, Georgia to initiate research on these advanced techniques aimed at the enhancement of the efficiency of plantain breeding schemes. For cowpea, IITA is collaborating with University of Minnesota, USA. For yam, IITA is collaborating with Prof. G. Kahl, University of Frankfurt, Germany to study amplified fragment length polymorphisms.

6.6. Cytogenetics

Only limited cytogenetic studies have so far been carried out on the crops treated in this paper. Because of their heterozygosity and the multigenic nature of many important traits (disease resistance), more profound studies would help in the formulation of efficient breeding strategies.

6.7. Virus diagnostic techniques

Recent developments in biotechnology permit detection of viruses by means of highly specific assays such as serological techniques using monoclonal antibodies. Another highly promising technique is based on nucleic acid hybridization. These modern techniques provide highly efficient tools for the diagnosis of viruses and viroids. The ultimate benefit to be drawn from these activities is a faster, safer and more efficient transfer of improved germplasm in vegetative form. Monoclonal antibodies used in ELISA or dot-blot immunoassays, and the so-called Western

blot methods are practical biotechnology innovations for improved diagnosis of virus diseases.

Hybridoma technology provides methods for producing monoclonal antibody preparations of molecular homogeneity. Monoclonal antibodies have been useful in differentiation of strains, quantification of viral antigens, and detection and diagnosis of virus infection. The ability to distinguish minor antigenic differences with monoclonal antibodies has made them useful probes to differentiate strains of a virus. Access to them would be very useful to IITA in order to identify quickly and reliably several of the viruses and their variants or strains, that infect mandate crops.

Detection techniques based on the use of cDNA are often more sensitive than serological methods and their use by IITA virologists will materially increase the capacity to detect virus infected plants. The cDNA probes, and especially non-radioactive ones, are bound to become more popular. A "broad-spectrum" probe could be developed for quarantine purposes and "narrow-spectrum' probes for epidemiological studies.

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Table 1: **Current and 'do-able' applications of biotechnology at IITA in various crops**

Cassava	Activity	IITA and Collaborators	Status
I	Thermotherapy meristem culture(Elimination of African Cassava Mosaic Virus).	IITA	On-going
II	Rapid multiplication (Virus-tested clones)	IITA	"
III	*In vitro* germplasm conservation (Clonal depository)	IITA	"
IV	Electrophoresis (To verify duplicate accessions and identify mutants)	IITA	New initiative
V	*In vitro* screening for stress tolerances (Al and salt)	IITA	"
VI	Somatic embryogenesis (Induce somatic embryo-genesis from leaf tissue)	IITA	"
VII	Anther culture (Haploid plant production	IITA	"
VIII	Embryo culture (culture of embryos of *Manihot* sp.)	IITA	"
IX	Resistance to virus disease	Washington Univ.	"
X	Breeding for low HCN (Enabling technique: RFLP)	IITA & advanced lab.	Exploring
XI	Monoclonal anti-bodies and cDNA for virus detection	Scottish Crop Research Institute	New initiative
XII	Genome mapping/ploidy determination	Cornell Univ. & IITA	"

Yams

I	Meristem culture/disease elimination (Elimination of Yam mosaic virus)		
(a)	Thermotherapy/meristem culture	IITA	on-going
(b)	Meristem culture and chemotherapy	IITA	"
II	*In vitro* germplasm conservation (clonal depository).	IITA	"
III	*In vitro* Micropropagation (Virus-tested clones)	IITA	"
IV	*In vitro* tuberization (virus-tested clones)	IITA	on-going
V	Electrophoresis (To verify duplicate accessions and identify mutants).	IITA	New Initiative
VI	Somaclonal variation	IITA	"
VII	Embryo culture	IITA	"
VIII	Monoclonal antibodies (Yam mosaic virus diagnostics)	IITA & USDA labs.	"
IX	Resistance to virus disease	IITA & Univ. of Wageningen	"

Plantain/ cooking bananas

I	*In vitro* micropropagation	IITA	on-going
II	Embryo culture	IITA	"
III	Somaclonal variation	IITA	"
IV	*In vitro* germplasm conservation (clonal depository)	IITA	"
V	Resistance to black Sigatoka disease Enabling technique: RFLP, cell culture	IITA & adv. labs	Exploring

Cowpea

I	Resistance to insects		
	a) Immature embryo culture/embryo rescue	IITA, Univ. of Napoli, Purdue Univ., Univ. of Ibadan.	New Initiative
	b) Resistance Gene transfer (gene insertion, protoplast fusion etc.)	IITA, Univ. of Napoli, Purdue University.	"
	c) Enabling technique: RFLP	Univ. of Minnesota	"
II	*In vitro* screening for stress tolerances (Al, and cold)	Univ. of Napoli	"
III	Monoclonal antibodies (For detection of various viruses and their strains)	IITA & Canada Agriculture	"
IV	Resistance to virus diseases	IITA, and Univ. of Wageningen	"

Maize

I	Maize downy mildew	IITA & Univ. of California	New initiative
II	Bt genes for borers	IITA & Advanced labs	Exploring
III	Striga resistance (Enabling technique: (RFLP)	"	"
IV	Resistance to virus diseases	"	"
V	Monoclonal antibodies for virus and fungal diseases	"	"
VI	Endophyte technology for control of borers	"	"

Rice

	a) Disease and insect Resistance (wide crosses with *O. glaberrima)*	IITA	New initiative
	b) Transfer of RYMV reistance gene(s) from *O. glaberrima*	"	"

c) Rice RFLP " "