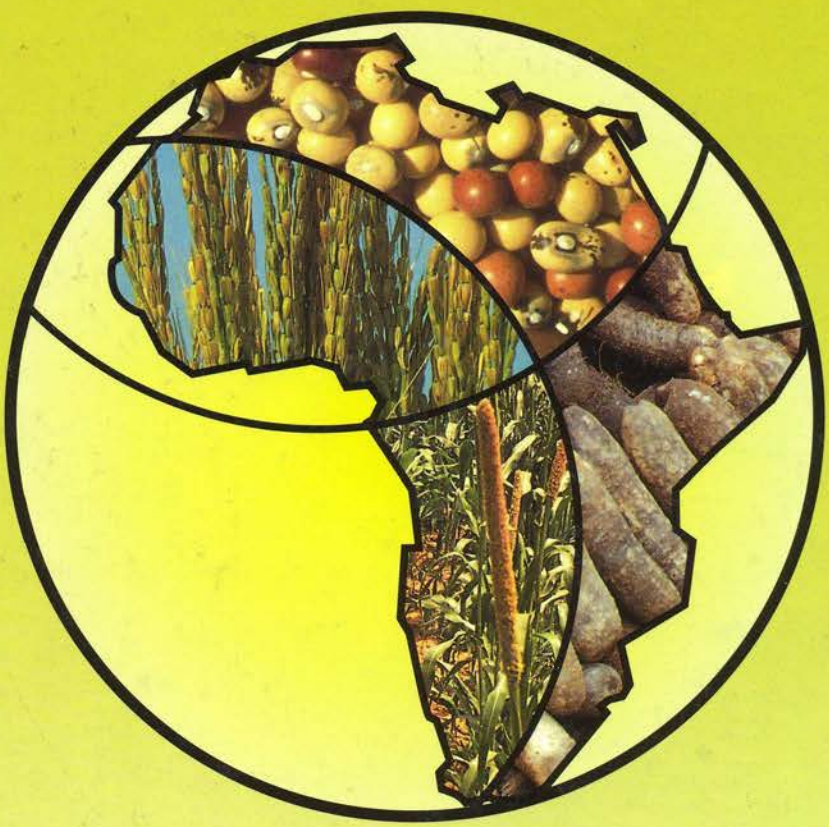
# CROP GENETIC RESOURCES OF AFRICA



## VOLUME II

Proceedings of an International Conference
on Crop Genetic Resources of Africa
17–20 October 1988 Ibadan, Nigeria

N. Q. NG, P. PERRINO, F. ATTERE and H. ZEDAN

# *Crop Genetic Resources of Africa*

*Volume II*

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*Proceedings of an international conference organized by the International Institute of Tropical Agriculture (IITA) and the National Research Council of Italy (CNR), in association with the International Board for Plant Genetic Resources (IBPGR) and the United Nations Environment Programme(UNEP), and held in Ibadan, Nigeria, 17-20 October 1988*

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**IBPGR**
International Board for Plant Genetic Resources

**UNEP**
United Nations Environment Programme

**CNR**
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# *Preface*

Worldwide, food crops are increasing their yields but from ever fewer varieties. The genetic base of the world's food supply is shrinking, at an ever faster rate. The dangers in this situation are the particular concern of plant geneticists, who act as the conservators of the world's genepool of agricultural species. The genetic resources saved from extinction today may provide the solutions for tomorrow's unexpected pests or calamities. Hence germplasm conservation is an imperative for society, having an inestimable but unquestioned value.

Many changes have occurred since the first meeting on Crop Genetic Resources of Africa was held in January 1978 in Ibadan, Nigeria. This meeting was organized by the International Institute of Tropical Agriculture (IITA) and the Association for the Advancement of Agricultural Sciences (AAASA) and brought together many research scientists from Africa and other parts of the world.

In order to review past achievements and develop future strategies for African plant genetic resources conservation and utilization, the International Board for Plant Genetic Resources (IBPGR), IITA and the United Nations Environment Programme (UNEP), in association with the Consiglio Nazionale delle Ricerche (CNR) of Italy, jointly organized two workshops.

The first workshop, held at UNEP in Nairobi, Kenya, was organized from 26 to 30 September 1988 by IBPGR and UNEP, in association with IITA and the CNR, and brought together representatives and scientists from national research programs in Africa to discuss recent developments in the field of plant genetic resources. In addition, participants discussed ways to further work on conserving, collecting, evaluating and using the wide range of plant diversity in Africa. The workshop was sponsored by the following donor agencies: Finnish International Development Agency (FINNIDA), Finland; Norwegian Agency for International Development (NORAD), Norway; Swedish International Development Authority (SIDA), Sweden; and Directorate General for International Cooperation (DGIS), Netherlands.

The second workshop was held at IITA in Ibadan, Nigeria, from 17 to 20 October 1988, to address and discuss those activities and strategies as they related to the crops of special interest to IITA: cassava, cowpea, maize, plantain, rice, soybean, yams and bambara groundnut. In addition, the issues of plant quarantine regulation and safe movement of germplasm were addressed. This workshop was organized by IITA and CNR in association with IBPGR and UNEP and was sponsored by the Ministry of Foreign Affairs of the Government of Italy.

The two meetings drew a combined participation of over 200 experts and observers who represented 38 African countries and 20 international and scientific institutions in Europe and the Americas. It is hoped that through the subsequent deliberations of many officials of African countries, representatives of international organizations and experts in plant genetic resources, the recommendations of the two workshops will promote the goals of effective use of plant genetic resources and preserve the fruits of evolution of African species for posterity.

Volumes I and II of this publication constitute the proceedings of the Nairobi and Ibadan workshops, respectively. The various chapters bring together the contributions of research workers in plant genetic resources collection, evaluation, conservation, management and survey, taxonomy, cytogenetics, plant breeding and plant health. They give a picture of current activities of the international and regional institutions that are involved in plant genetic resources in Africa. The recommendations from each workshop for future action by participants and country delegates are also reported. The status reports presented by country delegates at the two workshops are to be published in volume III.

Grateful acknowledgement is made of the generous contributions of the sponsors of the workshops, for financial support in organizing the workshops and for publishing the proceedings in the two volumes. The contribution of the Organization of African Unity (OAU) in preparing volume III for publication is also gratefully acknowledged.

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| IITA | International Institute of Tropical Agriculture |
| IBPGR | International Board for Plant Genetic Resources |
| UNEP | United Nations Environment Programme |
| CNR | Consiglio Nazionale delle Ricerche |

# *Summaries of Chapters*

## 1.1 Germplasm collection and conservation activities in Africa: The role of the International Board for Plant Genetic Resources (IBPGR)

IBPGR, established in 1974 with a mandate to further the study, collection, preservation, documentation and evaluation of the genetic diversity of important crop plants and their wild relatives, has been active in all these fields in Africa since that date. A total of 225 collecting missions have taken place and the main achievements of these missions are described in this paper. The current situation regarding germplasm conservation is outlined and some recent developments involving IBPGR are used to illustrate progress. IBPRG's operative structure in Africa during the period is described and future initiatives are briefly outlined.

## Les activités de collecte et de conservation du germoplasme en Afrique: Le rôle du Conseil international des ressources phtyogénétiques (CIRPG)

Le CIRPG a pour mandat d'encourager l'étude, la collecte, la conservation, la documentation et 'évaluation de la diversité génétique des principales plantes cultivées et de leurs congénères sauvages. Depuis son établissement en 1974, le Centre a participé activement à ces divers travaux en Afrique. Le présent ouvrage fait le point sur les principaux résultats des 225 missions de collecte organisees par le CIRPG. La situation actuelle en matière de conservation du germoplasme y est dressée, les progrès obtenus étant illustrés par les derniers développements auxquels le Centre a été associé. Par ailleurs, le lecteur trouvera une description du cadre des opérations menées en Afrique durant la période visée, ainsi qu'un exposé succinct des prochaines initiatives.

## 1.2 The genetic resources activities of the International Institute of Tropical Agriculture (IITA)

In 1975 IITA established its Genetic Resources Unit (GRU) to further the Institute's work on germplasm collection, characterization, evaluation, documentation and conservation. IITA, and particularly the GRU, work closely with other research institutes and genebanks worldwide. All IITA plant exploration trips are carried out in collaboration with national programs. To date, the GRU has undertaken 54 exploration trips to 28 African countries and collected 20,000 germplasm samples. IITA now has over 40,000 germplasm accessions.

Training is an increasingly important component of IITA's plant genetic resources activities. A number of courses on *in vitro* germplasm multiplication, conservation, disease elimination and distribution have been conducted, as well as individual on-the-job training. GRU, in collaboration with the IBPGR, has organized training courses in germplasm exploration and conservation, seed technology and genebank management.

GRU scientists are also involved in research to promote the usefulness of germplasm, to provide greater knowledge about taxonomy, genetics, interspecific relationships and variation of plant/crop species, as well as other related studies.

## Les activités de l'Institut international d'agriculture tropicale (IITA) dans le domaine des ressources génétiques

Le Département des ressources génétiques (DRG) de l'IITA a été créé en 1975 dans le but de faciliter les activités de collecte, de caractérisation, d'évaluation, de documentation et de conservation du matériel génétique entreprises par l'Institut. Celui-ci, et tout particulièrement le DRG, travaille souvent en étroite collaboration avec les programmes nationaux. Le DRG a effectué à ce jour 54 missions de prospection dans 28 pays d'Afrique et collecté 2000 échantillons de matériel génétique. L'IITA dispose actuellement de plus de 40,000 obtentions.

Une part croissante des activités de l'IITA est réservée à la formation dans le domaine des ressources phytogénétiques. Outre la formation individuelle sur le tas, l'Institut a organisé des stages sur la multiplication *in vitro*, la conservation, l'élimination des maladies et la distribution géographique des cultures. Le DRG a monté conjointement avec l'CIRPG des stages de formation sur la prospection et la conservation du matériel génétique, la technologie semencière et la gestion des banques de gènes.

Le DRG est actuellement engagé dans une recherche sur les utilisations du matériel génétique, la taxonomie, la génétique, les relations interspécifiques et les variations des espèces sauvages et cultivées, ainsi que dans d'autres domaines connexes.

## 1.3 West Africa Rice Development Association (WARDA) activities in rice germplasm collection, conservation and utilization

WARDA began its germplassm program in 1973 and started its own collection in 1978, employing a full-time scientist. In defining its objectives, WARDA has been careful not to duplicate the efforts of other international centers. WARDA has received several collections from IRAT/ORSTOM, IITA and many other institutes and national programs. It has collected many cultivars within West Africa through its ecologically based stations. For collections in Mali and Nigeria in 1979 and 1980, it used outside consultants.

The WARDA medium-term germplasm bank was installed and functional by 1982. It contains 5,327 registered accessions. Active rejuvenation of accessions with low viabilities was first undertaken in 1983. Some of the data on accessions have been computerized.

WARDA has given support to and collaborated with international institutes collecting in the WARDA region. Hundreds of its accessions have been sent out to requesting scientists or organizations throughout the world. Effort is being made to send duplicate samples of all accessions to IRRI and IITA for safe keeping.

## Les activités de collecte, de conservation et d'utilisation du germoplasme de riz à l'Association pour le développement de la riziculture en Afrique de l'Ouest (ADRAO)

L'ADRAO a lancé son programme de matériel génétique en 1973 et a commencé à constituer sa collection en 1978 avec le concours d'un spécialiste. Les objectifs de l'ADRAO ont été définis de façon à éviter que les activités de l'Association ne fassent double emploi avec celles d'autres centres internationaux. L'IRAT/ORSTOM, l'IITA et d'autres instituts ainsi que de nombreux programmes

nationaux lui ont fait don de plusieurs collections. Ses stations localisées dans diverses zones écologiques d'Afrique de l'Ouest lui ont permis de collecter de nombreux cultivars mais elle a eu aussi recours à des consultants au Mali et au Nigéria en 1979 et 1980.

Opérationelle dès son installation en 1982, la banque de matériel génétique à moyen terme de l'ADRAO dispose de 5,327 obtentions enregistrées. La régénération active des obtentions à faible viabilité a été entreprise pour la première fois en 1983. Un certain nombre de données les concernant ont été informatisées.

L'ADRAO fait bénéficier de son soutien des instituts internationaux collectant dans sa région et collabore avec eux. Elle a fait parvenir sur simple demande des centaines d'obtentions à des chercheurs et à des organisations du monde entier. Pour plus de sûreté, elle s'efforce de transmettre une reproduction de toutes ses obtentions à l'IRRI et à l'IITA.

## 1.4 Germplasm collection, conservation and utilization activities of the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM)

This paper synthesizes the bibliography of ORSTOM's work on genetic resources of coffee, okra, yam, cassava, *Pennisetum* spp., sorghum and rice over the past decade. Several tens of thousands of samples have been studied, thanks to isoenzyme electrophoresis and multivariate statistical analysis.

New species have been recorded and details of the structure of centers of origin and diversification have been provided.

Original evolutionary situations have been discovered, along with unexpected levels of introgression between cultivated and spontaneous forms. New sources of resistance to fungal, viral and bacterial diseases have been found, as well as more tolerant genetic combinations.

Accurate genetic identification of cultivars, the mastery of *in vitro* culture and methods to diagnose diseases will open the way to a new conception of conservation and utilization of genetic resources.

## Les activités de collecte, de conservation et d'utilisation du germoplasme à l'Office de la recherche scientifique et technique d'outre-mer (ORSTOM)

Cette publication fait la synthèse bibliographique des travaux de l'ORSTOM sur le café, le gombo, l'igname, le manioc, le mil, le sorgho et le riz dans le domaine des ressources génétiques au cours des dix dernières années. Plusieurs dizaines de milliers d'échantillons ont été étudiés grâce à l'électrophorèse des isoenzymes et à l'analyse statistique à plusieurs variables.

De nouvelles espèces ont été répertoriées et des précisions sur la structure des centres d'origine et de diversification ont été apportées.

Des situations évolutives originales sont mises en évidence, accompagnées de niveaux d'introgressions inattendus entre formes cultivées et spontanées. De nouvelles sources de résistance aux maladies fongiques, viroses et bactérioses sont dévoilées ainsi que des combinaisons génétiques plus tolérantes.

La parfaite identification génétique des cultivars, la maîtrise de la culture *in vitro* et des méthodes de diagnostic des maladies ouvrent la voie à une nouvelle conception de la conservation et de l'utilisation des ressources génétiques.

## 1.5 Plant genetic resources activities of the Food and Agriculture Organization of the United Nations (FAO)

This chapter traces the history of FAO's commitment to plant genetic resources from the earliest tentative meetings in the 1940s to the 1950s and 1960s when it provided assistance to plant exploration

missions and began publishing the precursor to the *Plant Genetic Resources Newsletter*. It follows through FAO's involvement in the establishment of the CGIAR (1971) and IBPGR (1974). FAO continues to collaborate with and provide support to these bodies, which have themselves acted as catalysts in setting up national and international programs.

In 1983 an International Undertaking and a Commission on Plant Genetic Resources was set up. About 115 countries adhered to the Undertaking and/or became members of the Commission. Recently, FAO has focused on developing national programs, their infrastructures, human resources and policies for better conservation and use of germplasm. Projects are funded through the regular program, the technical cooperation program, trust funds, UNDP and other sources. Future FAO work will concentrate on developing global mechanisms for the safe flow of germplasm, data, technology and funds.

## Les activités de l'Organisation des nations unies pour l'alimentation et l'agriculture (FAO) dans le domaine des ressources phytogénétiques

Ce chapitre retrace l'historique des actions menées par la FAO au profit des ressources phytogénétiques depuis les premières réunions expérimentales des années 40 jusqu'aux années 50 et 60 où elle a apporté une aide aux missions de prospection végétale et commencé à publier le précurseur du *Plant Genetic Resources Newsletter*. Le chapitre indique ensuite la part prise par la FAO dans la création du GCRAI (1971) et du CIRPG (1974 ).

La FAO poursuit sa collaboration et son soutien à ces organismes qui ont eux-mêmes servi de catalyseurs dans la mise en place de programmes nationaux et internationaux.

En 1983, un Engagement international et une Commission des ressources phytogénétiques ont été instaurés. Quelque 115 pays ont soit adhéré à l'Engagement et/ou sont devenus membres de la Commission. Les travaux récents de la FAO sont centrés sur l'élaboration de programmes nationaux, leur infrastructure, les ressources humaines et les politiques propres à améliorer la conservation et l'utilisation du matériel génétique. Des projets sont financés dans le cadre du programme ordinaire, du programme de coopération technique, des fonds fiduciaires, du PNUD et d'autres sources. Les travaux de la FAO seront à l'avenir orientés vers la mise au point de mécanismes globaux permettant d'assurer la bonne circulation du matériel génétique, des données, de la technologie et des fonds.

## 1.6 Plant germplasm collecting activities in Africa of the National Research Council (CNR) of Italy

The Germplasm Institute of the CNR of Italy, in collaboration with FAO, IBPGR, ICARDA, IITA and national institutes, has organized and conducted several expeditions to African countries to collect samples of crops considered to be at risk.

A brief account of a number of collecting missions, collaborative efforts, training, regions visited and plant germplasm collected in Africa and the distribution of plant genetic resources to African countries is given. Some comments and suggestions on evaluation and utilization to promote better use of germplasm collections are included.

## Les activités du Consiglio Nazionale delle Ricerche (CNR) dans le domaine de la collecte des ressources végétales en Afrique

L'Institut du matériel génétique du Conseil italien de la recherche (CNR) de Bari (Italie), avec le concours de la FAO, du CIRPG, de l'ICARDA, de l'IITA et d'instituts nationaux, a organisé et dirigé plusieurs expéditions dans des pays africains pour collecter des échantillons de cultures considérées en voie d'extinction.

Un bref compte rendu portant sur diverses missions de collecte, les efforts de collaboration, la formation, les régions visitées et le matériel phytogénétique collecté en Afrique septentrionale et méridionale est présenté, ainsi que la répartition des ressources phytogénétiques dans les pays africains. Des commentaires et suggestions sur l'évaluation et l'utilisation ont aussi été ajoutés pour améliorer l'emploi des collections de matériel génétique.

## 2.1 Characterization and evaluation of plant germplasm: A problem of organization and collaboration

Intensive collecting activities over the past few decades have resulted in the accumulation of valuable genetic stocks stored in various genebanks throughout the world. Although some sources of variation for useful traits have been identified and transferred to crops, most of the collections are waiting to be classified, characterized and evaluated. A tentative definition of characterization and evaluation work and/or data is given, but, in practice, it is almost impossible to draw a sharp distinction between the two kinds of activity.

Better evaluation techniques could enhance the use of genetic resources for crop improvement, but these cannot be oriented evaluation without collaboration between breeders and specialists. For this reason, examples of collaboration with national and international agricultural institutes and organizations in the field of evaluation for resistance to biotic and abiotic stresses are provided.

## La caractérisation et l'évaluation du germoplasme végétal: Une question d'organisation et de collaboration

Les opérations de collecte menées activement au cours des dernières décennies ont permis d'accumuler un précieux matériel génétique entreposé dans différentes banques de gènes réparties dans le monde entier. Certaines sources de variation permettant d'obtenir des caractères utiles ont été identifiées et appliquées aux cultures, mais l'essentiel des collections attend d'être classé, caractérisé et évalué. On a essayé de définir les opérations et/ou les données relevant de la caractérisation et de l'évaluation, mais dans la pratique, il est presque impossible de distinguer catégoriquement ces deux types d'activités.

De meilleures techniques d'évaluation pourraient promouvoir l'utilisation des ressources génétiques aux fins d'amélioration des cultures, mais il ne peut y avoir d'évaluation orientée sans collaboration entre sélectionneurs et spécialistes. C'est pourquoi des exemples de collaboration avec des instituts et organismes agricoles nationaux et internationaux dans le domaine de l'évaluation de la résistance aux agressions biotiques et abiotiques sont présentés dans ce chapitre.

## 2.2 Evaluation of cowpea germplasm for insect pest resistance

The identification of useful levels of pest and disease resistance precedes hybridization, selection and testing. This is the most important step in the development of pest-resistant varieties. Priorities regarding key pests must be set according to the damage they cause, research problems, and other practical control options.

The 'funnel and sieve' screening approach is usually adopted for large quantities of germplasm material. Field screening is used for most of the major field pests of cowpea at the initial stages of germplasm evaluation. However, it is often necessary to set up different trials for each pest species, or to use selective insecticides to eliminate those pests that might mask the presence of resistance to the target pests.

Laboratory and screenhouse testing is used to screen further for resistance. The process of screening-methodology development and subsequent improvements is greatly enhanced by the availability of a good resistant source.

A few examples are given in this paper to illustrate IITA's approach to germplasm evaluation.

### L'évaluation de la résistance du germoplasme de niébé aux ravageurs

L'identification des niveaux utiles de résistance aux ravageurs et aux maladies doit précéder l'hybridation, la sélection et les essais. C'est l'étape la plus importante dans la mise au point d'une variété résistante aux ravageurs.

Il convient de définir les priorités en tenant compte des ravageurs les plus nuisibles, des problèmes de recherche et d'autres possibilités de lutte.

Pour le criblage de grandes quantités de matériel génétique on adopte en général la technique de 'l'entonnoir avec tamis'. Le criblage en plein champ, utilisé aux stades initiaux du processus d'évaluation, permet d'identifier la plupart des principaux ravageurs des champs de niébé. Toutefois, il y a souvent lieu d'exécuter des essais adaptés à chaque espèce de ravageurs ou d'avoir recours à des insecticides sélectifs pour éliminer les nuisibles dont la présence pourrait dissimuler la résistance aux ravageurs ciblés.

Les essais exécutés en laboratoire et sous abri grillagé servent à affiner le criblage pour les résistances. L'existence ou l'identification d'une bonne source de résistance facilitent grandement la mise au point d'une méthodologie de criblage.

Quelques exemples illustrent l'approche de l'IITA en matière d'évaluation du matériel génétique.

## 2.3 *In vitro* conservation and distribution of root and tuber crop germplasm

Meristem-tip culture has been used to eliminate viruses from plants and to conserve germplasm collections. Tissue culture materials are the most suitable form for the international exchange of vegetative plant materials. This method is approved and accepted by plant quarantine authorities.

Two important *in vitro* techniques that can be used for the conservation of root crop germplasm are described and reviewed: reduced growth storage method for short- to medium-term storage; and cryopreservation for long-term storage. The former has been used successfully to conserve a wide range of crop species.

Clonal germplasm of cassava and sweet potato has been distributed in tissue culture form from IITA to many countries. A total of 2,000 accessions of different root crop species are maintained by the reduced growth storage method at IITA. More than 1,500 accessions of root crop germplasm can be stored in a 3.2 x 2.4 x 2.9 m culture room. Cultures can be maintained for 1-2 years before subculturing.

### La conservation *in vitro* et la distribution du matériel génétique de plantes à racines et tubercules

La culture de méristèmes est employée pour éliminer les virus des végétaux et conserver les collections de matériel génétique. Pour les plantes à multiplication végétative, le transfert international de matériel sous forme de cultures de tissus est le plus approprié. La méthode est approuvée et reconnue par les services de quarantaine.

L'auteur de cette communication examine deux techniques principales de conservation *in vitro* du matériel génétique des plantes à racines et tubercules: la conservation *in vitro* en conditions de croissance ralentie pour une période de courte ou de moyenne durée et la conservation à ultra-basse

température pour une période de longue durée. La conservation *in vitro* sous des conditions de croissance ralentie a donné de très bons résultats avec une très grande diversité d'espèces.

L'IITA a distribué dans de nombreux pays des clones de manioc et de patate douce sous forme de cultures de tissus. Deux mille obtentions de différentes espèces de plantes à tubercules sont conservées à l'IITA grâce à la culture *in vitro* en conditions de croissance ralentie. Une chambre de 3,2 x 2,4 x 2,9 m peut recevoir plus de 1,500 obtentions de matériel génétique de plantes à racines. Les cultures peuvent être conservées pendant 1 à 2 ans avant d'être régénérées.

## 3.1 Yam germplasm diversity, uses and prospects for crop improvement in Africa

Edible yams are an important food crop in the economy of Africa, particularly in sub-Saharan Africa. Only landraces are currently cultivated, but there is great diversity in the food and agronomic characteristics of the crop, and improvements are needed. Although yam is propagated vegetatively, hybridization occurs freely in nature and the seeds produced are viable. However, the forests where many yams grow are fast disappearing, and it is becoming increasingly urgent to collect and conserve these resources.

Germplasm collection and conservation is carried out in 11 institutions in eight African countries. Most of them receive support from IBPGR. Yam germplasm is held mainly in vegetative form, which is costly and increases the risk of loss. This paper discusses *in vitro* conservation as one method of saving costs and reducing the loss. Regional cooperation with international funding for storage and maintenance of germplasm is also a viable proposition.

### La diversité, les utilisations et les perspectives d'amélioration de l'igname en Afrique

L'igname comestible est une culture vivrière qui joue un rôle important dans l'économie africaine, et tout particulièrement en Afrique subsaharienne. Seules les races de pays sont actuellement cultivées, mais les caractéristiques alimentaires et agronomiques de cette culture sont très diverses et des améliorations sont nécessaires. Bien que l'igname se propage végétativement, l'hybridation se produit couramment dans la nature et les semences sont viables. Toutefois, les forêts où poussent de nombreuses espèces d'igname sont en voie de disparition rapide et il devient de plus en plus urgent de collecter et conserver ces ressources avant leur extinction.

La collecte et la conservation du matériel génétique sont effectuées par 11 instituts dans 8 pays africains. La plupart d'entre eux bénéficient d'un soutien du CIRPG. Le matériel génétique de l'igname est principalement détenu sous forme végétative, procédé coûteux qui accroît les risques de perte. Le présent document étudie la conservation *in vitro* en tant que méthode susceptible d'abaisser les coûts et de réduire les risques de pertes. Une coopération régionale avec financement international pour le stockage et l'entretien du matériel génétique constitue une autre proposition viable.

## 3.2 New trends for yam improvement in the *Dioscorea cayenensis-rotundata* complex

This paper presents the main results of the Côte d'Ivoire National Research Program on Yams, in which the Faculty of Science and Technology of Abidjan University has participated for some years. The subdivisions of both cultivated and wild West African yams are described, and the relationship between

the two examined, listing those cultivated yams which are related to wild annual, wild perennial or wild semi-perennial yams. A number of proposals are made for future work on yam improvement, including the hybridization of wild and cultivated yams to produce progeny, including clones bearing both male and female flowers.

### Les nouvelles tendances de l'amelioration de l'igname au sein du complexe *Dioscorea cayenensis-rotundata*

Ce chapitre présente les principaux résultats du Programme national de recherche de la Côte d'Ivoire sur l'igname, auquel la faculté des sciences et techniques de l'université d'Abidjan collabore depuis plusieurs années. La classification des ignames cultivées et sauvages de l'Afrique de l'Ouest y est indiquée ainsi que les rapports entre les deux catégories examinées, mentionnant les ignames cultivées qui sont apparentées à des espèces sauvages annuelles, vivaces ou semi-vivaces. Diverses propositions ont été faites au sujet des travaux futurs sur l'amélioration de l'igname, notamment l'hybridation d'espèces sauvages et cultivées pour obtenir une descendance comprenant des clones porteurs à la fois de fleurs mâles et femelles.

## 3.3 Cassava germplasm strategies for Africa

The importance of cassava as a staple crop in tropical Africa in the future warrants a selection of germplasm for sustained productivity with low inputs. The authors believe that the genetic erosion of cassava is minimal. The major world collections of cassava germplasm contain thousands of accessions which, because of duplication and other factors, may actually represent only a few hundred distinct varieties. Active collections are best enriched through collection and introduction. The use of wild species in genetic improvement programs is discussed.

Any germplasm strategy should match genotypes to specific local needs. Tropical Africa's needs for exotic germplasm of cassava may be met to some extent from South America, where the African and Asian cassava stocks originated. The four main agroecological zones of Africa are discussed in relation to similar zones in South America, with the emphasis on Brazil. These zones are: the tropical coastal belt; the tropical rainforest zone; the savannas; and the arid zones. Recommendations for meeting Africa's cassava germplasm needs are proposed.

### Les stratégies de l'exploitation du germoplasme de manioc en Afrique

En Afrique tropicale, l'importance future du manioc comme culture vivrière rend nécessaire une sélection de germoplasme pour une productivité viable et durable avec peu d'intrants. Les auteurs pensent que l'érosion génétique du manioc est minime. Les plus importantes collections mondiales de germoplasme de manioc comprennent des milliers de variétés qui, à cause de la duplication et d'autres facteurs ne représentent en fait que quelques centaines de variétés distinctes. Des collections actives sont mieux enrichies par la collection et l'introduction. Les auteurs abordent le problème de l'utilisation d'espèces sauvages dans des programmes d'amélioration génétique.

Toute stratégie axée sur le germoplasme devrait faire correspondre les génotypes aux besoins spécifiques locaux. On peut tenter de répondre aux besoins de l'Afrique tropicale en germoplasmes exotiques de manioc par des introductions en provenance d'Amérique du Sud, d'où sont originaires les variétés de manioc africaines et asiatiques. Les quatre principales zones agro-écologiques africaines sont décrites en parallèle avec des zones similaires d'Amérique du Sud, avec une attention particulière pour le Brésil. Ces zones sont: la bande côtière tropicale, la zone forestière humide tropicale, les savanes et

les zones arides. Les auteurs font des recommandations pour répondre aux besoins en germoplasme de manioc en Afrique.

### 3.4 Bananas in Africa: Diversity, uses and prospects for improvement

With few exceptions, African bananas belong to the genus *Musa*. The edible fruits can be grouped into three categories: plantain; highland beer and cooking bananas; and dessert bananas.

The black Sigatoka leaf spot disease, caused by the fungus *Mycosphaerella fijiensis*, is the major constraint to future plantain and banana production in Africa. Contact or systemic fungicides are too expensive and present a health hazard if used in backyards. The only feasible control method is the distribution of black Sigatoka-resistant bananas. Many accessions have been received as *in vitro* propagules through the INIBAP Transit Center in Belgium. The short-term campaign against black Sigatoka consists of identifying and rapidly multiplying *in vitro* black Sigatoka-resistant starchy alternatives for distribution. The long-term strategy focuses on the creation of black Sigatoka-resistant plantains through genetic improvement.

### Les bananiers en Afrique: Leurs diversité, leurs utilisations et les perspectives d'amelioration

A quelques exceptions près, les bananes africaines appartiennent au genre *Musa*. Les fruits comestibles se divisent en trois catégories: plantain, banane d'altitude à bière et à cuire, et banane de dessert.

La cercosporiose du bananier (maladie de Sigatoka), provoquée par le champignon *Mycosphaerella fijiensis*, est le principal obstacle au développement futur de la production de plantain et de banane en Afrique. Les fongicides de contact ou systémiques sont trop coûteux ou présentent des risques sanitaires quand ils sont utilisés à proximité des habitations. La seule méthode de lutte possible est la diffusion d'espèces résistantes à la maladie de Sigatoka. De nombreuses obtentions ont été reçues sous forme de propagules *in vitro* par l'intermédiaire du centre de transit de l'INIBAP en Belgique. Les moyens de lutte à court terme contre la cercosporiose du bananier consistent à identifier et à multiplier rapidement des bananes farineuses de remplacement résistantes à la maladie de Sigatoka aux fins de diffusion. La stratégie à long terme est axée sur la création d'espèces de plantain résistantes à la cercosporiose parle biais de l'amélioration génétique.

### 3.5 Cowpea genepool distribution and crop improvement

IITA, which holds the CGIAR global mandate for the improvement of cowpea, has a world collection of more than 15,100 cowpea accessions, including 560 of African wild endemic species. The collection is being used extensively in IITA's breeding programs.

The origin and taxonomy of cowpea and its closely related species has recently been reviewed, but it is not yet possible to pinpoint a specific area as the center of domestication. In the light of recent findings, the scheme of classification should probably be modified. The large and diverse cowpea germplasm collection available at IITA and elsewhere provides an opportunity for scientists around the world to improve cowpea cultivars. Germplasm resistant to insect pests has been identified and much progress has been made in screening for disease resistance. Several elite germplasm lines, called VITA lines, were directly selected from existing germplasm and have been adopted in many countries. They have good yield potential, resistance to multiple diseases and pests, and good adaptability to various ecological regions in the dry Sahel and humid tropics.

There is still much work to be done to further improve cowpea. So far, no high level of resistance has been found in cultivated cowpea to pod borers, pod-sucking bugs and cowpea coreid bug. Phytochemi-

cals and secondary plant metabolites in some of the wild species and genotypes should so be investigated.

### La distribution du pool génétique et l'amélioration du niébé

L'IITA détient le mandat mondial du GCRAI pour l'amélioration du niébé. Sa collection de plus de 15,100 obtentions, dont 560 espèces sauvages africaines, est largement utilisée par ses programmes d'amélioration variétale.

L'origine et la taxonomie du niébé et des espèces qui en sont très proches ont récemment fait l'objet d'une étude qui n'a pas encore permis d'identifier une zone spécifique de domestication. La classification de cette plante devra sans doute être corrigée à la lumière des nouvelles connaissances. Par son ampleur et sa diversité, la collection de matériel génétique disponible à l'IITA et dans d'autres centres donne aux spécialistes du monde entier la possibilité d'améliorer les cultivars de niébé. Du matériel génétique résistant aux insectes nuisibles a été identifié et de grands progrès ont été accomplis dans le domaine du criblage pour la résistance aux maladies. Plusieurs lignées de matériel génétique d'élite, dénommées lignées VITA, ont été sélectionnées directement à partir d'un matériel génétique existant et ont été adoptées dans de nombreux pays. Elles ont un bon potentiel de rendement, sont résistantes à plusieurs sortes de maladies et de ravageurs et présentent une bonne adaptabilité aux diverses zones écologiques du Sahel aride et des tropiques humides.

Il y a cependant encore beaucoup à faire pour parfaire l'amelioration du niébé. Jusqu'à présent, le niébé cultivé n'a pas fait preuve d'un degré élevé de résistance aux foreurs et aux punaises suçeuses des gousses ni aux coréïdes. Il faudrait étudier les composés phytochimiques et les métabolites secondaires des plantes dans certaines espèces et certains génotypes sauvages.

## 3.6 A *comprehensive breeding system for maize improvement in Africa*

Large increases in maize production in Africa can be achieved from improved hybrids and open-pollinated varieties developed with a comprehensive breeding system that includes: (a) selecting the best germplasm available for compositing into appropriate seed parent and pollen parent breeding populations; (b) using reciprocal recurrent selection-inbred tester with multi-stage selection for important agronomic traits to cyclically develop improved disease-resistant, insect-resistant, stress-tolerant and high-yielding breeding populations that respond to improved cultural practices; and (c) developing superior hybrids (or the advanced generation of the population cross) from these improved breeding populations for commercial use. Increases in maize production will be much greater from effective population improvement programs in a few populations than from ineffective programs in many populations.

### Un programme de sélection complet pour l'amelioration du maïs en Afrique

Un accroissement substantiel de la production maïsicole en Afrique est réalisable par la mise en oeuvre d'un programme complet d'amélioration génétique reposant sur des hybrides améliorés et des variétés à pollinisation libre, et englobant les aspects suivants: (a) la sélection des génotypes les plus adéquats en vue de leur introduction dans des populations de géniteurs mâles et de géniteurs femelles; (b) l'utilisation de la sélection récurrente réciproque et d'un testeur stable, associée à une sélection par étapes portant sur les principaux caractères agronomiques, dans le but de créer, à chaque nouveau cycle, des populations plus productives, dotées d'une meilleure résistance aux maladies et aux ravageurs et d'une plus grande tolérance aux contraintes, et qui répondent aux techniques culturales améliorées; l'elaboration d'hybrides élites (ou de la génération avancée du croisement entre populations) au départ

de ces populations en vue de leur exploitation commerciale. Les hausses de rendement obtenues à partir de programmes d'amélioration efficaces fondés sur un nombre limité de populations seront beaucoup plus importantes que celles pouvant découler de programmes inopérants, basés sur de nombreuses populations.

## 3.7 Germplasm diversity in bambara groundnut and prospects for crop improvement

Although the Bambara groundnut,*Vigna subterranea*, is of African origin and has a high nutritional value, very little research has been devoted to it. IITA, with nearly 2,000 accessions, is the major center for Bambara groundnut germplasm. This paper reviews the taxonomy, origin and geographical distribution of Bambara groundnut and describes the scope of variability and yield potential of the germplasm maintained at IITA. Bambara groundnut belongs to the family Leguminosae, subfamily Papilionoidae. Further refinement of its taxonomic position is highly controversial.

An impressive degree of variability has been found at both plant and seed level in the IITA accessions. Bambara groundnut could be a stable, low-cost and profitable food crop. Trials at IITA have produced yields of up to 3,000 kg/ha. There is considerable scope for the improvement of this crop. Efforts should be made to regroup ecotypes of comparable adaptability. A more objective system should be developed to classify the various morphotypes. When the genetic base is sufficiently wide — as in the case of Bambara groundnut — improvement is possible by means of selection among the existing landraces.

## La diversité génétique, les utilisations et les perspectives d'amélioration du voandzou

Bien que le voandzou soit originaire d'Afrique et ait une valeur nutritive élevée, très peu de recherches ont été consacrées à sa culture. L'IITA, avec près de 2,000 obtentions, est la principale banque de gènes pour le voandzou. Ce chapitre présente la taxonomie, l'origine et la répartition géographique du voandzou et décrit le potentiel de variabilité et de rendement du matériel génétique conservé à l'IITA. Le voandzou, *Vigna subterranea*, appartient à la famille Leguminosae, sous-famille Papilionoidae. Les précisions taxonomiques plus poussées sont hautement controversées.

Les obtentions de l'IITA ont montré un degré de variabilité remarquable à la fois au niveau de la plante et de la semence. Le voandzou pourrait être une culture vivrière stable, peu coûteuse et rentable. Les essais menés à l'IITA ont donné des rendements pouvant atteindre jusqu'à 3,000 kg/ha. Les perspectives d'amélioration de cette culture sont très prometteuses. Il faudrait s'efforcer de regrouper des écotypes d'adaptabilité comparable. Un système plus objectif devrait être mis au point pour classifier les différents types morphologiques. Quand la base génétique est suffisamment large — comme dans le cas du voandzou — les améliorations sont possibles par voie de sélection parmi les races de pays existantes.

## 3.8 Soybean germplasm diversity, uses and prospects for crop improvement in Africa

The great diversity in soybean germplasm will be very useful for plant breeders in Africa. Significant improvements are needed in the best soybean varieties that are presently available in Africa. Available traits which are important for Africa include resistance to Cercospora leaf spot, bacterial pustule, pod-

sucking bugs, leaf defoliators, shattering and lodging. More research is needed to find a good source of resistance to red leaf blotch. Despite advances, more work on the incorporation of high and stable yields, promiscuous nodulation and good seed storability into improved varieties is needed. Soybean breeding programs in Africa have developed varieties that can produce good yields without the need for insecticides, fungicides or high rates of fertilizer. The challenge to breeders is to stay one step ahead of the pest and disease complex. The development of varieties with the nil-lipoxygenase genes will improve taste and thus make soybeans easier to introduce into traditional foods. Plant quarantine services should be cautious about seed from Asia and South America as it could introduce new diseases, such as soybean rust, into Africa.

## La diversité génétique, les utilisations et les perspectives d'amélioration du soja en Afrique

La grande diversité du matériel génétique de soja sera très utile aux sélectionneurs opérant en Afrique. Il est nécessaire d'améliorer sensiblement les meilleures variétés de soja qui sont actuellement disponibles en Afrique. Les caractères existants présentant une importance pour l'Afrique comprennent la résistance à la cercosporiose, aux pustules bactériennes, aux punaises des gousses, aux défoliants, à la déhiscence des gousses et à la verse. Il faut mener des recherches plus poussées pour trouver une bonne source de résistance aux taches rouges des feuilles. Malgré les progrès réalisés, il faut s'efforcer davantage d'incorporer aux variétés améliorées des rendements stables et élevés et de bonnes qualités de conservation des semences. Des programmes de sélection du soja menés en Afrique ont mis au point des variétés capables de donner de bons rendements sans insecticides, fongicides ou emploi intensif d'engrais. Le défi auquel sont confrontés les sélectionneurs consiste à toujours conserver une longueur d'avance sur les ravageurs et les maladies. La création de variétés dépourvues du gène de la lipoxygénase améliorera le goût et facilitera par conséquent l'introduction du soja dans l'alimentation traditionnelle. Les services de quarantaine phytosanitaire devront être vigilants à l'égard des semences provenant d'Asie ou d'Amérique du Sud, car elles pourraient introduire de nouvelles maladies en Afrique, comme la rouille du soja.

## 3.9 African rice diversity: Conservation and prospects for crop improvement

Africa contains representatives of four of the six known genomes in the genus *Oryza*. The indigenous domesticated species, *O. glaberrima*, has been established and cultivated in West Africa for thousands of years. Although much work has been carried out on evaluating and making use of African rice germplasm for the improvement of Asian rice cultigens, it might be better to select superior cultivars of *O. glaberrima* from existing collections and then to improve them by combining better characteristics from *O. sativa* for African conditions. Africa has great potential for the expansion of rice cultivation, but the varieties chosen, drawn from existing germplasm or developed by combining desired characters from various germplasm soruces, must be suitable. Genetic resources centers play a vital role in collecting and preserving these important resources and making them available for use by researchers. Nineteen landraces and one wild *Oryza* population was involved in the development of IR64 at IRRI. IRGC distributes between 40,000 and 50,000 samples of rice a year.

## La diversité du riz africain: Conservation et perspectives d'amélioration

Quatre des six génomes connus du genre *Oryza* sont représentés en Afrique. L'espèce indigène acclimatée, *O. glaberrima* , a été introduite et cultivée en Afrique de l'Ouest depuis des millénaires. Bien que de nombreux travaux aient été consacrés à l'évaluation et à l'utilisation de matériel génétique

de riz africain pour améliorer des cultigènes de riz asiatique, il serait peut-être préférable de sélectionner des cultivars supérieurs de *O. glaberrima* à partir des collections existantes, puis de les améliorer en leur incorporant les caractéristiques de *O. sativa* les mieux adaptées aux conditions africaines. L'Afrique est dotée d'un important potentiel d'expansion de la riziculture, mais les variétés choisies, tirées du matériel génétique existant ou conçues par combinaison de caractères souhaités provenant de diverses sources de matériel génétique, doivent être appropriées. Les centres de ressources génétiques jouent un rôle vital dans la collecte et la conservation de ce matériel important qu'ils mettent en outre à la disposition des chercheurs. Dix-neuf races de pays et une espèce sauvage de *Oryza* ont contribué à la création de IR64 à l'IRRI. L'IRGC distribue entre 40,000 et 50,000 échantillons de riz par an.

## 4.1 Plant quarantine and the global transfer of plant genetic resources

This paper provides background information on the interaction of plant quarantine with the transfer of plant genetic resources. A description of the historical bases of plant quarantine is followed by an analysis of the legal basis and the procedures set up under the International Plant Protection Convention (1951), including procedural aspects of the Phytosanitary Certificate.

The geographical basis for quarantine regulations to protect pest movement along man-made pathways is analyzed and the biological questions involved in the entry status of plant material are considered, including the exceptions made for scientific materials.

The author draws attention to the three factors required for the establishment of a pathogenic organism, and shows the role of quarantines in breaking up this disease triangle.

### La quarantaine phytosanitaire et les échanges internationaux de ressources phytogénétiques

Ce chapitre décrit de façon générale l'interaction entre la quarantaine phytosanitaire et le transfert des ressources phytogénétiques. Un rappel de l'historique du système de quarantaine est suivi d'une analyse du dispositif et des procédures officielles établies dans le cadre de la Convention internationale sur la protection phytosanitaire (1951), y compris les aspects administratifs concernant le Certificat phytosanitaire.

Les aspects géographiques permettant au dispositif de quarantaine de contrôler le déplacement des ravageurs le long des voies de communication créées par l'homme sont analysés et les questions biologiques liées aux conditions d'entrée du matériel végétal sont examinées, notamment les exceptions concernant le matériel scientifique.

L'auteur attire l'attention sur les trois facteurs nécessaires à l'installation d'organismes pathogènes et montre le rôle joué par les services de quarantaine pour briser ce triangle de la maladie.

## 4.2 The role of plant quarantine in Nigerian agricultural development

An effective agricultural development program necessarily involves importations from all over the world. This increases the risk of introducing devastating pests, diseases and noxious weeds. This paper discusses a number of cases of such infestations.

The recent cassava bacterial blight, mealybug and red spider mite infestations have cost Nigeria over 1 billion naire (US$ 200 million). Statistics show that the country is still free of over 452 dangerous pests and diseases occurring elsewhere in the world, the introduction of which would be a serious threat to Nigeria's agricultural development. The paper highlights the potential implications of such risks, together with the legal obligations of the Plant Quarantine Service.

Consideration is given to the contributions made to date in the enforcement of regulations and pre-entry quarantine procedures with regard to prohibitions, entry conditions as specified on import permits, plant treatments, source of original health inspections and certificates and additional declarations required regarding the virulence, mode of transmission and global distribution of such major pests and diseases as rusts, bunts, smuts, bacterial blights, downy mildews, wilts, insects, spiroplasmas, mycoplasma organisms, nematodes and even virulent strains of some pests and diseases already found in Nigeria. There is also reference to the post-entry quarantine processing of imported plants.

The Nigerian Plant Quarantine Service has succeeded in keeping out more than 98% of foreign pests and diseases, processing over 63,000 selections of different varieties of about 82 agricultural crops.

## Le rôle de la quarantaine phytosanitaire dans le développement agricole du Nigéria

Tout programme efficace de développement agricole implique nécessairement des importations provenant du monde entier. Ceci augmente le risque d'introduire des ravageurs et des maladies dévastatrices ainsi que des mauvaises herbes. Ce chapitre examine divers cas d'infestations de ce type.

Les récentes attaques de bactériose du manioc, de cochenilles et d'acariens rouges ont au Nigéria plus d'un milliard de nairas (200 millions de dollars E.-U.). Les statistiques montrent que le pays est encore exempt de plus de 452 maladies et ravageurs dangereux présents dans d'autres parties du monde, dont l'introduction constituerait une sérieuse menace pour le développement agricole du Nigéria.

Le chapitre souligne les implications éventuelles d'un tel risque, ainsi que les obligations officielles du service de quarantaine phytosanitaire.

Sont également examinées les contributions faites à ce jour pour respecter les règlements et les procédures de quarantaine préalables à l'entrée, notamment les interdictions, les conditions d'entrée telles que spécifiées sur les permis d'importation, les traitements intéressant les plantes, l'origine des inspections sanitaires initiales et des certificats, ainsi que les déclarations complémentaires nécessaires relatives à la virulence, au mode de transmission et à la répartition générale des principaux ravageurs et maladies tels que rouilles, caries, charbons, bactérioses, mildiou, flétrissements, insectes, spiroplasmes, mycoplasmes, nématodes et même certaines souches virulentes de plusieurs ravageurs et maladies déjà présents au Nigéria. Il est également fait référence au traitement de quarantaine après l'entrée des plantes importées.

Les services nigérians de quarantaine phytosanitaire ont réussi à empêcher l'entrée de plus de 98% des maladies et ravageurs étrangers, traitant plus de 63,000 sélections de différentes variétés d'environ 82 cultures.

## 4.3 Kenyan Plant Quarantine Service: Its role and responsibilities in the East African region

Kenya, Tanzania and Uganda are still free from some major crop pests and diseases. As far back as 1930, the three East African countries realized the importance of setting up a regional plant quarantine service to ensure that new pathogens and pests were not introduced. The regional plant quarantine service was sited at the Muguga Station in Kenya and remained operational until July 1977, when regional cooperation was abandoned.

The collapse of the East African Community left Tanzania and, more particularly, Uganda without any plant quarantine system and hence vulnerable to the introduction of various new pests and diseases. This fear has been confirmed by the introduction of the greater grain borer, *Prostephanus truncatus* Horn, to Tanzania in 1982 and the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, in 1987. These pests have wrought havoc in Tanzania and are likely to have a profound effect on social development in the whole region. The greater grain borer has already gained entry to Kenya, and it seems likely that the cassava mealybug will also spread to Kenya eventually.

There is a critical need for East African countries to cooperate in plant quarantine systems. The Plant Quarantine Station at Muguga has facilities for germplasm conservation (*in vitro*, *ex situ* and as seed). The station has close working relations with the National Genebank in the maintenance of active and base collections.

### Le service de quarantaine phytosanitaire du Kenya: Son rôle et ses responsabilités dans l'Est africain

Le Kenya, la Tanzanie et l'Ouganda sont encore épargnés par certains ravageurs et maladies importants. Dès 1930, les trois pays de l'Afrique de l'Est avaient compris l'importance de mettre en place un service régional de quarantaine phytosanitaire pour veiller à ce que de nouveaux ravageurs et organismes pathogènes ne soient pas introduits. Ce service régional était installé dans la station de Muguga au Kenya et a fonctionné jusqu'en juillet 1977, date à laquelle la coopération régionale a pris fin.

L'effondrement de la Communauté de l'Afrique de l'Est a laissé la Tanzanie et plus particulièrement l'Ouganda sans aucun système de quarantaine phytosanitaire et par conséquent exposés à l'introduction de divers ravageurs et maladies. Cette crainte a déjà été confirmée par l'introduction du grand capucin des céréales, *Prostephanus truncatus*, en Tanzanie en 1982 et de la cochenille du manioc, *Phenacoccus manihoti* Matile-Ferrero, en 1987. Ces ravageurs ont provoqué des désastres en Tanzanie et sont susceptibles d'avoir un effet profond sur le développement social de l'ensemble de la région. Le grand capucin a déjà fait son entrée au Kenya; il semble que la cochenille du manioc risque également de se propager dans le pays.

Il est vital que les pays de l'Afrique de l'Est coopèrent dans le cadre de systèmes de quarantaine phytosanitaire. La station de quarantaine de Muguga dispose d'installations permettant de conserver le matériel génétique (*in vitro*, *ex situ* et sous forme de semence). Elle maintient des rapports de travail étroits avec la banque nationale de gènes pour l'entretien des collections actives et des collections de base.

## 4.4 Quarantine aspects of the international transfer of plant genetic resources from the International Institute of Tropical Agriculture (IITA)

It is essential that the correct balance be maintained between the benefits of strict quarantine regulations and those of the international adaptive testing programs. With regard to viruses in legumes, the emphasis at IITA is on prevention of seedborne infection. In vegetatively propagated crops, a zero tolerance approach is adhered to. *In vitro* culture techniques are very important here. At IITA, a plant germplasm health certificate is attached to each consignment of cowpea seed exported, and the receiving party is informed of the pest-risk status of any viruses involved.

There are different quarantine arrangements regarding breeders' seeds of leguminous crops and the genetic resources of these crops emanating from germplasm banks. For breeders seed, a 'sanitation' approach that involves keeping seed infection rates down to a certain maximum acceptable level is generally sufficient. Seeds from germplasm banks generally belong to a higher risk group and, for such materials, total elimination of seedborne viruses is recommended.

### Les divers aspects de la quarantaine et les échanges internationaux de ressources génétiques issues de l'Institut international d'agriculture tropicale (IITA)

Il est essentiel de maintenir un juste équilibre entre les avantages provenant des règles strictes de quarantaine et ceux issus des programmes internationaux d'essais adaptatifs. En ce qui concerne les virus des légumineuses, l'IITA met l'accent sur la prévention des infections transmises par les

semences. Pour les cultures à propagation végétative, il doit y avoir absence totale de virus. Les techniques de culture *in vitro* sont essentielles à cet égard. A l'IITA chaque expédition de semences de niébé pour l'exportation est accompagnée d'un certificat phytosanitaire et le destinataire est informé des risques éventuels entraînés par les virus concernés.

Les semences de pré-base 1 de légumineuses et les ressources génétiques fournies par les banques de gènes ne sont pas soumises aux même dispositifs de quarantaine. Une méthode 'sanitaire' veillant à maintenir les taux de contamination à un certain niveau minimum acceptable est suffisante pour les semences de pré-base 1. Les semences issues des banques de gènes appartiennent souvent à un groupe à risques plus élevés. Pour ce type de matériel végétal, il est indiqué d'éliminer totalement les virus transmis par les semences.

## 4.5 Plant health research at the International Board for Plant Genetic Resources (IBPGR)

The transfer of germplasm, especially of vegetatively propagated crops, involves serious quarantine hazards unless appropriate precautions are taken. The major factors influencing the level of risk involved with the international movement of plant genetic resources are reviewed and recommendations for improvement are made.

*In vitro* transfer of germplasm can greatly reduce risks if good *in vitro* practices are adopted. The application of therapy techniques to eliminate virus and virus-like diseases contributes substantially to reducing the hazards involved in germplasm movement. In all cases, adequate virus indexing is an essential step in the process of germplasm transfer.

IBPGR has initiated a research program on disease indexing and therapy based on the most up-to-date biotechnology in order to develop rapid and sensitive techniques that will facilitate the safe movement of germplasm, especially of vegetatively propagated crops. Research on the development of therapy and indexing methods that are carried out *in vitro* will provide a contained system that will offer maximum safety for the international transfer of plant genetic resources that cannot be moved as seeds.

## La recherche phytosanitaire au Conseil international pour les ressources phytogénétiques (CIRPG)

Les transferts de matériel génétique, notamment des cultures à multiplication végétative, présentent des risques phytosanitaires sérieux si l'on ne prend pas toutes les précautions nécessaires. L'auteur passe en revue les principaux facteurs ayant une incidence sur le degré de risque associé au transfert international des ressources phytogénétiques et formule des recommandations en vue d'améliorer les conditions.

Le transfert *in vitro* de matériel génétique est susceptible de réduire considérablement les risques à condition d'adopter de bonnes pratiques *in vitro*. Les techniques d'élimination des viroses ou maladies analogues contribuent fortement à la réduction des risques. En tout état de cause, la détection des virus est une étape essentielle du processus de transfert.

L'CIRPG a lancé un programme de recherche sur les systèmes de détection et d'élimination des maladies; il se fonde sur la biotechnologie la plus avancée afin de mettre au point des techniques rapides et sensibles permettant de transférer en toute sécurité du matériel génétique et notamment les cultures à multiplication végétative. Le développement de méthodes de thérapie et de détection, elles-mêmes effectuées *in vitro*, doit déboucher sur un système parfaitement maîtrisé présentant des conditions optimales de sécurité pour le transfert international des ressources phytogénétiques inaptes à voyager sous forme de semences.

## 5.1 The implications of biotechnology in germplasm conservation and utilization

The genetic resources of plant species producing recalcitrant seed or vegetatively propagated species present delicate problems for medium- and long-term conservation. Solutions may be found by using biotechnology techniques based on the multiplication of plants *in vitro*. Significant results have already been obtained for the conservation and distribution of root and tuber species in the form of virus-free plantlets reproduced *in vitro* and maintained at slow growth.

A new stage has been reached in associating cryogeny with *in vitro* culture of meristems and zygotic or somatic embryos. The first applications of this technique permit the evaluation of the possibilities and limitations of cryoconservation.

## Les conséquences de la biotechnologie pour la conservation et l'utilisation du germoplasme

Les ressources génétiques des espèces végétales à semences récalcitrantes ou reproduites par voie végétative posent de délicats problèmes de conservation à moyen et long terme. Des solutions peuvent être trouvées grâce aux biotechnologies fondées sur la multiplication des plantes *in vitro*. Des résultats significatifs ont déjà été obtenus pour la conservation et la diffusion des espèces à racines et tubercules sous forme de plantules indemnes de virus reproduites *in vitro* et maintenues en croissance ralentie.

Une nouvelle étape a été franchie en associant la cryogénie à la culture *in vitro* de méristèmes et d'embryons zygotiques ou somatiques. Les premières applications de cette technique permettent d'évaluer les possibilités et les limites de la cryoconservation.

## 5.2 Taxonomy and wide crosses of pulse crops, with special reference to *Phaseolus* and *Vigna*

Although food legumes are a valuable source of protein in the tropics, their yields and resistance levels remain low. Breeders are constantly looking for new germplasm and tend to look to landraces and wild conspecific materials in preference to alien germplasm composed of related taxa. This paper discusses the problems of interspecific hybridization and describes experiments carried out on two genera, *Phaseolus* and *Vigna*.

A discussion of biosystematic and genepool considerations is followed by a description of how interspecific crosses are obtained, the obstacles to hybridization and the approaches used to breed the resulting materials. In order to obtain crosses, a comprehensive knowledge of the taxonomy, phyletic relationships and genetic components of a botanical group is necessary. To increase fertility, yield and quality once the crosses have been obtained, cumulative selection is recommended.

## La taxonomie et les croisements interspécifiques chez les légumineuses à graines avec mention particulière de *Phaseolus* et *Vigna*

Quoique les légumineuses alimentaires soient une source précieuse de protéines dans les pays tropicaux, leurs rendements et leurs niveaux de résistance restent bas. Les sélectionneurs sont constamment à la recherche de nouveau matériel génétique et tendent à s'intéresser aux races de pays et au matériel sauvage apparenté, de préférence au matériel génétique éloigné composé d'unités taxonomiques apparentées. Ce chapitre étudie les problèmes de l'hybridation interspécifique et décrit les expériences menées sur deux genres: *Phaseolus* et *Vigna*.

Des considérations sur la biosystèmatique et le pool génétique précèdent une description des procédés d'obtention de croisements interspécifiques, des obstacles à l'hybridation et des méthodes

employées pour sélectionner le matériel obtenu. Pour réussir les croisements, il est nécessaire d'avoir une connaissance complète de la taxonomie, des rapports phylétiques et des composantes génétiques du groupe botanique. Afin d'accroître la fertilité, le rendement et la qualité, une fois les croisements obtenus, il est recommandé de procéder par sélection cumulative.

# INTRODUCTION

Welcome Address

Opening Remarks

Keynote Address

# *Welcome Address*

K. S. FISCHER
*Deputy Director General (Research), International Institute of Tropical Agriculture (IITA)*

Cet atelier de quatre jours s'inscrit dans le prolongement de celui qui a eu lieu à Nairobi en septembre. Il en est attendu un succès tout aussi fructueux. Cette fois-ci on arbordera questions spécifiques aux ressources phytogénétiques des principales cultures vivrières dans les systèmes agricoles africains des régions tropicales humides et subhumides.

On behalf of the Director General of the International Institute of Tropical Agriculture (IITA) and as co-organizer with the National Research Council of Italy (CNR), the International Board for Plant Genetic Resources (IBPGR) and the United Nations Environment Programme (UNEP), it is my pleasure to welcome you to this workshop on genetic resources of selected crops in Africa. I would particularly like to welcome guests from our host country, Nigeria — the Director of the Department of Agricultural Sciences in the Federal Ministry of Science and Technology, and the Vice Chancellor of the University of Ibadan — and the representative of the Department for Cooperation and Development in the Ministry of Foreign Affairs, Italy, which has sponsored this event.

This workshop is a sequel to the workshop held in September 1988 in Nairobi, Kenya. That event was successful in addressing general issues on plant genetic resources in Africa. We expect this one to be equally successful in addressing more specific issues for the major food crops in Africa's humid and subhumid regions — cassava, maize, cowpea, yam, plantain, rice and soybean — which have been selected by IITA for genetic improvement.

We welcome our colleagues and partners from the national agricultural research systems of Africa and eminent scientists from elsewhere, and promise you four days of hard work that will ensure the success of this workshop. We are aware of the strong commitment by governments to the preservation and free exchange of Africa's genetic resources. IITA continues to be committed to assisting national programs in this endeavor.

In 1978, IITA and the Association for the Advancement of Agricultural Sciences in Africa (AAASA) jointly organized the first workshop on African plant genetic resources. Since then, IITA has constructed a new storage and processing laboratory. Through continued exploration, characterization, evaluation and documentation activities, IITA has acquired more than 40,000 accessions of various crops. This period has seen the development of new techniques for the conservation of vegetatively propagated plants and radical changes in our opportunities for genetic manipulation. It is timely to hold this workshop a decade later.

Your contribution to this workshop is essential in order to identify:

- gaps in collections, to ensure the full diversity of genetic resources from all ecosystems;
- gaps in the capacity of national programs, to strengthen their resources;
- gaps in the diversity of species, which, with new techniques of genetic manipulation, have increased their economic relevance;
- gaps in the long-term security of genetic resources, so that they are available for all to use;
- gaps in evaluation and documentation procedures, to maximize the use of resources;
- gaps in the safe and rapid movement of material to users;
- gaps in our basic knowledge of the mechanism of co-evolution of crops with pests and diseases.

The workshop has been structured for you to address these issues. I wish you success in your deliberations on how to use scarce funds and human resources most effectively to ensure Africa's food security now and in the future.

# *Opening Remarks (1)*

P. PERRINO
*On behalf of the Department for Cooperation and Development, in the Ministry of Foreign Affairs of Italy, and of the National Research Council (CNR), Italy*

Le Département Italien de la Coopération et du Développement et le Conseil National Italien pour la Recherche, en collaboration avec l'IITA, le CIRPG et le PNUD, ont parrainé cet atelier tenu comme très important pour améliorer les ressources génétiques des principales cultures vivrières africaines. Un atelier similaire d'une journée est prévu dans quelques mois pour présenter les résultats obtenus sur le niébé pendant la période 1985-88. Ce sera le fruit d'un effort conjoint de l'Italie et de l'IITA.

Mr Chairman, Ladies and Gentlemen,

It is a great honor for me to speak on behalf of the Italian Department for Cooperation and Development in the Ministry of Foreign Affairs and the Italian National Research Council (CNR).

The Department for Cooperation and Development, aware of the importance of plant genetic resources in the world, is sponsoring the International Institute of Tropical Agriculture (IITA) and other international organizations of the Consultative Group on International Agricultural Research (CGIAR) to undertake the collection, conservation and evaluation of plant genetic resources. For this reason, the Department is proud to sponsor this workshop, which will deal mainly with the major food crops in Africa.

In 1970, CNR founded the Germplasm Institute in Bari, Italy, for collecting, storing and distributing plant genetic resources, with its main objective being to improve agriculture in the Mediterranean region. The Germplasm Institute, in collaboration with the Food and Agriculture Organization of the United Nations (FAO), the International Board for Plant Genetic Resources (IBPGR) and national institutes, initially placed more emphasis on the collection of wheat, other cereals and pulses in the Mediterranean countries and Ethiopia.

This workshop has been organized within the framework of a collaborative project set up between various Italian institutions and IITA with the aim of improving African agriculture through the utilization of cowpea, one of IITA's most important mandated crops. The project began by helping IITA to improve its facilities for the conservation of existing germplasm collections, by basing a plant explorer at IITA with responsibility for collecting germplasm in African countries, and by starting upstream research studies on cytogenetics, species relationships, *in vitro* culture, nutritional value and disease resistance in cowpea. These

studies are being carried out in Italy at the Universities of Naples and Viterbo, the Institute of Nutrition in Rome and the CNR Germplasm Institute. The Institute has also been involved in collecting cowpea in the Mediterranean countries and wild species of *Vigna* in some Southern African countries, and for this reason was asked to participate in organizing this workshop in collaboration with IITA, IBPGR and the United Nations Environment Programme (UNEP).

In a few months time, IITA and the Italian institutes involved in the collaborative project will be organizing a one-day workshop to be held here at IITA to present the results of the research carried out on cowpea between 1985 and 1988. This workshop will also be sponsored by the Italian Department for Cooperation and Development.

On behalf of the Department for Cooperation and Development and my colleagues from Italy, especially of the project manager, Professor Luigi Monti of the University of Naples, I will close with best wishes for the success of this scientific meeting which we believe will provide valuable information for better management and utilization of plant genetic resources in Africa.

# *Opening Remarks (2)*

D. H. VAN SLOTEN
*Deputy Director, International Board for Plant Genetic Resources (IBPGR)*

Le séminaire de Nairobi consistait en sept sessions techniques couvrant la diversité génétique, les études écogéographiques, la conservation ex situ, la conservation in situ, l'évaluation et l'utilisation de la diversité génétique des espèces sauvages, et les programmes de collaboration. Les sept groupes de travail ont émis une série de recommandations qui concernent cinq domaines principaux: renforcement des programmes nationaux, formation, enquête sur les collections existantes, évaluation des collections existantes, et enquête sur les programmes nationaux en cours.

Ladies and Gentlemen,

It is my pleasure to welcome you all here on behalf of the International Board for Plant Genetic Resources (IBPGR) and to provide the link with the workshop on plant genetic resources in Africa, held in September 1988 in Nairobi, Kenya, and organized by the IBPGR, the United Nations Environment Programme (UNEP), the International Institute of Tropical Agriculture (IITA) and the National Research Council (CNR) of Italy.

The workshop in Nairobi took place as scheduled at the United Nations Conference Center, UNEP Headquarters, and was attended by 164 participants. Among the participants were representatives of the most important international organizations active in the field of plant genetic resources, as well as representatives of 40 African nations. The workshop agenda consisted of seven technical sessions covering genetic diversity, ecogeographical studies, *ex situ* conservation, *in situ* conservation, evaluation and utilization of genetic diversity in cultivated crops, evaluation and utilization of genetic diversity in wild species, and collaborative programs. The formal sessions were followed by seven Working Group sessions. Each Working Group produced a set of recommendations, which all touched on the five major areas which need to be addressed: strengthening national programs, training, a survey of existing collections, evaluation of existing collections and a survey of current national programs.

The major recommendations of the Nairobi workshop can summarized thus:

*On general aspects*

to create a greater public awareness of the importance of plant genetic resources among policy-makers and others who influence policy-making at a national level

| | |
|---|---|
| *On surveys* | to collate data on existing collections of germplasm and on the distribution of plant species to give a consolidated picture of the diversity of genetic resources in Africa |
| *On collection of germplasm* | to use ecogeographic methods to facilitate germplasm collection, particularly with respect to wild species |
| *On training* | to improve training in all aspects of plant genetic resources work, both *in situ* and *ex situ*. Training should be available at all levels, from technical assistants to research workers, and should cover all areas of expertise, including taxonomy, experimental botany, ecology and genetics |
| | to make training available in all major languages used in plant genetic resources work in Africa, namely Arabic, English, French and Portuguese |
| *On collaboration* | to exchange germplasm freely between government and private institutions, universities and international institutes, both within and between countries |
| | to develop information networks for the exchange of data |
| | to prepare comprehensive catalogues for all major crops |
| | to stimulate affiliation of centers of excellence in Africa with relevant institutions in other parts of the world |

I would like to express IBPGR's gratitude to the co-sponsors of the Nairobi workshop: the Finnish International Development Agengy (FINNIDA), the Directorate General for International Cooperation (DGIS, Netherlands), the Norwegian Agency for International Development (NORAD) andthe Swedish International Development Agency (SIDA).

The Nairobi workshop and this workshop at IITA both commemorate the first workshop on plant genetic resources in Africa, organized by IITA and the Association for the Advancement of Agricultural Sciences in Africa (AAASA) and held at IITA in January 1978, just over 10 years ago. The aim of the Nairobi workshop was to identify the strengths and weaknesses of national, regional and, eventually, continental activities on plant genetic resources in Africa and possible solutions. We expect this workshop at IITA to focus on the more technical aspects of plant genetic resources activities on IITA mandate crops.

Initially, the intention was to publish the proceedings of the Nairobi workshop as a joint IBPGR/UNEP publication. Following the agreement with IITA and CNR on organizing the two workshops, it was agreed to publish the proceedings of both workshops as companion volumes. During the Nairobi workshop, Dr A. O. Williams of the Organization of African Unity (OAU) expressed great interest in the country reports and offered OAU's assistance in publishing these. In subsequent discussions, it was agreed to publish all country reports prepared for the two workshops in one volume. Thus, the final publication will consist of three volumes, and we hope this publication will serve as a standard reference on plant genetic resources in Africa.

On this note, I would like to wish you success in your deliberations during this workshop.

# *Opening Remarks (3)*

H. ZEDAN
*Senior Program Officer, United Nations Environment Programme (UNEP)*

Considérant le taux de destruction alarmant atteint par les divers écosystèmes tropicaux, le PNUD attache un importance particulière à cet atelier qui doit contribuer de manière significative à la conservation des ressources génétiques de l'Afrique et à améliorer sa sécurité alimentaire. Le moment est particulièrement bien choisi pour organiser cette manifestation en réponse à la Réunion des Ministres Africains sur l'Environnement qui a eu lieu au Caire, afin de mettre en place un réseau continental pour les ressources génétiques.

Mr Chairman, Distinguished Participants, Ladies and Gentlemen,

I very much appreciate the opportunity to address this important meeting. I bring you greetings from Dr M.K. Tolba, our Executive Director, and Dr R. Olembo, both distinguished scientists with a deep interest in what this workshop represents. The United Nations Environment Programme (UNEP) is pleased to be associated with this workshop, together with the International Institute of Tropical Agriculture (IITA), the International Board for Plant Genetic Resources (IBPGR) and the National Research Council (CNR) of Italy.

Since its establishment in 1972, UNEP has been concerned with the conservation of plant genetic resources and their utilization for sustainable development. The world's population is growing at an ever-increasing rate, which not only creates imbalance in the relationship between people, resources, environment and development but also increases the pressure on natural resources. The conservation of plant genetic resources and their natural ecosystems constitutes one of the major issues of our time. Countless plant species are still undiscovered or undescribed. Most of them are in the tropics, and many may be of significant value as sources of food, energy, fiber, drugs, chemicals or other materials, as well as sources of genes for genetic selection and improvement of crop plants and for the biotechnology applications.

Yet these species are being exterminated at an unprecedented rate, essentially as a result of human activity. The rapid destruction of the world's most diverse ecosystems, especially in the tropics, has led most experts to conclude that perhaps one quarter of the world's total biological diversity, amounting to a million species or more, is at serious risk of extinction within the next 20 to 30 years. An average loss of 100 species per day during this period exceeds the historical rate of extinction by a factor of perhaps 1,000.

Despite such measured warnings, the emerging awareness of the need for urgent action to conserve and develop Africa's plant genetic resources is not yet reflected in well-organized

national and regional programs. Conservation programs including planning, training, education, research and cooperation at national, regional and international levels are rare. National efforts for conservation are still sectoral and poorly organized, and local people are seldom involved. Few countries have prepared conservation strategies or guidelines to meet conservation requirements. Assistance and guidance available to African countries wishing to start such programs have been modest or even absent, and the magnitude of the problem is still far from fully appreciated by decision-makers.

There is a clear need for additional efforts to safeguard Africa's genetic resources, but much useful work is already being done. This includes the coordinating activities of UNEP, IBPGR, IITA, the Food and Agriculture Organization (FAO) and other United Nations and non-governmental organizations which aim to:

- promote measures which maintain plant genetic diversity and ecological processes, while incorporating these measures into all development activities;

- develop measures leading to rational, sustainable use of plant genetic resources.

The urgent need for action to halt and reverse the degradation of the African resource base led the African Ministerial Conference on the Environment in Cairo in 1985 to adopt the Cairo Program for African Cooperation. The Conference decided at its first meeting to establish eight regional technical cooperation networks for the Conservation and Management of Genetic Resources to help the African continent integrate conservation with environmentally sound management strategies and sustainable utilization of resources. It is timely that this workshop will be discussing technical and scientific aspects of IITA-mandated crops in response to the call to establish a continental network for genetic resources. We all expect recommendations from the workshop on how to maximize benefits and minimize losses of these resources and how to ensure their sustained availability to all users, making the most effective use of available local financial and human resources.

I regard this workshop as a timely follow-up to the IBPGR/UNEP/IITA/CNR workshop on plant genetic resources in Africa, held in September 1988 in Nairobi. At that workshop, African scientists were able to review both past achievements and the need for further work. They called for action to increase public awareness of the importance of plant genetic resources, to strengthen training and capabilities in all areas related to the conservation and management of plant genetic diversity, to better document the distribution and diversity of African plant species, to assess the implications of current agricultural and sociological practices for the conservation of plant genetic resources, and to evaluate and document the existing situation of plant genetic resources work in Africa and disseminate the resulting information widely. They also called for greater African cooperation through networking on germplasm research, collection, conservation and utilization, the transfer of information and technology, the creation of regional and national genebanks for use by all African countries, ecogeographic studies, floristic surveys and inventories, greater cooperation between national and international organizations in the exchange of materials and information, and the development of activities to conserve plant species of potential value.

UNEP is convinced that, in the years to come, African countries will develop national genetic resources committees and programs. The socioeconomic impact of such programs cannot be underestimated. I hope that you will derive maximum benefit from the exciting developments which await you.

# *Keynote Address*

A. BANJO
*Vice-Chancellor, University of Ibadan, Nigeria*

Non seulement les pressions de la démographie croissante, de l'urbanisation et de l'agriculture à grande échelle entraînent des pertes irréversibles parmi les ressources génétiques natives de l'Afrique, mais de plus, la collecte, la conservation et l'évaluation du matériel génétique sont pratiquement inexistantes dans de nombreux pays africains. L'objectif premier de ce séminaire est ce de relever ce défi et d'examiner comment renforcer les programmes nationaux relatifs au matériel génétique.

Ladies and Gentlemen,

I welcome this opportunity to address this gathering of eminent scientists, funding agencies and agricultural policy-makers on this most important issue of ensuring food security for the African continent. I join the Director General of the International Institute of Tropical Agriculture (IITA) in welcoming you to the Ibadan academic community and hope that you will find the serene environment of IITA an ideal spot for successful deliberations.

African governments, realizing the importance of an adequate food supply for social stability and the well-being of their citizens, have directed their efforts to food production as one of the major strategies for the development of their countries. However, the rapid population growth rate in Africa — one of the highest in the world — is placing unprecedented demands on available food and other scarce resources. The need to feed a rapidly expanding population has prompted the development of new and more efficient technologies in agricultural production. These new technologies have permitted vast expanses of arable land to be brought under cultivation. Concurrently, the aggregation of people in urban areas has created additional demand for land to make possible the construction of such infrastructures as highways, industrial plants and dwelling places. Large-scale farming, urbanization and other land uses that destroy natural vegetation are major causes of the loss of genetic diversity in native plants. Ironically, one of the most spectacular agricultural technological breakthroughs of modern times — the development of high-yielding cultivars — is also respons-ible for the rapid disappearance of valuable germplasm in the developing world. These highly productive new cultivars are rapidly replacing landraces of major economic importance, landraces that have often evolved in stressful environments and therefore contain genetic systems that equip them to withstand adverse climatic, soil or other growth constraints.

In Africa, the problem of genetic erosion is acute. Indiscriminate destruction of the natural habitats to which wild species are adapted, lack of a clear perception of the importance of wild relatives of crop species by policy-makers and inadequate resources are some of the factors that have contributed and are still contributing to the loss of genetic diversity in crop plants. Viewed against this background, an international workshop such as this, which focuses specifically on the genetic resources of selected crops in Africa, appears extremely relevant to the present situation in Africa. The continent is rich in economically important crops, but the full potential of many of these crops has not been exploited. Also, in some cases, crops introduced from other countries are replacing the indigenous species, with the consequent neglect of the native plants. Such under-utilized and under-exploited native species should not be allowed to become extinct and thus unavailable for use by future generations.

One of the most critical problems facing Africa today is how to feed its people. Food shortages, with attendant famine and misery, are common in many African countries. In order to solve the problem of hunger and malnutrition in Africa, the yields of crop plants must be increased, and cultivars adapted to a wide range of environments and more resistant to pests and diseases must be developed. To meet these breeding objectives, a much wider range of germplasm, consisting of standard cultivars, breeding lines, landraces and related wild and weedy species, is needed to broaden the genetic base of breeding populations. However, it is not enough to collect and conserve germplasm. Objective evaluation of this germplasm is necessary if it is to be effectively utilized. Such an evaluation should be made in collaboration with the users of germplasm, especially the plant breeders, and according to the breeding objectives set down by the breeders.

Germplasm collection, conservation and evaluation activities are, in many African countries, either non-existent or in their infancy and, as a result, most African national crop improvement programs depend on genetic resources units — such as the one here at IITA — for most of their needs. It is doubtful, however, whether such international genetic resources units can satisfy completely the particular needs of individual African countries. This workshop may wish to consider and make recommendations on how to strengthen national germplasm collection and conservation programs.

It is gratifying to note that many national, regional and international organizations — all with vested interests in global genetic diversity conservation — are participating in this workshop. In addition, representatives of individual African nations will have the opportunity to report on their activities and exchange ideas and information on the prospects and problems of germplasm conservation in their respective countries. The high caliber of the organizations and scientists participating in this workshop and the impressive turnout of African delegates attest to the importance of the collection, conservation and evaluation of germplasm in increasing and sustaining food production in Africa.

Because the primary objective of this workshop is to review past achievements and assess the need for further work in plant genetic resources collection, conservation, research and utilization for the improvement of agriculture, I venture to suggest that at the end of the meeting, the workshop should be able to:

- highlight the present genetic status of important native African crop plants and their wild relatives;

- provide recommendations on the collection, conservation and evaluation of the germplasm of important African crops not in the mandate of international agencies;

- propose coordination strategies between African genetic resources units which will reduce duplication of efforts and maximize utilization of available resources;

- impress upon participating countries the urgent need for expansion of training opportunities for indigenous genetic resources staff;

- find ways to ensure easy access to genetic resources by crop improvement practitioners in national crop improvement programs and other institutions engaged in food-crop research;

- consider the need to set up special genebanks for genetic marker stocks of important food crops, and offer suggestions as to how this should be done.

I wish you fruitful deliberations at this very important workshop.

# PART 1

## Germplasm Collection and Conservation Activities in Africa

# 1.1

# *Germplasm Collection and Conservation Activities in Africa: The Role of the International Board for Plant Genetic Resources (IBPGR)*

R. Reid, F. Attere and J. Toll

The genetic diversity of crops in Africa was, for a long time, naturally preserved by virtue of the continent's relatively traditional agriculture. However, during the past few decades there has been a rapid deteriotation of natural resources and a resultant loss in genetic diversity. The rapid increase of population throughout the continent has resulted in an ever-increasing pressure on the environment, and to feed these growing numbers of people new and better plant varieties have been introduced. The acceptance of these cultivars has led to an abandonment of the traditional varieties at the expense of the rich genetic diversity that they contained.

At the same time there have been some significant changes in traditional African farming practices, and the adoption of monocultural practices has become more widespread. In addition, large areas of forest have been cleared in order to provide land for new farms, and this has resulted in the loss of genetic diversity in wild species. Overgrazing of most grasslands, an increase in both the number and frequency of bush fires, and the insidious spread of soil erosion have all played a major part in the reduction of the continent's genetic resources.

Much of the above has been under way, albeit at a slow pace, for many centuries. Then, in the late 1960s and early 1970s, much of Africa was affected by prolonged drought. These catastrophioc climatic events evidently brought not only increasing pressure on land use, but also heightened the perception that the genetic diversity, so necessary for sustainable agriculture, was also being rapidly eroded.

The International Board for Plant Genetic Resources (IBPGR), based in Rome, Italy, was established in 1974 with a mandate to further the study, collection, preservation, documentation and evaluation of the genetic diversity of important crop plants and their wild relatives. IBPGR has acted as a catalyst in stimulating the action needed to sustain a continued interest for the conservation of these threatened genetic resources.

## GERMPLASM COLLECTION

Prior to the 1970s, very few collecting expeditions took place in which the conservation of the collected material was the primary goal of the expedition. Most countries were keeping some collections, composed mainly of exotic material and a few indigenous crop cultivars; there were few, if any, wild or weedy relatives in the collections. The composition of collections tended to reflect the interest of the breeders, their preoccupation at the time and the resources which they had at their disposal (Attere, 1988). Most expeditions were undertaken to search for specific plants or plant characters, and were typified by the occasional and often opportunistic collecting of forage grasses in East Africa.

This type of collecting continues, but increasingly the trend has been toward the collection of specific crops in relation to set priorities, or collecting to fill diversity gaps and/or gather endangered germplasm.

Spurred on by the severe and prolonged drought which affected large areas of Africa, collecting activities intensified during the early 1970s. These activities tended to concentrated on a few apparantly endangered crops, with the emphasis on cereals and grain legumes, following priorities set during the early years of IBPGR involvement. With experience and the resultant corporate knowledge of the real situation, IBPGR revised its crop priorities in 1981; this revision has provided the basis for IBPGR activities ever since (*see* Table 1).

Initially, the major collections were made in close collaboration with regional and international organizations, such as the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) and the United Nations Environment Program (UNEP), and some of the international agricultural research centers, including the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), the International Rice Research Institute

**TABLE 1 Regional priorities in Africa**

| Region | Priority | Crops |
|---|---|---|
| Ethiopia | 1 | coffee, millets, sorghum, wheat |
| (Priority 1) | 2 | banana, chickpea, cotton, cowpea |
| | 3 | *Brassica* spp., barley, *Pisum* spp., *Vicia faba* |
| | 4 | teff |
| East Africa | 1 | millets, *Phaseolus* spp., sorghum |
| (Priority 3) | 2 | banana, cassava, cotton, rice, groundnut |
| | 3 | maize, pigeonpea, yam |
| | 4 | *Dolichos* spp., *Lablab* spp. |
| West Africa | 1 | pearl millet, rice, sorghum |
| (Priority 2) | 2 | cassava, cotton, cowpea, groundnut |
| | 3 | maize, yam, African oil-palm |
| North Africa | 1 | wheat, *Vicia faba, Brassica* spp., barley |
| (Priority 1) | 2 | forage grasses and legumes |
| | 3 | *Avena* spp. |
| | 4 | millets |

(IRRI), the Centro Internacional de Agricultura Tropical (CIAT), the International Livestock Center for Africa (ILCA) and IITA. Emphasis was placed on acquiring crop landraces and primitive cultivars, and some significant collections were assembled. Of particular note were the representative collections of sorghum and pearl millet from West and East Africa and of barley from North Africa. By the mid-1980s over 130 missions had been carried out by non-national institutes, usually with a national counterpart as part of the collecting team. Duplicate samples of the collected material were left with the collaborating institutions of the national program.

The materials that have been collected with IBPGR support since 1974 are presented in Table 2 (North Africa), Table 3 (West Africa), Table 4 (East Africa) and Table 5 (Ethiopia) (*see overleaf*). Table 6 summarizes the situation throughout Africa on the basis of the main crop groups, while Figure 1 gives the number of IBPGR collecting missions in Africa, according to country (*see page 22*).

## MAIN ACHIEVEMENTS IN GERMPLASM COLLECTION

During the late 1970s and the 1980s, considerable progress was made in germplasm collection in Africa, much of it as a result of international collaborative efforts. Some of the main achievements are outlined here.

An IBPGR/ICRISAT Sorghum and Millets Germplasm Advisory Committee established in 1976 reviewed gaps in the collections, and put forward recommendations for further action by IBPGR and ICRISAT. Also, in 1976, FAO sub-contracted ORSTOM, using UNEP funds, to collect sorghum in the Sahel; this work was later taken over by IBPGR and ICRISAT. Numerous missions visited different coutries in Africa until 1981, when the IBPGR/ICRISAT Committee revised it priorities. Explorations continued thereafter in yet more countries, with increasing emphasis on wild and weedy species, but sorghum still has to be obtained from Angola, Chad, Madagascar and Sierra Leone on the basis of the 1981 priorities.

As with sorghum, the recent droughts in Africa have led to a considerable loss of pearl millet diversity. In 1984 IBPGR published a summary of the genetic resources work in sorghum and reported that significant collections were held by ORSTOM and ICRISAT (IBPGR, 1984a). In 1985, IBPGR collaborated with the International Union for the Conservation of Nature (IUCN)/ World Wildlife Fund (WWF), ORSTOM and the national research organizations in surveying and collecting wild species of *Pennisetum* (and sorghum) in Mali and Niger. The current requirement is for more wild and weedy material to be collected in Burkino Faso, Niger and Sudan. Top-priority countries requiring urgent attention as far as cultivars are concerned are the Central African Republic and Sierra Leone.

Rice germplasm has been collected extensively in Africa by IBPGR, ORSTOM/IRAT/IDESSA, the West Africa Rice Development Association (WARDA) and IITA, often in collaboration with national programs and universities. IBPGR has funded a substantial part of these activities. Rice has now been collected from 23 countries in Africa, but many remote regions within these countries have not been visited. IBPGR has recently carried out ecogeographically-based missions to obtain wild and weedy species of rice from the inland delta of the Niger river.

Cowpea is one of the mandate crops of IITA. An IBPGR Working Group on *Vigna* concluded that collections should be made in a number of countries for cultivated cowpea and in the East African and Zambian phyto-geographical zones for wild forms (IBPGR, 1982).

**TABLE 2 Crop samples from North Africa (1974-87)**

| | |
|---|---|
| **Cereals** | |
| *Avena* | 120 |
| barley | 634 |
| millets | 93 |
| sorghum | 203 |
| *Triticum/Aegilops* | 1,037 |
| maize | 234 |
| other | 23 |
| **Forages** | |
| grass | 144 |
| legumes | 2,653 |
| other | 21 |
| **Industrial** | |
| *Beta* | 61 |
| **Legumes** | |
| *Lens* | 88 |
| *Vicia* | 296 |
| *Pisum* | 73 |
| *Lupinus* | 50 |
| **Vegetables** | |
| *Brassica* | 105 |
| *Allium* | 134 |
| Cucurbits | 148 |

**TABLE 3 Crop samples from West Africa (1974-87)**

| | |
|---|---|
| **Cereals** | |
| millets | 4,826 |
| rice | 4,316 |
| sorghum | 4,148 |
| maize | 570 |
| **Forages** | |
| grass | 293 |
| legume | 219 |
| other | 17 |
| **Industrial** | |
| coffee | 160 |
| **Legumes** | |
| *Vigna* | 2,544 |
| *Arachis* | 166 |
| *Phaseolus* | 618 |
| **Roots** | |
| *Dioscorea* | 1,955 |
| *Ipomoea* | 117 |
| cassava | 100 |
| **Vegetables** | |
| *Solanum* | 867 |
| *Abelmoschus* | 1,071 |

**TABLE 4 Crop samples from East Africa (1974-87)**

| | |
|---|---|
| **Cereals** | |
| millets | 3,533 |
| rice | 1,404 |
| sorghum | 4,715 |
| maize | 585 |
| **Forages** | |
| grass | 734 |
| legume | 773 |
| other | 17 |
| **Industrial** | |
| cotton | 270 |
| **Vegetables** | |
| *Abelmoschus* | 402 |
| *Solanum* | 149 |
| Cucurbits | 1,332 |
| *Capsicum* | 176 |
| **Roots** | |
| cassava | 189 |
| **Legumes** | |
| *Vigna* | 1,720 |
| *Arachis* | 766 |
| *Cajanus* | 350 |
| *Phaseolus* | 738 |

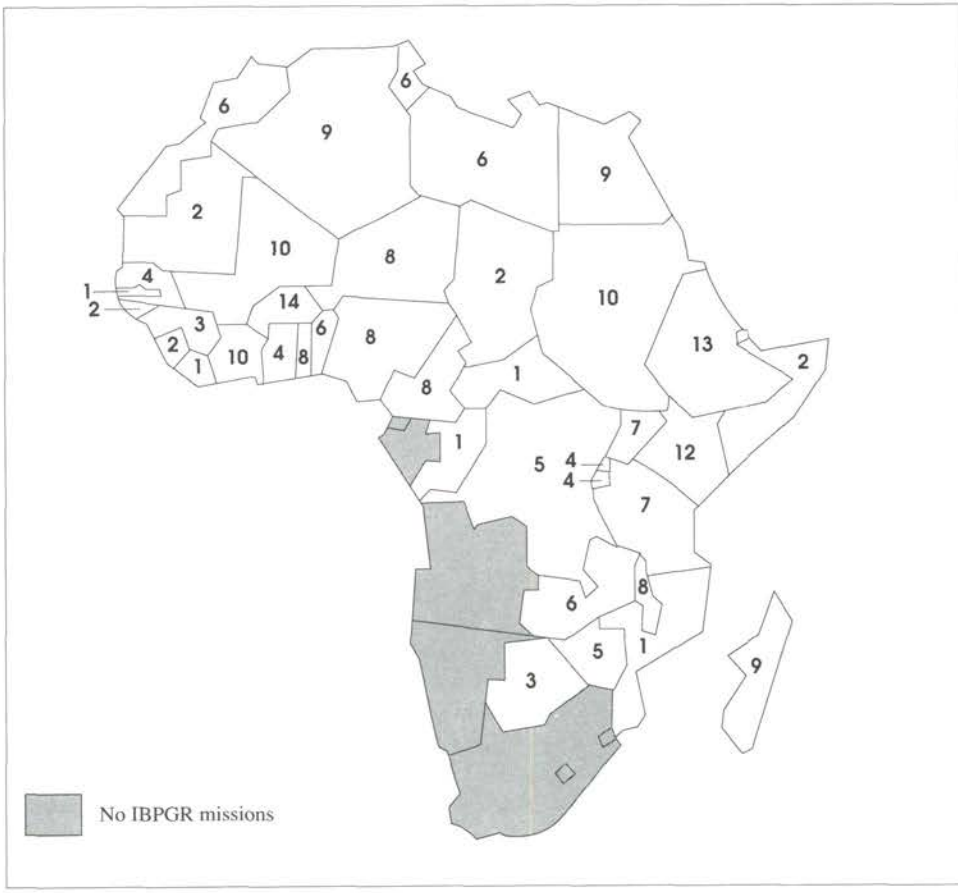
**TABLE 5 Crop samples from Ethiopia (1974-87)**

| | |
|---|---|
| **Cereals** | |
| barley | 485 |
| millets | 30 |
| sorghums | 329 |
| **Forages** | |
| grass | 61 |
| legumes | 1,116 |
| other | 1 |
| **Legumes** | |
| *Cicer* | 220 |
| *Pisum* | 61 |
| *Vicia* | 51 |
| **Vegetables** | |
| *Brassica* | 80 |

**TABLE 6 Crop samples from Africa (1974-87)**

| Region | Cereal | Legume | Forage | Fruit | Industrial | Root | Veg | Other |
|---|---|---|---|---|---|---|---|---|
| N. Africa | 2,344 | 621 | 2,818 | 6 | 172 | — | 702 | 20 |
| E. Africa | 10,303 | 3,785 | 1,524 | 120 | 798 | 270 | 2,716 | 276 |
| W. Africa | 13,875 | 3,416 | 529 | 24 | 213 | 2,214 | 2,074 | 81 |
| Ethiopia | 1,004 | 372 | 1,178 | — | 28 | — | 126 | 54 |

**FIGURE 1 Number of IBPGR collecting missions in Africa**



IBPGR missions collected cowpea germplasm between 1981 and 1983 in Burkina Faso, Ethiopia, Mali, Mozambique, Niger, Sudan, Zambia and Zimbabwe.

Priorities for the collection of tropical and subtropical forage crops were defined at IBPGR Working Group meetings in 1979 and 1983. A plan of action for forage genetic resources was developed after the 1983 meeing (IBPGR, 1984b). Many forage legumes are indigenous to Africa, and ILCA has systematically collected *Trifolium* species in the Ethiopian highlands with IBPGR support. Amongst the numerous forage missions supported by IBPGR in conjunction with scientists from international centers are two of particular note: an IBPGR-funded mission by ILCA and CIAT, in association with national scientists, to collect forage legumes and grasses in Tanzania in 1985; and IBPGR support for a CIAT collector working in collaboration with the national institutes to collect grass and legume samples in Burundi and Rwanda.

Since the early 1980s, some 74 collecting missions have been almost entirely proposed and carried out by national institutions. The emerging and existing national programs in Africa will clearly play a far greater role in the initiation and organization of collecting missions in the future. The trend in recent years towards specialized collecting and the acquisition of the wild relatives of crop species will continue as plant breeding techniques become more advanced and the value of these wild relatives is increasingly recognized. Also, while many of the early priorities were dictated by emergency situations, attention is now being increasingly focused on a regional or country basis. This approach has several advantages: the need for collecting can be more easily assessed, professional links can be developed with a range of national organizations, and local scientists will become more involved with the entire process.

## GERMPLASM CONSERVATION

Conservation is the vital link between the acquisition and utilization of plant genetic resources, and includes all the ways in which plant germplasm is stored and preserved. The needs of conservation and the the resources devoted to it vary widely with the crop. Germplasm can be conserved as seed, as vegetative material, as tissue cultures or as living plants *in situ* and *ex situ*. One or more of these methods may be used for any crop (IBPGR 1985, 1986).

The standards and strategies of conservation vary greatly between countries and institutions in Africa, from those of high quality — such as IITA, ILCA and the Plant Genetic Resources Center/ Ethiopia (PGRC/E) — to non-existent. Until recently, most germplasm was kept under extremely poor conditions. Indeed, many of the existing cold stores for seeds in Africa do not work and there is often inadequate management of the germplasm.

IBPGR has put major emphasis on providing facilities and equipment of an appropriate technology, such as driers and freezers for seed storage, and has assisted many countries in establishing cold stores for seeds. These countries include Botswana, Burkina Faso, Burundi, Côte d'Ivoire, Ethiopia, Ghana, Kenya, Madagascar, Mauritania, Mozambique, Niger, Nigeria, Senegal, Sudan, Tanzania, Togo, Zaire, Zambia and Zimbabwe. The Genetic Resources Unit of IITA was established in 1975 with the initial financial assistance from IBPGR, and ILCA and PGRC/E have also received IBPGR support for their conservation efforts.

There are currently too few well-equipped and adequately funded long-term storage facilities in Africa. However, some progress has been made, as shown by the following recent developments assisted by IBPGR.

| | |
|---|---|
| *Burundi, Rwanda, Zaïre* | A genetic resources program has been established with IRAZ and there are now long-term storage facilities in Burundi. |
| *Côte d'Ivoire* | A genetic resources center is being established in Bouaké. |
| *Egypt* | Small items of equipment have been provided to facilitate the continued smooth running of the genebank. |
| *Nigeria* | A new institute dealing the crop genetic resources, the NBPGRVC, has recently been established in Nigeria, with assistance from IBPGR and the United Nations Development Program (UNDP). Its establishment follows several years of cooperation between IBPGR, FAO and NIHORT, which was the IBPGR regional centre for vegetables such as okra, *Amaranthus* species and *Celosia argentea*. The institute's new mandate covers a wide range of crops. |
| *SADCC countries* | The Nordic Genebank and IBPGR are assisting in the establishment of a regional genebank in Lusaka, Zambia, to serve the Southern African Development Coordination Conference (SADCC) countries. The objective of the project is to strengthen national capabilities by establishing strong national programs and basic conservation structures. The project will extend over a 20-year period and the Nordic countries have already pledged $US 10 million. |
| *Senegal* | At present, genetic resources work in Senegal is located at several different places: Bambey (millet, groundnut, cowpea and sorghum); Djibelor (rice), Nioro (maize); Kaolack (cotton); and Camborene (horticultural crops). Plans are being drawn up to develop a centralized genetic resources center, probably in Bambey. |
| *Uganda* | A cold store room has been built at Serere Research Station. |
| *Zimbabwe* | A long-term conservation center has been established at Gwebi. |

## IBPGR'S OPERATIVE STRUCTURE IN AFRICA

As IBPGR grew over the years and the overall African genetic resources work expanded, it became necessary for IBPGR representation on the continent. Field Offices were established in 1981 to cover West Africa (initially based in Burkino Faso, but now in Niger), in 1982 to

cover East and Southern Africa (based in Nairobi) and in 1984 to cover North Africa and South-West Asia (based in Rome). These Field Offices perform a vital coordinating role in ensuring that IBPGR and the genetic resources community maintain a close working relationship.

An IBPGR collector was based in Niamey for a year, from 1987 to 1988. A resident IBPGR collector, based in Cyprus, conducts missions into North African countries. Since 1986, an IBPGR collector has been operating from Harare, Zimbabwe, and is responsible for germplasm collection activities in the countries of the SADCC region. IBPGR also supported an intern to collect forage plants in Ethiopia during 1985, and an intern was based in Togo from 1984 to 1985 to assist national scientists in germplasm collection, characterization and documentation.

Recently, IBPGR commissioned a study, using Kenya as a model, to evaluate the feasibility of monitoring genetic erosion on a systematic basis. A model is being developed for monitoring genetic erosion in which causal agents are identified and quantifiable parameters formulated. Factors taken into account include development shemes, urbanization, human and animal migrations, and even climatic changes. It is hoped that the results of this study, once they are published, will prove useful to IBPGR Field Officers in planning future collecting.

## FUTURE INITIATIVES

Many of the projects discussed above are clearly ongoing. There are, however, a number of new initiatives now under way that deserve mention.

In 1987, the database on germplasm collected with IBPGR support was updated and reorganized, and was distributed to IBPGR Field Offices in Nairobi and Niamey. In addition to the passport data on existing collections, this database is being supplemented by literature surveys and distribution maps are being prepared. Mapping work will receive high priority in the coming years, as part of the planning for the germplasm collection program. These studies are an important means of obtaining better data on the distribution of genetic resources and of ensuring that a range of variation is available and is described in the collections.

Projects that are wholly or partly African in origin include a database of 21,400 accessions of a Mediterranean forage species conserved in genebanks, a study on the African *Vigna* genepool, a study of species diversity in the African *Gossypium* complex and a study of the *Corchorus* and *Hibiscus* genepools.

## CONCLUSION

Since its establishment, IBPGR has played an active role in the conservation of African plant genetic resources. A great deal has already been accomplished, particularly in the collection and preservation of landraces and primitive cultivars. More emphasis is now being placed on the wild relatives of crops.

In order to strengthen the efforts of the national programs, IBPGR Offices on the continent are fully operational and IBPGR continues to be committed to ensuring the long-term conservation of African plant genetic resources.

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# 1.2

# *The Genetic Resources Activities of the International Institute of Tropical Agriculture (IITA)*

N.Q. NG

The International Institute of Tropical Agriculture (IITA) deals with three different groups of crops, divided according to the Institute's crop improvement mandate or the place of origin of the crops and their related species:

- IITA mandated crops which are indigenous to Africa: cowpea,*Vigna unguiculata*; rice, *Oryza glaberrima*; and yam, *Dioscorea* species);

- IITA mandated crops which are not indigenous to the African continent: Asian rice, *Oryza sativa*; maize, *Zea mays*; cassava, *Manihot esculenta*; starchy banana, *Musa* species; soybean, *Glycine max*; and sweet potato, *Ipomoea batatas*. The mandate for sweet potato has recently been transferred to the Centro Internacional de la Papa (CIP), based in Peru;

- food legumes indigenous to Africa which, although not within IITA's mandate, are collected and preserved by IITA: bambara groundnut,*Vigna subterranea*; African yam bean, *Sphenostylis stenocarpa*; and miscellaneous minor legumes.

Since the establishment of its crop improvement programs in 1970, IITA has devoted considerable resources to collecting the germplasm of crops within its mandate. The germplasm is preserved, used in crop improvement programs and distributed to national plant breeders and to scientists and agricultural workers worldwide (IITA, 1988a). In order to expand its germplasm collection, characterization, evaluation, documentation and preservation activities, IITA established its Genetic Resources Unit (GRU) in October 1975 (Ng, 1982, 1987).

## PLANT EXPLORATION AND COLLECTION

The GRU systematically explores and collects the germplasm of IITA's mandated crops and their wild relatives on the African continent and assembles cowpea germplasm from research institutes and genebanks worldwide. Its aim is to collect and preserve the world collection of cowpea germplasm not only for the IITA cowpea improvement program and scientists worldwide, but also for posterity. In recent years, we have placed great emphasis on exploring and collecting wild cowpea and other wild *Vigna* species.

IITA explorers also collect all African indigenous rice species as well as landraces of Asian rice in Africa (Ng et al., 1983). In addition, the GRU and the Rice Research Program assemble, from other continents, some rice germplasm which is superior in yield, resistant to pests and diseases and tolerant to physiological stresses, for immediate use in crop improvement. Our responsibility is to collect and preserve the African regional rice germplasm as well as to maintain a good collection of currently useful varieties for plant breeding. Similarly, the GRU has placed greater emphasis in recent years on the collection of wild *Oryza* in Africa (IITA, 1988b).

The GRU and the IITA's Root and Tuber Improvement Program jointly collect and assemble yam germplasm, mainly in Nigeria, but recently also from countries in West and Central Africa. The goal is to collect germplasm for use in plant breeding and to preserve the African indigenous yam germplasm for posterity. We will continue to collect and assemble germplasm from national programs, particularly collections made recently with funding from the International Board of Plant Genetic Resources (IBPGR). IITA has arranged to collect yam germplasm in Ghana and to duplicate the collection maintained by the University of Abidjan in Côte d'Ivoire. We count on the collaboration of these and other national institutions to collect and conserve yam germplasm. It is a big task, and I invite IBPGR and other international organizations to work together to collect, evaluate, maintain and conserve yam germplasm on this continent.

The GRU also collects maize, cassava, indigenous food legumes (such as bambara groundnut), African yam beans and other African indigenous crops which are of major importance, either for utilization and preservation at IITA or for distribution to national or other international organizations. With the exception of bambara groundnut and African yam bean, those crops which are not in IITA's mandate receive minor or no attention during plant exploration.

Most of the soybean and sweet potato germplasm at IITA was introduced from other parts of the world, mainly for crop improvement. Materials brought to IITA are maintained and preserved by the GRU and IITA tissue culture laboratory in the same way as IITA-collected material.

All IITA plant exploration trips are carried out in collaboration with national programs and sometimes also with IBPGR. A duplicate set of all collected germplasm samples is given to the relevant national organization, as specified by the host government or organization. The other set of collected samples is brought back to IITA, through Plant Quarantine Service of the Federal Republic of Nigeria. After multiplication and registration, this set of materials will always be accessible to researchers in the country where the germplasm was collected. The local names or the original accession numbers (if available) and the precise location where the material was collected or assembled are documented. Thus, the country which either provided the collection or from which the materials were collected can always request the materials from IITA.

To date, the GRU has undertaken 54 exploration trips to 28 African countries and has collected some 20,000 samples of various types of germplasm (*see* Figure 1). In addition, we also receive samples donated by national programs, IBPGR and individuals.

IITA now holds over 40,000 accessions of germplasm, over 30,000 of which are grain crops (*see* Table 1 *overleaf*). These are maintained and preserved by GRU. Other germplasm materials, consisting mainly of root and tuber crops and plantain, are maintained and preserved by the tissue culture laboratory and in the field collection (*see* Chapter 2.3). The GRU will assist in the maintenance and preservation of this germplasm.

**FIGURE 1** **Countries in which IITA has launched over 50 plant exploration missions since 1976**

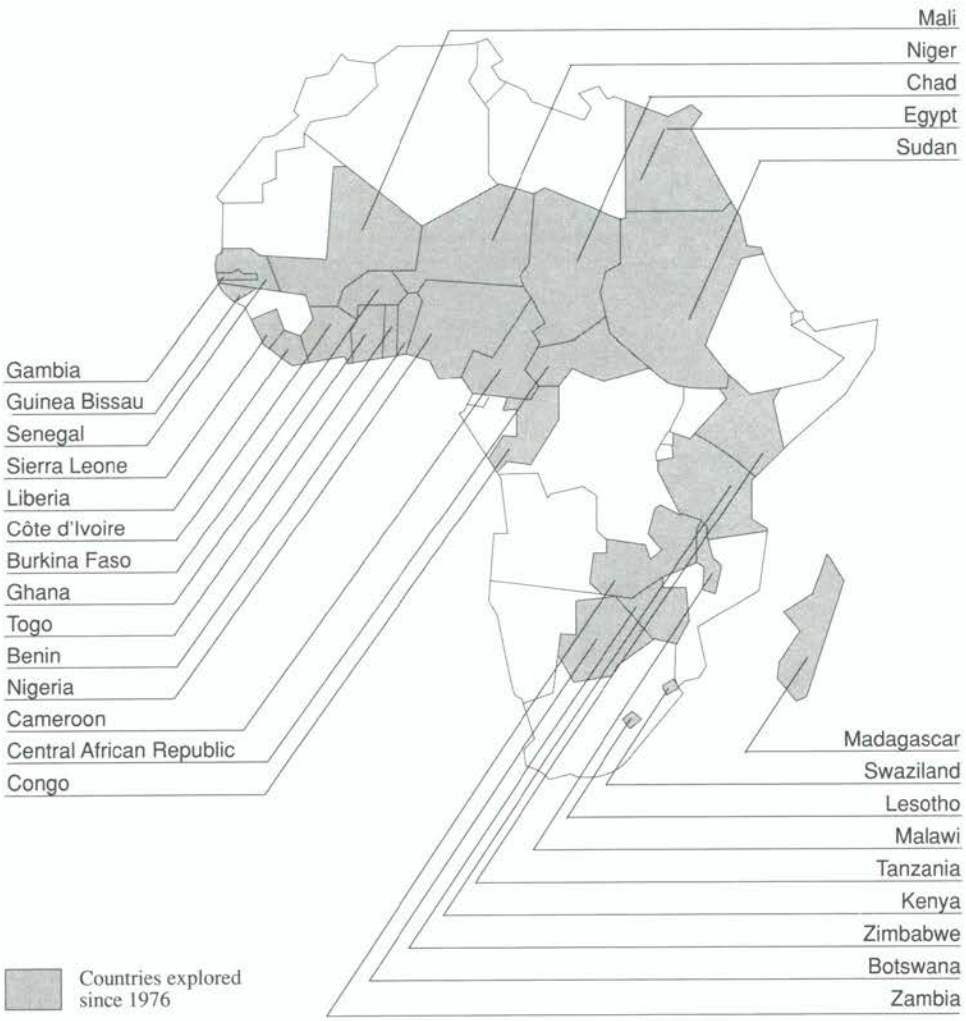

**TABLE 1 Germplasm materials held in the IITA Genetic Resources Unit as of mid-1988**

| Crop | Species | No. of accessions |
|---|---|---|
| **Grain crops** | | |
| cowpea | *Vigna unguiculata* | 15,100 |
| | wild *Vigna* | 560 |
| rice | *Oryza sativa* | 8,901 |
| | *Oryza glaberrima* | 2,557 |
| | wild *Oryza* | 183 |
| maize | *Zea mays* | 5,000 [1] |
| soybean | *Glycine max* | 1,448 |
| bambara groundnut | *Vigna subterranea* | 1,996 |
| African yam bean | *Sphenostylis stenocarpa* | 123 |
| winged bean | *Psophocarpus tetragonolobus* | 27 |
| lablab bean | *Lablab purpureus* | 28 |
| Kersting's groundnut | *Kerstingiella geocarpa* | 7 |
| pigeonpea | *Cajanus cajan* | 8 |
| jack bean | *Canavalia ensiformis* | 5 |
| sword bean | *Canavalia gladiata* | 4 |
| green gram | *Vigna radiata* | 100 |
| rice bean | *Vigna umbellata* | 1 |
| Mexican yam bean | *Pachyrhizus tuberosus* | 3 |
| **Tree and cover** | | |
| *Leucaena* | *Leucaena leucocephala* | 4 |
| *Mucuna* | *Mucuna pruriens* | 3 |
| *Gliricidia* | *Gliricidia sepium* | 4 |
| **Roots, tubers, and plantain** [2] | | |
| sweet potato | *Ipomoea batatas* | 1,000 |
| yam | *Dioscorea* spp. | 1,000 |
| plantain | *Musa* spp. | 250 |
| cassava | *Manihot esculenta* | 2,000 |
| | wild *Manihot* | 48 |
| Total | | 40,360 |

1 Mainly breeding lines developed by IITA's Maize Research Program, which also keeps about 50,000 breeding lines in short-term seed storage.

2 Roots, tubers and plantains are preserved in the tissue culture laboratory of the Root, Tuber and Plantain Improvement Program and in field genebanks.

## GERMPLASM CONSERVATION AND DISTRIBUTION

The system of seed preservation at IITA for long-term preservation (base collection) and storage facilities have has been described by Ng (1987). Seeds for the base collections are sealed in aluminum cans, each with a 0.033-liter or 0.0175-liter capacity, for storage at -20°C. Newly harvested seeds with a viability greater than 90% are dried to a moisture content of 6% in a drying cabinet (<10% relative humidity) at 20°C, before being sealed in cans for

storage. Under these conditions, we can preserve the viability of cowpea and rice for more than 100 years, and of soybean and bambara groundnut for probably up to 50 years.

Seeds for the active collections are preserved in two store units, maintained at 5° ± 1°C and 30-35% relative humidity. At present, seeds are stored in screw-top plastic jars which are not airtight, but in future airtight containers will be used. Cowpea and rice seeds can be preserved under the present conditions for 20-40 years. Requests for seeds from IITA scientists and researchers around the world are met from this collection. Between 1978 and 1987, IITA distributed over 33,000 germplasm samples (*see* Table 2).

**TABLE 2 Germplasm distribution from IITA' Genetic Resources Unit[1] to more than 80 countries 1978-87**

| Region | Cowpea | Rice | Bambara groundnut | Winged bean | Others | Total |
|---|---|---|---|---|---|---|
| | Number of samples distributed [2] | | | | | |
| Africa | 7,371 | 4,016 | 390 | 545 | 333 | 12,655 |
| Asia | 4,573 | 5,541 | 285 | 135 | 1,070 | 11,604 |
| Europe | 1,583 | 1,881 | 80 | 155 | 716 | 4,415 |
| N. and S. America | 2,580 | 1,327 | 198 | 189 | 920 | 5,214 |
| Total | 16,107 | 12,765 | 953 | 1,024 | 3,039 | 33,888 |

1 These figures do not include the thousands of samples of breeding lines distributed directly by IITA's plant breeders.

2 The Unit held 1,000 accessions as of October 1989.

## GERMPLASM CHARACTERIZATION, EVALUATION AND DOCUMENTATION

Germplasm is useful to scientists and plant breeders only if it has been properly characterized and evaluated, as this enables scientists to study the diversity of a species, to search for material for direct introduction as cultivars, or to provide genetic variability in breeding programs. At IITA, many germplasm accessions are grown out every year for characterization and evaluation in IITA's laboratories and experimental fields. GRU staff members are responsible for the characterization of cowpea, rice, bambara groundnut and yam. They collaborate with scientists from IITA's crop improvement programs and national scientists to evaluate germplasm for resistance to insect pests and diseases and physiological stresses.

A computer file is kept for each accession, and is divided into four categories:

- descriptive data recorded at the time of collection during exploration (passport data);
- data on agrobotanical characters recorded during field and laboratory evaluation, with 44 characters for rice, 30 for cowpea, 46 for bambara groundnut and 49 for yam;
- data on disease and pest resistances and on resistance to physiological stresses;
- information on seed store, date the sample entered the store and the seed viability percentage.

In addition, as new information emerges in the form of either published research or feedback from scientists, it is recorded in the appropriate form. The computerized retrieval system will identify accessions that possess specified characteristics. The genetic resources information stored in the computer file can be analyzed so that the variation of a character can be studied and pairs of characters can be easily correlated.

## TRAINING

Training is an important component of the plant genetic resources activities at IITA. Between 1981 and 1988, five group-training courses on *in vitro* germplasm multiplication, conservation, disease elimination and distribution of the root and tuber crops were conducted by the tissue culture laboratory. Many scientists and research technicians from developing countries benefited from these courses. The laboratory now provides individual on-the-job training for a small number of national scientists annually.

In collaboration with IBPGR, the GRU organized two group-training courses in germplasm exploration and conservation in 1980 and 1982 and one training course in seed technology and genebank management in 1983. In addition, in response to a request by national scientists, the GRU conducted another group-training course in seed technology and genebank management in 1988, in which some 60 scientists and technicians from national programs participated. The GRU has also provided individual on-the-job training for several young African scientists, and this type of training is expected to increase in the future.

## RESEARCH

GRU scientists and research students have conducted research to promote the usefulness of germplasm and to expand our knowledge about the taxonomy, genetics, interspecific relationships and variation of plant/crop species, as well as other related studies (IITA 1988b). In recent years the GRU has conducted research in the following areas:

- the diversity and yield potential of bambara groundnut;
- interspecific hybridization between cowpea and wild relatives;
- diversity of *Vigna* species in section *Catjan*, closely related to cowpea,*V. unguiculata*;
- interspecific hybridization between *Oryza sativa* and *O. glaberrima* to study genetic differentiation of the African collection;
- survey of distribution of *Vigna* species in Africa;
- resistance of *Vigna* and *Oryza* germplasm to insect pests and diseases, in collaboration with other IITA scientists;
- cowpea and bambara groundnut seed storability;
- biotechnological research, in collaboration with advanced laboratories in Europe and the USA.

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# 1.3

# *West Africa Rice Development Association (WARDA) Activities in Rice Germplasm Collection, Conservation and Utilization*

A. O. ABIFARIN

The West Africa Rice Development Association (WARDA) started its germplasm program on a small scale in 1973. The program was strengthened in 1978 with the addition of a full-time scientist, and since then its activities in germplasm collection and conservation have grown steadily.

## OBJECTIVES OF THE WARDA GERMPLASM PROGRAM

Aware of the contributions and activities of the other international centers, including the International Institute of Tropical Agriculture (IITA), the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) and the International Rice Research Institute (IRRI), which have collected many cultivars in West Africa in collaboration with national programs, WARDA has defined its objectives in such a way as to avoid duplication of effort and to ensure efficient management of its resources. These objectives are:

- to acquire, collect and conserve West African indigenous cultivars, landraces and wild species;
- to maintain a medium-term genebank with a temperature of 1-5°C and sample size of 30-300 g each;
- to conserve collections from other African countries outside the WARDA region and accessions of specific interest outside Africa;
- to conserve under short-term storage conditions 500-2,000 g per sample of all important commercial varieties and promising cultivars in West Africa;

- to collect in certain key areas of West Africa where other organizations have never collected, not collected recently or not collected adequately;
- to extend, when necessary, logistic or administrative support to international agricultural research centers such as IRRI, IRAT/ORSTOM and IBPGR for germplasm collection, conservation, evaluation, utilization and training in the WARDA region;
- to evaluate, in cooperation with IRAT, the collected African rice germplasm;
- to encourage the utilization of the collected germplasm in the region;
- to make available any cultivars in its germplasm bank to any scientists or institutes requesting these materials;
- to store duplicate samples of WARDA accessions at other germplasm banks, such as that at IRRI

## ACTIVITIES OF THE WARDA GERMPLASM PROGRAM

The approaches adopted by the WARDA germplasm program and the activities it has carried out since its inception in order to meet these objectives are outlined briefly here.

### Acquisition of collections

Between 1973 and 1977, WARDA received collections from national and international research stations, mostly in Africa. These were kept in a cold room, at about 21°C and 60% relative humidity. Although WARDA started its own active collections in 1978, it continued to acquire accessions from collaborating national programs and international centers. For example, in 1978 it received 283 accessions from IITA and some other accessions from the Central Agricultural Research Institute in Liberia. Between 1974 and 1982, IRAT/ORSTOM sent collections made in 11 African nations, and in 1981 WARDA received 369 accessions for medium-term storage from collections made in Chad, Guinea, Côte d'Ivoire and Mali by IRAT. In addition, samples from national collections have been acquired from several countries, including Burkina Faso and Nigeria. In 1985 WARDA received 23 collections made in Liberia by Kogoshima University, Kogoshima , Japan (Nakagama et al., 1988).

### Direct-collection activities

WARDA has adopted three approaches to rice germplasm collection in West Africa. The first is the collection by WARDA's ecologically based research stations. The mangrove swamp rice station in Rokupr, Sierra Leone, has collected landraces from the mangrove swamp ecologies; the irrigated rice station at Saint-Louis, Senegal, has collected lowland rices; the former deepwater/floating rice station in Mopti, Mali, collected deepwater and floating rice; and the upland rice station in Bouaké, Côte d'Ivoire has collected some upland landraces. Most of these have been sent to the WARDA central germplasm bank at Fendall, Liberia. A few of the collections made by two of these stations are outlined here.

In 1978, the mangrove swamp rice station collected 350 accessions. By 1980, the collection of traditional mangrove swamp varieties stood at 507, the largest number coming from Sierra Leone, with some from The Gambia and Guinea Bissau (WARDA, 1980). In 1981, 13 newaccessions from Guinea were added. In 1987, 51 accessions (34 collected from Guinea and 17 from Sierra Leone) were added. The Guinean varieties came from different ecologies, but those from Sierra Leone all came from mangrove swamp areas in the south of the country. By the end of 1982, the collection stood at 639 accessions (WARDA, 1982). More accessions were added between 1983 and 1985. In 1986, 18 accessions were collected, nine each from Guinea Bissau and The Gambia, bringing the total number to 754.

From 1978 to 1979, the irrigated rice research station carried out two explorations to the Casamance area in Senegal. In 1978, 164 accessions were collected; in the following year a further 81 (62 of *Oryza sativa* and 19 of *O. glaberrima.*) were collected.

The second approach WARDA employs in germplasm collection is to use consultants for special collections. In 1979 WARDA carried out rice germplasm explorations in two member countries, Nigeria and Mali (WARDA, 1979). The explorations were led by Dr T.A. Thomas and Dr M. Kazim, who had been made available to WARDA for three months by the Indian Council of Agricultural Research, New Delhi.

The Mali collection, made by Dr Kazim in late 1979, covered deep-flooded and floating rice in three areas of the country: Mopti (Kouna, Koloni, Bussoura, Segue, Ngomo, Sareseni, Taikri and Diambacourou), Gao (Bara, Gargouna and Goutchien) and Tenèkou (Daga, Boukari, Diondioni, Diafarabé, Kara and Koubi). A total of 104 collections were made, and each was accompanied by the following information: source, soil type, plant structure, maturity type, panicle type, grain types, grain color, kernel color and awn condition. The collection was made up of *Oryza sativa, O. glaberrima, O. barthii* and *O. stapfii* species.

Between October 1979 and January 1980, Dr Thomas, in cooperation with Dr N. Q. Ng of IITA and staff from Nigeria's National Cereals Research Institute (NCRI), explored many parts of Nigeria extensively, particularly around the Niger and Benue rivers and the northern parts of the country. Table 1 shows the number of types of rice collected in Nigeria and Mali.

The third approach employed by WARDA is for its scientists to acquire germplasm when they are visiting trial sites and farmers' fields in the region.

**TABLE 1 Rice germplasm collected by WARDA's explorers to Nigeria and Mali during 1979 and 1980**

| Species | No. of accessions | |
|---|---|---|
| | Nigeria[1] | Mali |
| *O. glaberrima* | 308 | 6 |
| *O. sativa* | 237 | 95 |
| *O. longistaminata* | 9 | 1 |
| *O. barthii* | 6 | 1 |
| *O. stapfii* | 2 | 1 |
| Subtotal | 562 | 104 |
| Total | 666 | |

1 In collaboration with IITA and NCRI.

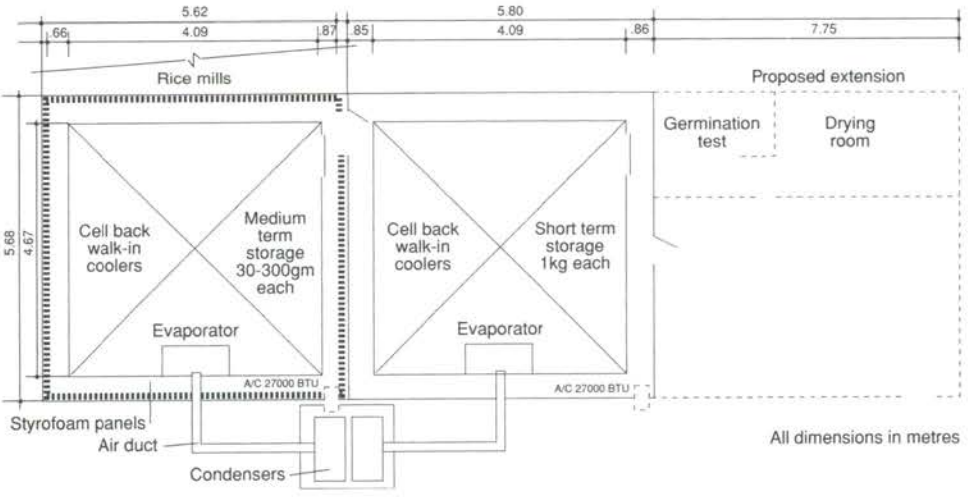
## Conservation activities

*Germplasm bank construction*

The two main activities undertaken by WARDA to conserve germplasm are: the construction of a germplasm bank; and viability determination and rejuvenation.

Until 1981, collections were kept in paper bags and aluminum foil envelopes in an air-conditioned room. In that year, however, WARDA installed walk-in cold rooms for its germplasm collections. A temperature of 1-5°C and relative humidity of 60-70% is maintained for medium-term storage. The size of each accession varies from 30 to 300 g. They are kept in universal bottles with rubber-lined metal caps. There are now 5,357 registered accessions in medium-term storage. In addition, a short-term storage unit was set up to conserve 500-1,000 g of all important cultivars recommended in West Africa and promising entries in yield trials in the region. Storage conditions are similar to medium-term storage rooms, but the temperature ranges from 5 to 10°C and seeds are stored in larger containers. There are 217 cultivars in the short-term storage. Figure 1 shows the floor plan of the WARDA germplasm bank building.

**FIGURE 1 WARDA germplasm bank floor**




Most of the collections received before the germplasm bank became functional in 1982 have lost their viability. Consequently, since 1983 WARDA has systematically rejuvenated many accessions with less than 85% viability. In 1983, 528 collections were rejuvenated and multiplied (*see* Table 2).

**TABLE 2 Germplasm collections multiplied in 1983**

| Country of origin | Number multiplied |
|---|---|
| Nigeria | 248 |
| Côte d'Ivoire | 129 |
| Mali | 78 |
| Senegal | 34 |
| Guinea Bissau | 31 |
| Gambia | 4 |
| Sierra Leone | 3 |
| Liberia | 1 |
| Total | 528 |

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# 1.4

# *Germplasm Collection, Conservation and Utilization Activities of the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM)*

A. CHARRIER and S. HAMON

The Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) began its activities in the field of genetic resources during the 1960s with studies on *Panicum* (Combes, 1975; Pernès, 1975). During the past 10 years new studies have been carried out on rice, okra, coffee, cereals, and root and tuber crops. Funding for ORSTOM collecting missions has come from many sources, including the International Board for Plant Genetic Resources (IBPGR), the European Economic Community (EEC) and the French Ministry for Research and Cooperation. From the outset, our goal has been to understand genepool organization, including cultivated and wild species. The study of gene flow and ecological and genetic barriers are fundamental for plant improvement.

Over the past decade we have added isoenzymatic variability to morphological evaluation (Second and Trouslot, 1980). The new biochemical markers now available will certainly increase our understanding of evolution. Microcomputer and multivariate analysis provide a good tool for managing and using a large amount of data. Special attention is also paid to controlled crosses in intra- and interspecific situations.

We present here an overview of the collecting missions carried out during the past decade.

## OKRA

ORSTOM's program on okra, *Abelmoschus* species, was sponsored by IBPGR. Following the basic work carried out by Siemonsma (1982) in Côte d'Ivoire, a worldwide survey of okra genetic resources was conducted (Charrier 1984), as a result of which numerous okra samples were sent to us from different parts of the world. In association with IBPGR, we sent a mission

to collect only okra in Togo and Benin (Hamon and Charrier, 1983). Great variability was found and an ecological partition of the main cultivated types was made. Another collection was undertaken in Guinea (Hamon et al., 1986). Outside Africa, a collecting mission was recently carried out in Thailand (Hamon et al., 1987). The total number of samples is now nearly 2,000 (*see* Tables 1 and 2).

**TABLE 1 Global collection of okra, *Abelmoschus* species**

| | *A. esculentus* | *A. caillei* | *A. moschatus* | *A. manihot* |
|---|---|---|---|---|
| West Africa | 873 | 673 | 17 | |
| East Africa | 251 | 1[1] | | |
| America | 8 | | | |
| Asia | 113 | | 35 | 36 |
| Europe | 129 | | | |
| Total | 1,374 | 673 | 52 | 36 |

1 Spontaneous hybrid with *A. esculentus*

**TABLE 2 ORSTOM/IBPGR collecting missions of okra, *Abelmoschus* species**

| | *A. esculentus* | *A. caillei* | *A. moschatus* | *A. manihot* |
|---|---|---|---|---|
| Benin | 213 | 64 | 12 | |
| Guinea | 97 | 94 | | |
| Niger | 31 | | | |
| Togo | 206 | 165 | 6 | |
| Thailand | 6 | | 35 | 36 |
| Total | 547 | 323 | 53 | 36 |

Our results show that two species, partly sympatric, are cultivated in West Africa. One is a previously undescribed species, *A. caillei* (Stevels, 1988), which is endemic and probably native to West Africa. The two species exhibit differences in ecological affinities; they are mainly autogamous and their hybrids are strongly sterile. They coexist but without significant gene exchange (Hamon, 1988). For both species, there is greater morphological diversity in West Africa than elsewhere. Unlike some other species, there is no correlation between enzymatic and morphologic variability. For the cultivated species, easily recognizable by their isozymic patterns (Hamon and Yapo, 1985), global polymorphism is limited because of its particular speciation process and high polyploidy status. In contrast, wild species collected in Thailand have a higher level of isoenzymatic polymorphism.

We have considerably improved crossing techniques in our laboratory and are now able to predict the rate of hybrids in intraspecific as well as interspecific crosses. We have also observed that the reproductive potential varies within cultivated species such as *A. esculentus*. Useful genes can be transferred through hybridization.

The basic collection is located in Côte d'Ivoire. Duplicate samples (100 g, 30 g) are sent to the US National Seed Storage Laboratory in Fort Collins and to ORSTOM headquarters in France, repsectively. Our data evaluation also allows us to distribute a package of 180 samples (20 g) containing a high level of diversity, which, in our opinion, is a good way to start a breeding program (Hamon and van Sloten, 1989).

Unfortunately, 'economically minor crops' are often not considered in international programs and scant attention is paid to them.

## COFFEE

To increase the diversity of the existing coffee collection, the Food and Agriculture Organization of the United Nations (FAO) and various French organizations have intensified their efforts over the past 25 years to collect wild coffee trees. Initially, emphasis was given to *Coffea arabica* because of its economic importance and to *C. canephora, C. congensis* and *C. eugenioides*, presumed to be the progenitors of *C. arabica*. Since 1978, ORSTOM's most important collecting missions in the African forests were made in Cameroon (Anthony et al., 1985), Congo and Guinea (*see* Table 3).

**TABLE 3 Coffee surveys since 1978**

| | Number of species collected | Number of genotypes |
|---|---|---|
| Before 1978 | 13 | 4,850 |
| Cameroon | 6 | 1,375 |
| Congo | 5 | 1,080 |
| Guinea | 5 | 269 |
| Tanzania | 3 | 820 |
| Total | 15+ | 3,544 |

The African coffee species are kept in living collections at Divo, Man, in Côte d'Ivoire. Over 15 species are represented by hundreds of genotypes collected from natural populations (Anthony and Mercier, 1987).

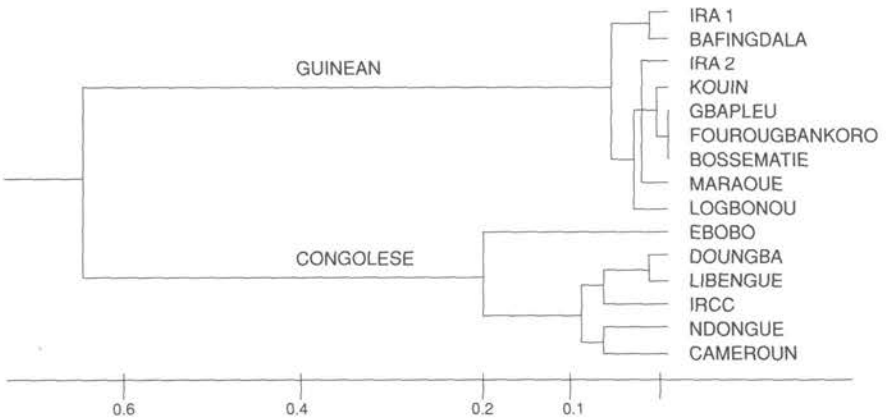
Botanists and geneticists have used numerous methods to describe the variability in wild coffee populations, including morphological and agronomic observations, enzymatic variability, and genetic analysis with progenies of controlled crosses within and between species. Some of the major achievements are outlined here.

Previously, the lack of caffeine was known only in those coffee species growing naturally on the islands of Madagascar, Comoros, Mauritius and Bourbon and belonging to the section *Mascarocoffea*. Coffee species collected in Kenya by Berthaud et al. (1980) and evaluated by Hamon et al. (1984) show that a new species, *C. pseudozanguebariae*, produces caffeine-free seeds.

Natural populations of *C. canephora* were collected in Côte d'Ivoire, Cameroon, Central African Republic, Congo and Guinea. Observations on morphological characteristics and

floral biology indicate a clear geographical differentiation into two groups — West Africa (Guinean) and Central Africa (Congolese). This was confirmed by electrophoretic analysis (Berthaud, 1986). Each group shows a specific combination of alleles (*see* Figure 1). Hybrid vigor and productivity have been observed in the progenies obtained by crossing these two groups.

**FIGURE 1 Genetic organization of *Coffea canephora* species based on Nei's genetic distance (the dendogram was drawn up using genetic distances between populations distributed over the distribution area)**




The great variability which exists in natural populations of *C. arabica* is clearly demonstrated by a hierarchical variance of morphological characters. A new source of useful genes for resistance to leaf rust, *Hemileia vastatrix*, and coffee berry disease, *Colletotrichum coffeanum*, was found.

The large number of taxa in East Africa (Bridson, 1982) and Madagascar correspond to allopatric populations with distinct morphological characters but without strongly developed genetic barriers.

The genetic evaluation of *Coffea* species should be continued, but special attention should be paid to the duplication of collections using *in vitro* culture techniques and long-term preservation, such as cryopreservation (*see* Chapter 5.1).

## RICE

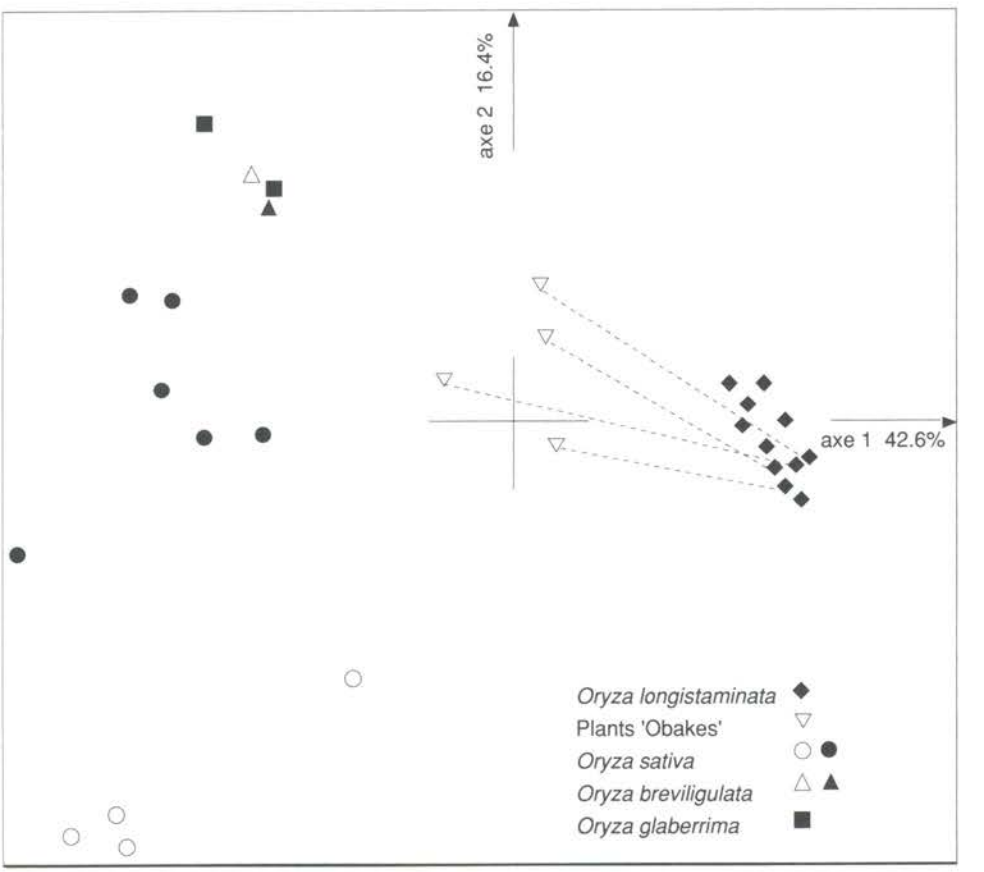
ORSTOM's geographical survey of rice genetic resources in Africa has now been completed. Collecting missions were undertaken in West Africa (Guinea) (Bezancon et al., 1984), East and Southern Africa (Tanzania, Zambia, Zimbabwe) and Madagascar (De Kochko, 1985). The ORSTOM collection consists of four main species, two cultivated and two wild: *Oryza sativa*, the worldwide cultivated species; *O. glaberrima*, the African endemic; *O. breviligulata* (*barthii*), the wild progenitor of *O. glaberrima*; and *O. longistaminata*, the perennial allogamous wild species (*see* Table 4).

**TABLE 4** **Rice (*Oryza* species) collecting missions**

| | *O. sativa* | *O. glaberrima* | *O. breviligulata* | *O. longistaminata* | *Others* |
|---|---|---|---|---|---|
| Before 1978 | 1,375 | 549 | 305 | 120 | 25 |
| Guinea Bissau | 134 | 47 | 2 | 11 | |
| Guinea Conakry | 573 | 172 | 7 | 18 | |
| Madagascar | 661 | | | 2 | |
| Tanzania | 53 | 3 | 6 | 12 | 11 |
| Zambia, Malawi | | | | | |
| Botswana | 20 | | 4 | 10 | |
| Total | 1,441 | 222 | 19 | 53 | 11 |

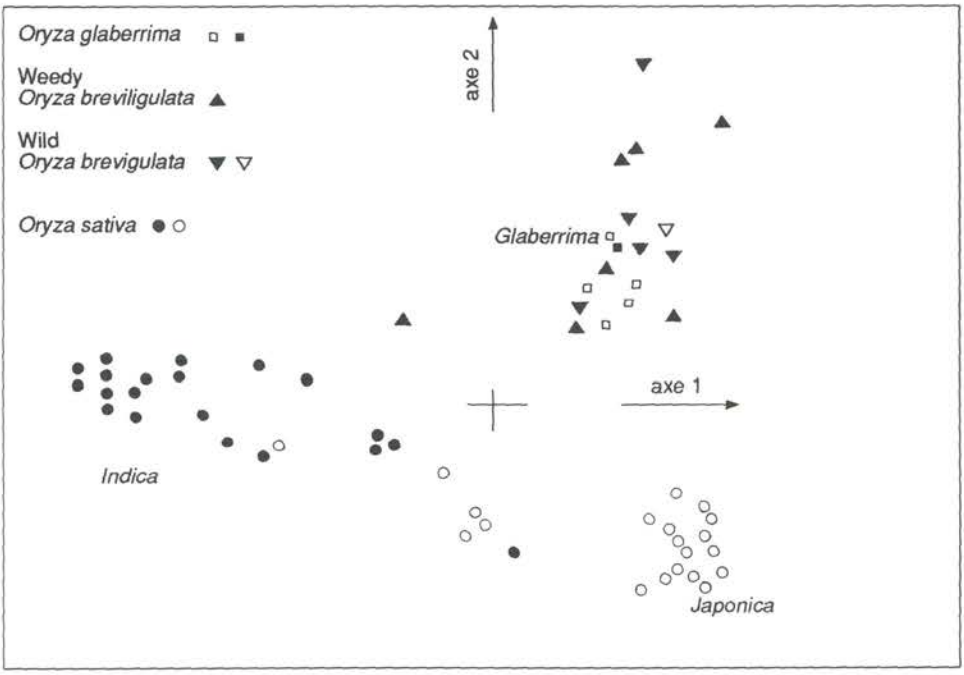
Mainly using isoenzymatic electrophoresis, we have shown that the diversity of *O. sativa* in Asia is also found in Africa. However, in Africa new polymorphism exists (De Kochko, 1987) and introgression with *O. longistaminata* (Ghesquière 1988) is not rare (*see* Figure 2).

**FIGURE 2** **Genetic diversity of rice**



Regarding the two types of *O. sativa* (*japonica, indica*) (Second, 1984), we have shown that even if some intermediate forms exist, they can be easily recognized by using electrophoretic patterns. We have also shown that the *O. glaberrima-O. breviligulata* complex is isolated (*see* Figure 3). These two groups exhibit variable levels of intercrossing but, using electrophoresis, we are able to find atypical varieties which have a high level of compatibility with any other genitors. Nevertheless, a good correlation exists between ecological constraints, the two *indica-japonica* groups (*indica,* irrigated, phenol+, *japonica,* upland, phenol-) and the phenol reaction (De Kochko, 1987).

**FIGURE 3 Genetic diversity of cultivated rice**




Breeding and improving rice directly with *O. glaberrima* will be difficult, for several reasons. As shown by Bezancon et al. (1977), the variability of *O. glaberrima* is included completely in the spectrum defined by its progenitor, *O. breviligulata*. By itself, yield production is low. It is likley to be useful only in hybridization with *O. sativa*.

The use of *O. longistaminata* merits special attention. In spite of its strong sterility barrier, the gene flow has been thoroughly analyzed (Ghesquière, 1985, 1988). The use of this species as a tool for introducing new variability in upland rice, as shown by Causse (1989), must be considered.

In conclusion, none of the above discoveries would have been possible if we had not had access to collected material. ORSTOM has tested the evolutionary hypotheses now validated by the use of chloroplastic DNA (Dally, 1988).

## MILLETS, SORGHUM AND *DIGITARIA* SPECIES

During the past 10 years pearl millet, *Pennistetum* species, sorghum and *Digitaria* species have been collected in the Sahelian region (*see* Tables 5 and 6).

**TABLE 5** ***Pennisetum*** **collecting missions**

| | Cultivated | Wild | Intermediate |
|---|---|---|---|
| Before 1978 | 2,151 | 91 | 347 |
| Burkina Faso | 211 | 13 | |
| Benin | 137 | 1 | 10 |
| Mali/Niger | 574 | 124 | |
| Mauritania | | 40 | |
| Senegal | | 9 | |
| Total | 922 | 187 | 10 |

**TABLE 6** **Sorghum and** ***Digitaria*** **collecting missions**

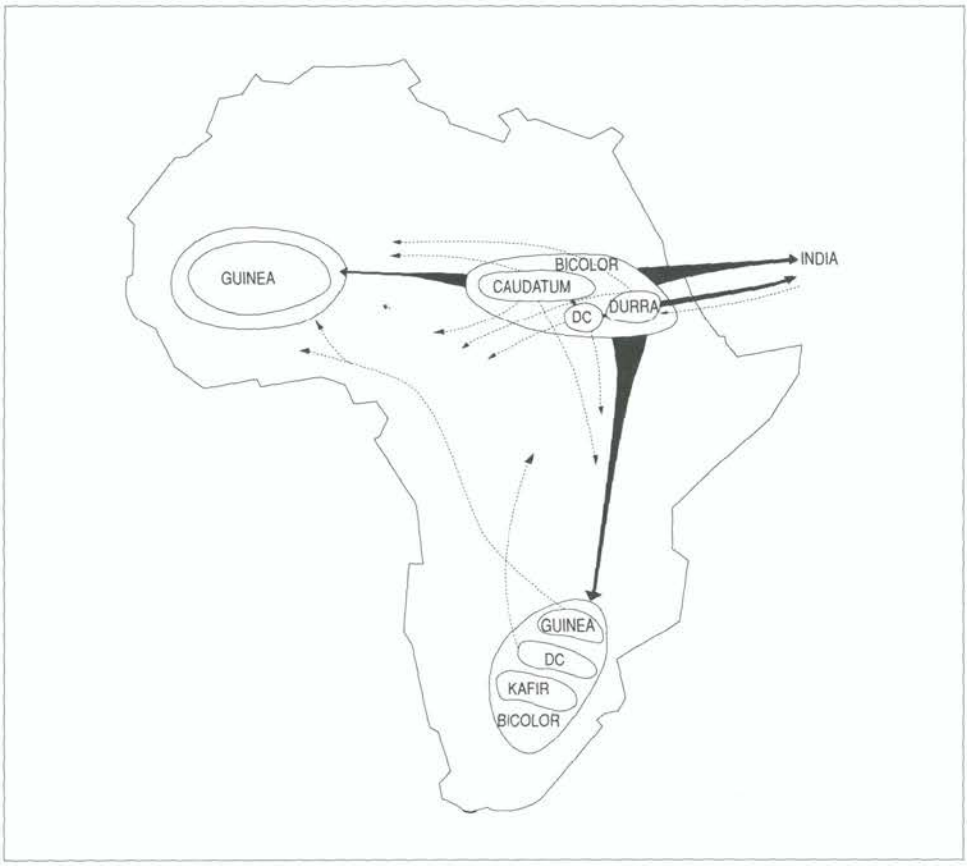
| | Sorghum | | *Digitaria* |
|---|---|---|---|
| | Cultivated | Wild | |
| Before 1978 | 1,305 | 12 | 61 |
| Benin | 512 | 11 | 12 |
| Burkina Faso | 390 | | 21 |
| Mali | 1,146 | | 139 |
| Total | 2,048 | 11 | 172 |

Over 3,000 samples of pearl millet were collected (Clément, 1985) and their diversity evaluated (Tostain and Marchais, 1989). Wild samples collected recently in Mauritania, Mali and Niger (Tostain et al., 1986) were evaluated using morphological, agronomic and electrophoretic analysis (Marchais and Tostain, 1988). From the results it appears that the genetic structure is composed of two main groups, the Mauritanian and the Niger families; the former is characterized by greater plant height, later flowering and specific electrophoretic patterns (Marchais and Tostain 1988). A strong correlation within each family exists between types associated with pasture or field crop, the former exhibiting lower vigor and enzymatic diversity in its progenies (Marchais and Tostain, 1988). A surprising observation is the lower level of enzymatic polymorphism found in wild species than in the cultivated forms.

The results of morphological evaluation of sorghum (Ollitrault, 1987) fit well with Harlan and de Wet's evolution hypothesis, but not so well with the electrophoretical data. A study which was carried out using 24 loci shows that the genetic structure is correlated with three geographic poles (East, West and Southern Africa), within which population diversity is high

(30%) and seems to be maintained by migrating processes, allogamic reproduction and heterosis (*see* Figure 4). This knowledge will be useful in breeding and in improving sorghum yield.

**FIGURE 4 Domestication of *Sorghum bicolor***




## ROOTS AND TUBERS

Yams and cassava are studied in close cooperation with the genetic laboratory of Abidjan University, Côte d'Ivoire.

The main purpose of ORSTOM's work on yam, *Dioscorea* species, is to assemble a virus-free collection. Virus diseases not only reduce yield but also lead to a loss of genetic material. The genetic structure of the *D. cayenensis-rotundata* complex is now better known (*see* Chapter 3.2) and we can propose a better rationalization of the composition of collections. Sanitation and indexing using *in vitro* culture and the enzyme-linked immuno-sorbent assay (ELISA) test are now in progress (Malaurie and Thouvenel, pers. comm.). We are also

developing all the techniques which permit micropropagation, such as somato-embryogenesis and meristem culture. We will soon be able to distribute a truly representative collection, miniaturized and disease-free, throughout the world for breeding purposes.

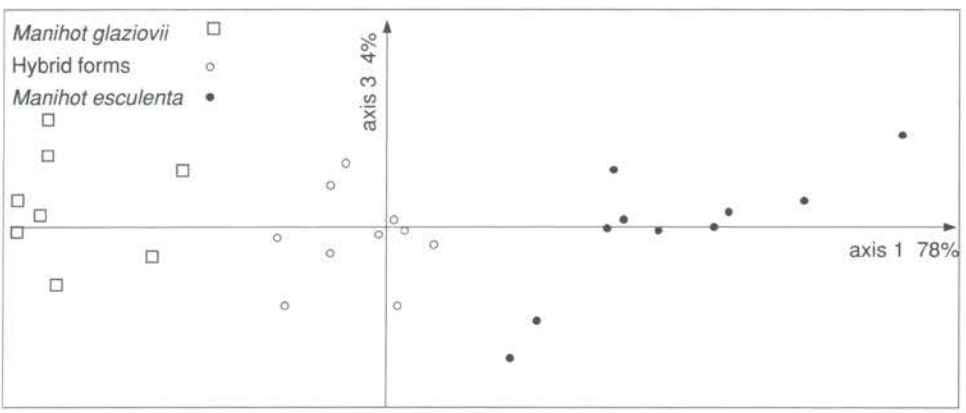
ORSTOM's work on cassava, *Manihotis* species, is connected with the international Cassava Network. Although cassava is not indigenous to Africa, African farmers maintain a great diversity of cultivars well adapted to local diseases and selected according to food preferences (Charrier and Lefèvre, 1988).

We now have a field collection of 356 clones of *M. esculenta* and 109 wild types of *M. glaziovii* or hybrids. Morphological analysis of such material, often misshapen as a result of virus diseases, is difficult (Zoudjihekpon, 1986). A technique using starch gel electrophoresis was adapted by Zoudjihekpon and Touré (1983), and four systems were revealed. Currently, 10 systems are available (Lefèvre, 1988a, 1988b), and thus we are now able to identify a variety by its pattern and to control or eliminate duplicates in a collection.

In the field we observed that *M. glaziovii* exhibits good resistance to disease. Lefèvre (1988a) has shown that in Côte d'Ivoire, and probably throughout West Africa, different levels of backcross between *M. esculenta* and *M. glaziovii* can be found. In many interspecific combinations female fertility exists and spontaneous intercrosses are relatively frequent (*see* Figure 5).

Future work will be aimed at producing virus-free material and intraspecific crosses using *M. esculenta*. Virus-resistant plants must be obtained, using *M. glaziovii*, but we need also to consider obtaining transgenic plants.

**FIGURE 5 Natural hybrids between *Manihot esculenta* and *Manihot glaziovii***




## CONCLUSION

ORSTOM's aim is not to produce improved varieties but to test hypotheses and to give guidelines to breeders. Most of our samples are duplicated and, in accordance with our mandate, are transferred to international institutes, such as the International Institute of Tropical Agriculture (IITA), the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), the International Rice Research Institue (IRRI) and the West Africa Rice

Development Association (WARDA). When possible, and where the necessary facilities exist, we also send samples to national organizations, such as the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD).

In collaboration with IBPGR in Côte d'Ivoire and Niger and with the Agence de Cooperation Culturelle et Technique (ACCT) in Tunisia, we try to transmit our philosophy, results and techniques to African students attending short courses. The building of a new ORSTOM center in the Agropolis complex at Montpellier, France, will be of great help for this purpose.

During the past 10 years ORSTOM has carried out a number of original studies on the evolutionary process, plant population polymorphism and genetic barriers, using the material that has been collected. In the next decade more emphasis will be placed on genetic evaluation and conservation. Molecular biology will certainly produce new tools which are easier to use and manage in developing countries. Nevertheless, the greatest problem is still the cost of conservation and rejuvenation. Detailed consideration needs to be given to the miniaturization of the size occupied by each genotype, the selection of materials for conservation and long-term conservation procedures.

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# 1.5

# *Plant Genetic Resources Activities of the Food and Agriculture Organization of the United Nations (FAO)*

N. M. ANISHETTY and J. T. ESQUINAS-ALCAZAR

The Food and Agriculture Organization of the United Nations (FAO) has for many years been concerned about the consequence of the loss of genetic variability of crops. It past, current and planned activities in this field are outlined here.

## HISTORICAL BACKGROUND

In 1947 and 1948 an FAO subcommittee on plant and animal stocks considered setting up a clearing house for information on germplasm, cooperation in plant exploration, the recording of collections and the removal of artificial barriers to the interchange of stocks. The major accomplishment during the early years was the publication of world catalogues on the genetic stocks of rice, wheat, barley and grain legumes (FAO, 1950a, 1950b, 1958, 1959) and the provision of assistance in the exchange of germplasm.

During the 1950s and early 1960s FAO collaborated with and/or provided logistic and other support to several plant exploration and collecting missions, including: the Australian pasture species collection in the Mediterranean region; the Swedish collection of *Brassica*, *Beta*, *Sinapsis* and grasses in Italy, Greece, Turkey and Yugoslavia; and the Japanese collection of wheat and its wild relatives in the Mediterranean and Middle East and wild rice in the Sahel and East Africa. In addition, FAO field officers made extensive collections of wild and primitive forms of various crops for use in the breeding programs of the countries to which they were assigned and for distribution to specialists in other countries (Whyte and Julén, 1963). Unfortunately, a large part of these collections was lost because no germplasm preservation strategy had been established.

Over the years FAO has developed a seed exchange program in response to a continuous stream of enquiries for samples of seed and other propagating material for use by breeders

and agronomists. The FAO *Plant Introduction Newsletter* was first published in November 1957, and in 1971 was renamed the *Plant Genetic Resources Newsletter*, which since 1978 has been published jointly with the International Board for Plant Genetic Resources (IBPGR).

### FAO Technical Meeting, 1961

In 1959 the Tenth Conference of FAO, recognizing the importance of, and danger to, genetic diversity, passed a strongly worded resolution requesting the Director General of FAO to provide assistance to the Organization's member nations, especially in coordinating plans for plant exploration, collection and storage of material.

The Conference also recommended that FAO should assist in developing international co-operation and coordination by convening an international meeting on plant exploration and introduction, and this meeting took place in July 1961. In the words of Sir Otto Frankel, 'it was the first major international conference dedicated to germplasm issues and blazed the trail for the 1967 Conference which was to inaugurate the genetic resources movement' (Frankel, 1986).

The main concern of the 1961 meeting was to discuss ways in which the world's germplasm could be surveyed, explored and made widely available for plant introduction. The meeting recommended the establishment of national and regional plant introduction stations and of exploration centers in the regions of genetic diversity with associated conservation areas for *in situ* preservation (Whyte and Julén, 1963). Because such exploration centers were expensive and difficult to set up, only one was established, in Izmir, Turkey, with funding from the United Nations Development Program (UNDP) and the Government of Sweden. In 1976, plant genetic resources centers were established in Ethiopia and Costa Rica, with support from the Federal Republic of Germany.

### FAO/IBP Technical Conference, 1967

The 1967 Conference jointly organized by FAO and the International Biological Program (IBP) was a landmark in developing scientific background and methodology and in defining goals and strategies for all phases of work in genetic resources. It was the first attempt to assemble all information relating to genetic diversity in economic plants and the preservation of this diversity. It produced a program of work for international action to be organized by FAO (Bennett, 1968; Frankel and Bennett, 1970).

### FAO/IBP Survey, 1971

In 1971, FAO and IBP jointly sponsored a field survey of crop genetic resources in the centers of diversity. Professor J. R. Harlan and Professor H. Kuckuck visited various parts of Africa and the Middle East, respectively. Their work, which was published by FAO (Frankel, 1973), provided much needed information on endangered genetic resources in the centers of diversity and helped to determine the priorities among regions and crops for germplasm collection.

## FAO Panel of Experts

One of the recommendations of the 1961 Technical Meeting led to the establishment, in 1965, of a Panel of Experts on Plant Exploration and Introduction to advise FAO on plant genetic resources and to develop international guidelines for exploration, collection, preservation and exchange of materials and information. A similar Panel of Experts on Forest Genetic Resources was established in 1968.

The Panel on Plant Exploration and Introduction held its first two meetings, in 1966 and 1967, in conjunction with the IBP genepools committee. Subsequently, there were four other meetings, in 1969, 1970, 1973 and 1974; all of these meetings were chaired by Sir Otto Frankel (FAO, 1969, 1970, 1973, 1975). During the earlier meetings the Panel elaborated upon the major recommendations of the 1967 Conference. It named priority targets for exploration; proposed a survey of threatened resources; drafted proposals for an international network of genetic resources centers; and urged FAO to initiate international cooperation in seed conservation, including the development and adoption of appropriate documentation systems.

During the later meetings, the structure and functions of genetic resources centers were defined and such terms as 'base collections', 'active collections' and 'working collections' were introduced. The Panel discussions also led to the idea of a global network of genetic resources centers including the areas of responsibilities of different institutions. The discussions on education and training at the Panel meetings and other bodies helped to initiate, in 1969, a postgraduate training course at MSc level on Germplasm Conservation and Utilization at the University of Birmingham, UK.

As a follow-up to the Panel discussions, a survey of seed storage facilities was undertaken and provided much needed information on existing collections and storage facilities. This information was published in the *Plant Introduction Newsletter*. At the request of the Panel, Professor E. H. Roberts of the University of Reading, UK, prepared a report on standards and procedures for seed storage installations used for long-term conservation (FAO 1970, 1973). These recommendations formed the basis for procedures and standards for the genebanks developed by IBPGR in the 1980s.

FAO played a central role in all these events. It provided the sponsorship and patronage for the cause of plant genetic resources conservation when no one else recognized the need.

The Panel of Experts charted the course for many years. The Technical Advisory Committee (TAC) of the Consultative Group on International Agricultural Research (CGIAR) had expected that, after the establishment of IBPGR, the Panel would be retained as a technical advisory body because of its expertise and professional experience. However, because the expertise needed was available in IBPGR, the role of the Panel diminished. Officially, FAO has not taken any decision to disband the Panel but there has been no effort to contact or reconvene it.

The Panel of Experts on Forest Gene Resources has held six meetings to date, and its main function has been to review activities in the field of forest genetic resources by various national and international organizations. It established priorities among various species at national and regional levels, most of which are carried out by the relevant national institutions, sometimes with funding and/or technical assistance from bilateral or multilateral agencies, especially through the FAO Forest Resources Division. The United Nations Environment Program (UNEP) has been a principal supporter of some of the collection, conservation and evaluation activities.

### FAO/IBP Technical Conference, 1973

The 1973 Technical Conference, sponsored by FAO and IBPGR, dealt with the scientific, technical and practical problems of exploration, conservation and use of germplasm but was more action-oriented than the previous conference. The meeting also resulted in a publication of a book, *Crop Genetic Resources for Today and Tomorrow* (Frankel and Hawkes, 1975).

### FAO Crop Ecology and Genetic Resources Unit, 1968

In 1968, FAO established a Crop Ecology and Genetic Resources Unit in the Plant Production and Protection Division to formulate a plan of action for the collection, conservation and documentation of genetic resources. This plan was submitted to UNDP but failed to receive full support. However, FAO's continued emphasis on the urgent need to collect and conserve the genetic resources of major crops threatened with extinction was reflected in one of the recommendations of the United Nations Conference on the Human Environment, held in Stockholm in 1972, which gave FAO the responsibility of helping to establish an international genetic resources program, gave UNEP the partial responsibility for plant resources and called for participation from all countries (IBPGR, 1979).

### Beltsville Meeting, 1972

Following the establishment in 1971 of the CGIAR, which is co-sponsored by FAO, UNDP and the World Bank, FAO submitted a proposal to TAC that a network of genetic resources centers be established. TAC convened a Working Party, which met at Beltsville, Maryland, in March 1972. The meeting was chaired by Sir Otto Frankel and recommended the creation, over a five-year period, of a global network of nine regional genetic resources centers located in the centers of genetic diversity, each to maintain a genebank and to be responsible for a network of collaborating national centers as well as a series of crop-specific centers, primarily the international agricultural research centers (IARCs).

The general recommendations of the Beltsville report were endorsed by TAC and the CGIAR, with modifications regarding: the number of centers (TAC felt that the establishment of nine centers over a five-year period was ambitious and reduced this number to three in the first instance, with the need to review at a later date); documentation and information exchange; and annual reports to TAC and the CGIAR.

As the coordinating functions outlined in the Beltsville report were so closely related to FAO's basic responsibilities, the CGIAR asked FAO to provide the central coordinating staff from its Regular Program budget. At the request of the Director General, the FAO Conference at its 17th meeting, in 1973, agreed to provide three additional professional officers to its Crop Ecology and Genetic Resources Unit and the Unit Program was reoriented to enable it to undertake the coordinating functions proposed by the Beltsville meeting.

### Creation of IBPGR, 1974

Further negotiations between the CGIAR and FAO resulted in an agreement to establish IBPGR as an independent entity, reporting to the CGIAR through TAC and receiving funds

through the CGIAR system. IBPGR headquarters were to be in FAO headquarters, Rome; and FAO was to provide the Secretariat to IBPGR and would have a non-voting seat on the Board of the IBPGR. In addition, FAO agreed to administer the funds of IBPGR as a trust fund without any overheads (although this agreement was initially for one year, it was operational until the end of 1987). IBPGR assumed responsibility for the planning and coordination of genetic diversity conservation, including field work, research and training. The interrelation of FAO and IBPGR introduced a new dimension into the international activities in plant exploration and introduction which had been in progress for a number of years.

During the formative years, IBPGR needed support from FAO for most of its field activities. In addition to providing the Secretariat to the IBPGR, through the Crop Ecology and Genetic Resources Unit (later renamed the Crop Genetic Resources Centre) the FAO Country Representatives offices provide much needed administrative support for the IBPGR field program. Although FAO's overall assistance to IBPGR assistance cannot be quantified, we believe that without it IBPGR would not have been able to develop the effective field program that exists today. This cooperation and assistance is continuing.

## RECENT DEVELOPMENTS: FAO GLOBAL SYSTEM

FAO and IBPGR efforts, coupled with the activities of the CGIAR commodity centers, have created the precedent for a major international effort to collect, conserve and utilize the germplasm of major economic species. They have also inspired great interest and support among many national, regional and international organizations.

Through such efforts, large germplasm collections of major crops were accumulated in genebanks, which in turn triggered concern and dissension over such issues as the safety of material stored, the quality of collections, and the availability and distribution of germplasm. Many of these issues, especially the free exchange of germplasm, emerged as a major theme in the debates during the FAO Conferences in 1979, 1981 and 1983. At the request of the member countries, in 1983 FAO passed two important resolutions:

- to formulate an International Undertaking on Plant Genetic Resources, an articulated and flexible legal framework which would ensure that plant genetic resources would be explored, preserved, evaluated and made available without restrictions for plant breeding and other scientific purposes;

- to set up a Commission on Plant Genetic Resources (CPGR), an intergovernmental body, which would provide a forum for donors and users of germplasm, funds and technology to discuss the conservation and use of germplasm and would monitor the implementation of the principles contained in the International Undertaking.

Through the Commission, which meets every two years, activities can be coordinated and responsibilities delegated among those concerned. Besides members, a number of other agencies, including development banks, international organizations and non-governmental organizations (NGOs) attend these meetings as observers.

To date, 91 member nations have joined the CPGR and 84 have adhered to the International Undertaking. More countries are expected to finalize their decision on the CPGR and International Undertaking by the time of the next meeting of the commission in April 1989.

### International Fund for Plant Genetic Resources

In order to implement the principles of the International Undertaking, FAO established an International Fund on Plant Genetic Resources (IFPGR) in 1987. This body provides the mechanism for the users of germplasm to compensate the donor nations of germplasm for their contribution to world agricultural development by conserving, improving and making available such resources over generations. In October 1987, the FAO Director General sent a Circulate State Letter to the member nations, intergovernmental organizations, NGOs, private foundations and enterprises requesting contributions.

Although response has been slow, we are pleased to note that already some private foundations, NGOs and member nations have expressed their interest in supporting IFPGR and have sent contributions. Some NGOs, especially the International Coalition for Development of Agriculture (ICDA), started a special campaign to mobilize funds for the FAO International Fund. Another NGO, the CS Fund of California made a small contribution, which is being used for the evaluation and utilization of teff germplasm in Ethiopia. The Government of Spain is providing approximately US$ 200,000 for three regional training courses in Latin America which will focus on the utilization of crops of agricultural interest in different regions.

## CURRENT FAO ACTIVITIES IN PLANT GENETIC RESOURCES

FAO's main objective is to help its member nations strengthen their capacity to conserve and use plant genetic resources through the development of infrastructures, facilities and human resources. FAO and other UN agencies provide technical support and assistance to countries in the form of projects, irrespective of political, ideological or market considerations. All the programs and projects are developed jointly with the respective governments, at their explicit request, in accordance with national priorities and within the context of overall development plans.

Through the Secretariat, the Commission continues to promote, stimulate and support activities relating to species and regions that are not adequately covered by other agencies working in the field of plant genetic resources, with emphasis on local species of social and economic importance. It is in contact with other organizations involved with plant genetic resources, especially IBPGR and other CGIAR organizations.

FAO activities aimed at the conservation and better utilization of plant genetic resources form part of a large number of field projects which involve many FAO divisions and services within the Departments of Agriculture and Forestry, as well as in the legislation branch. These projects are financed through the regular program, the technical cooperation program, trust funds, UNDP, UNEP, the Tropical Action Plan and, more recently, the IFPGR.

Although a large number of these projects can clearly be considered genetic resources projects, in many others the plant genetic resources component cannot be separated from more general agricultural research and development, as in the Plant Production and Protection and Research divisions of FAO, where germplasm acquisition (including plant introduction) and the evaluation and use of elite materials in breeding programs is relevant. In some cases, storage facilities (medium- to long-term seed stores) are also provided (for example, the national facility in Nigeria). In addition, most FAO projects contain an element of human resources development.

The Forest Resources division is supporting national programs in exploration, evaluation and conservation of genetic materials of socially and economically important species of interest to a range of countries. It is also involved in the establishment of *in situ* conservation and associated research. The Tropical Forestry Action Plans for individual countries are placing great emphasis on ecosystem and *in situ* conservation.

The Legal Council of FAO continues to assist in the development of necessary legal instruments for seed and genetic resources programs at national and international levels.

FAO also serves as a clearing house for information on major activities involving plant genetic resources. It prepares information bulletins and other materials to raise awareness at decision-making, technical and grass-roots levels, and disseminates information through publications and through arranging or co-sponsoring meetings, workshops and training courses.

The FAO seed laboratory facilities have made a substantial contribution to seed exchange and plant introduction. During 1987 alone, a total of 34,604 seed samples of various crops were despatched to 101 countries.

## FUTURE PERSPECTIVES

Over the next decade, FAO and its Commission will concentrate on establishing a long-term stable and equitable global system, through the implementation of the various articles of the International Undertaking. Article 8 (Financial Security) has been partially implemented with the establishment of the FAO International Fund. Article 9 (Monitoring of Activities and Related Action by FAO) will be covered by this program of work.

Some progress has also been achieved with respect to other articles. Article 7 deals with international cooperation. Accordingly, a Circular State Letter has been sent to member countries and organizations, enquiring about their readiness to participate in the network of base collections under FAO auspices or jurisdiction. The Circular State Letter provides several possible models which allow different degrees of commitment by genebanks and collections. A number of positive replies from countries and institutions have already been received. The establishment of a global information system on plant genetic resources, under the coordination of FAO in close cooperation with other organizations, particularly with IBPGR and the IARCs, is under discussion.

With reference to Article 10, on phytosanitary measures, and within the context of the International Plant Protection Convention (adopted in Rome in December 1951), FAO and IBPGR have agreed to initiate a program for the safe and expeditious exchange of germplasm through the publication of a series of crop-specific protocols to be used when germplasm is exchanged; these will describe indexing and other necessary procedures. FAO and IBPGR, in close cooperation with the specialized research institutions and IARCs involved, also plan to convene expert consultations on specific crops and their pathogens to develop appropriate protocols; these will be published jointly as a series of crop-specific guidelines.

Many other articles of the International Undertaking refer to national and regional activities for the conservation (exploration, collection, maintenance, evaluation and documentation) and utilization (plant breeding and seed production) of germplasm. Training in all aspects of the conservation and use of plant genetic resources is also provided for.

These articles aim to ensure that plant genetic resources are well documented and adequately conserved for the use of present and future generations, especially in developing

countries. A number of technical organizations, such as IBPGR, the IARCs and the International Union for the Conservation of Nature (IUCN), are already working in these fields, and FAO will attempt to avoid any duplication of activities. FAO will concentrate its technical work on developing national and regional strategies for the effective conservation and optimal use of plant genetic diversity, and the necessary policies and legislation to achieve this. It will pay special attention to local crosses which are not covered under the mandate of the IARCs and IBPGR, or which are given low priority by these organizations.

To implement Article 9, FAO, through the Commission, will prepare biannual reports in cooperation with the other institutions involved in the status of plant genetic resources worldwide. These reports will provide information on what is being done, and by whom, with the aim of identifying gaps and suggesting ways and means of filling such gaps. They will take into consideration the work of FAO and other organizations working in this field, as well as the national reports received from the countries in accordance with the Article 11 of the International Undertaking.

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# 1.6

# *Plant Germplasm Collecting Activities in Africa of the National Research Council (CNR) of Italy*

P. PERRINO

The Germplasm Institute of the National Research Council (CNR) of Italy was established in Bari in 1970 to collect, conserve, evaluate and distribute germplasm of crops which are important in the Mediterranean region. The CNR has conducted several missions in the Mediterranean, mainly in North Africa, for collecting crops believed to be threatened. It has also provided short courses for training undergraduate and graduate students in plant exploration and collection, as well as in multiplication and conservation activities.

Some of these activities are summarized here, with the aim of stimulating further collecting and preservation of plant genetic resources in Africa and promoting further studies in the light of information presented during this workshop.

## COLLECTING MISSIONS

Twenty of the CNR's 49 collecting missions between 1971 and 1988 were conducted in eight African countries/regions (*see* Table 1 *overleaf*). Collecting work was very active in Algeria (Porceddu and Olita, 1974) and Ethiopia (Porceddu and Perrino, 1973) between 1973 and 1978, with two missions to Tunisia and one to Egypt during this period period (Perrino et al., 1976a, 1976b). Between 1980 and 1985, missions were sent to Egypt, Libya and Morocco (Ali et al., 1982; Perrino et al., 1984; Hammer and Perrino, 1985; Perrino et al., 1986). After a break of three years, exploration resumed in 1988 when a mission was sent to southern Africa, mainly for wild cowpea (Padulosi et al., 1988b).

## COLLABORATION AND TRAINING

All the collecting missions conducted in Africa have been organized in collaboration with several international and national organizations. The list reported in Table 2 *(overleaf)*,

**TABLE 1** **Number of missions from 1971 to 1988**

| Country/years | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Greece | - | - | - | - | - | - | 1 | 1 | 1 | 1 | - | - | - | - | - | - | - | - | 4 |
| Italy | 1 | 1 | - | 1 | 1 | 1 | 1 | - | 1 | 3 | 1 | 1 | 2 | 1 | 2 | 2 | 2 | 1 | 22 |
| Spain | - | - | - | - | 1 | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | 3 |
| Subtotal | | | | | | | | | | | | | | | | | | | 29 |
| Algeria | - | - | 1 | - | 1 | 1 | 1 | 1 | - | - | - | - | - | - | - | - | - | - | 5 |
| Egypt | - | - | - | - | - | - | - | 1 | - | 1 | 1 | 1 | - | - | - | - | - | - | 4 |
| Ethiopia | - | - | 2 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 4 |
| Libya | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 | - | - | - | - | 2 |
| Morocco | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | - | 1 |
| Southern Africa | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 1 | 1 |
| Somalia | - | - | - | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | 1 |
| Tunisia | - | - | - | - | 1 | 1 | - | - | - | - | - | - | - | - | - | - | - | - | 2 |
| Subtotal | | | | | | | | | | | | | | | | | | | 20 |
| Total | | | | | | | | | | | | | | | | | | | 49 |

**TABLE 3** **Number of samples collected in African countries since 1972 by the Germplasm Institute, CNR, Bari, Italy**

| Country | Wheat | Barley | Maize | Faba bean | Pea | Chickpea | Lentil | Cowpea | Others | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Algeria | 371 | 110 | 21 | 27 | 21 | 9 | 1 | - | 220 | 780 |
| Egypt | 125 | 78 | 82 | 82 | 19 | 15 | 13 | 7 | 653 | 1,074 |
| Ethiopia | 349 | 143 | 11 | 16 | 95 | 25 | - | 2 | 189 | 830 |
| Libya | 94 | 80 | 11 | 7 | 6 | - | 3 | 1 | 202 | 404 |
| Morocco | 55 | 92 | 15 | 9 | 1 | - | 11 | 1 | 119 | 303 |
| Somalia | - | - | 28 | - | - | - | - | - | 1 | 29 |
| Tunisia | 117 | 52 | 5 | 36 | 17 | 14 | 10 | - | 120 | 371 |
| Total | 1,111 | 555 | 173 | 177 | 159 | 63 | 38 | 11 | 1,504 | 3,791 |

though far from complete, gives an idea of the efforts made by the organizers to make contacts with local and national organizations, break through bureaucratic obstacles and reach the goal of collecting germplasm threatened with extinction.

**TABLE 2 Collaboration in exploration, collection and training activities in Africa**

| Period | Country | Collaborating institutes |
|---|---|---|
| **Exploration, collection and training** | | |
| 1972-73 | Ethiopia | CNR (Italy), Faculty of Agriculture of Diredawa, Ethiopia |
| 1973-76 | Algeria | FAO, IBPGR, ARS (Tunisia) |
| 1975-76 | Tunisia | FAO, IBPGR, ARS (Tunisia) |
| 1977-79 | Egypt | FAO, IBPGR, ICARDA, Faculty of Agriculture, (Egypt) |
| 1981-83 | Libya | FAO, IBPGR, ZIGUK (GDR), ARS (Libya) |
| 1984 | Morocco | FAO, IBPGR, ICARDA, INRAT, Rabat (Morocco) |
| 1985-88 | Southern Africa | IITA, Ministry of Foreign Affairs |
| **Training courses** | | |
| 1971-88 | UK, Italy | IBPGR, FAO, University of Birmingham, UK (including PhD and MSc), Turkey, IITA (Nigeria) |
| **Evaluation and research studies** | | |
| 1984-88 | Syria, Tunisia, Egypt, Ethiopia, Kenya, Italy, etc. | ICARDA (Aleppo, Syria), IITA (Nigeria), University of Viterbo (Italy), University of Naples (Italy), Istituto della Nutrizione (Rome), etc. |

During collecting missions, undergraduate and graduate students from the University of Birmingham, UK, joined the teams to learn collecting strategies. Two of the students received a PhD in plant genetic resources and are now working with ICARDA and IBPGR in the Middle East and African countries. Other students trained in Bari for shorter periods are working in Africa with other international organizations, including ILCA, or with national institutes, such as the PGRC/E in Addis Ababa.

## REGIONS VISITED AND PLANT GERMPLASM COLLECTED

The regions and areas covered during 20 missions between 1971 and 1988 are shown in Figures 1 and 2 (*overleaf*). They include Ethiopia, mountainous zones and oases of North Africa and several countries of southern Africa.

In Ethiopia, Somalia and North Africa, the main emphasis was on collecting landraces of wheat, barely, oats, other Gramineae and grain legumes. In all, 3,791 samples were collected (*see* Table 3). Most were collected in Egypt, Ethiopia and Algeria (that is, in countries where more vegetables and cereals are cultivated). High genetic erosion was found in all regions, but this was less evident in Ethiopia (Porceddu and Perrino, 1973; Porceddu et al., 1973), Hoggar and Tassili (Polignano, 1978), and in remote areas of the Atlas Mountains (Porceddu and Olita, 1974; Perrino et al., 1976a, 1976b), where wheat and barley fields with an appreciable variability were found and sampled (*see* Figures 3, 4, 5 and 6). The reason for this loss of genetic diversity was the introduction of a few new high-yielding varieties from the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT). An interesting approach to this aspect of genetic erosion of wheat in North Africa may be found in Porceddu (1979).

**FIGURE 1** **Areas visited during collecting missions in North Africa**

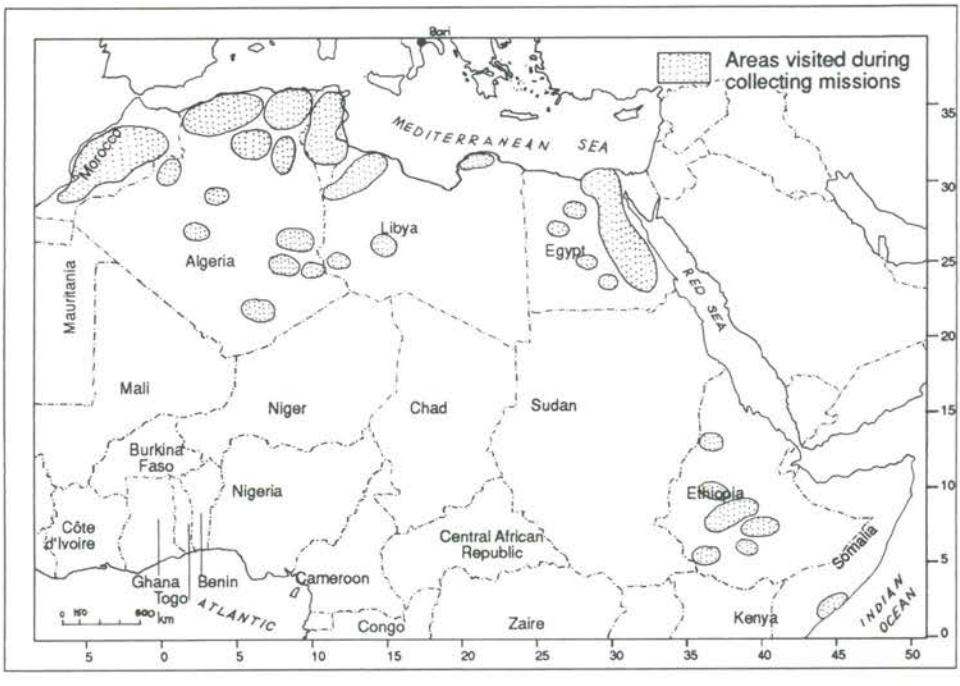


**FIGURE 2** **Areas visited during collecting missions in southern Africa**

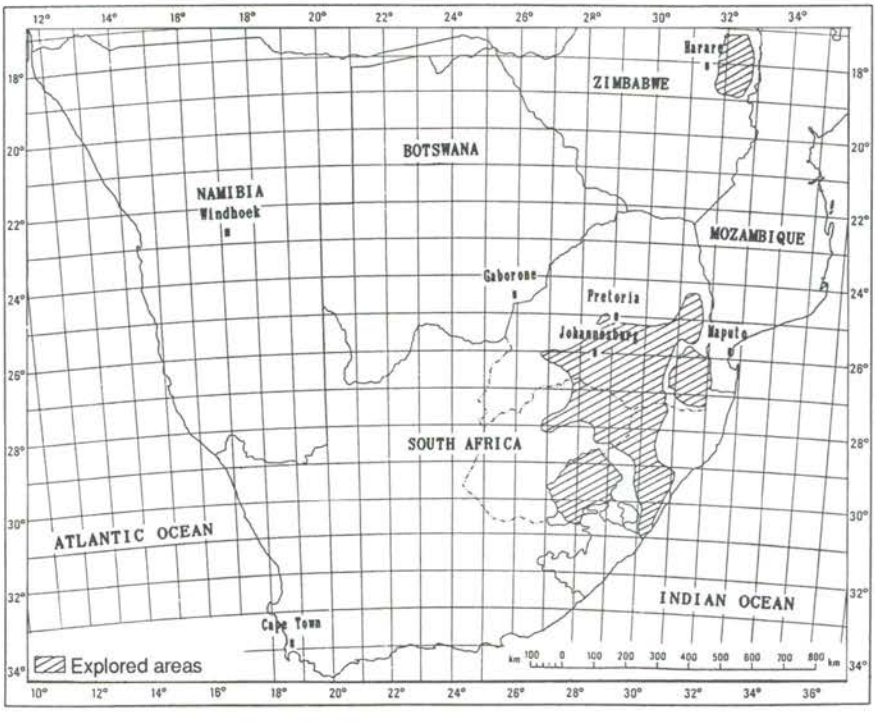

**FIGURE 3**
**Variability in a wheat field in Ethiopia**

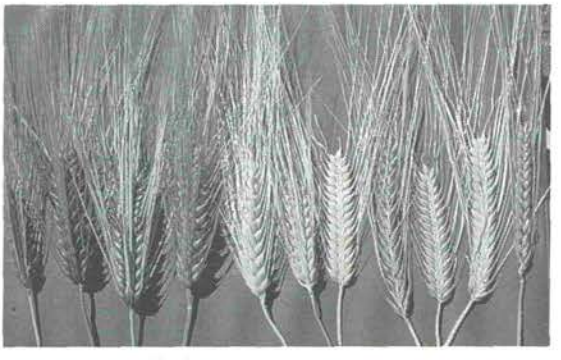

**FIGURE 4**
**Variability in a barley field in Ethiopia**

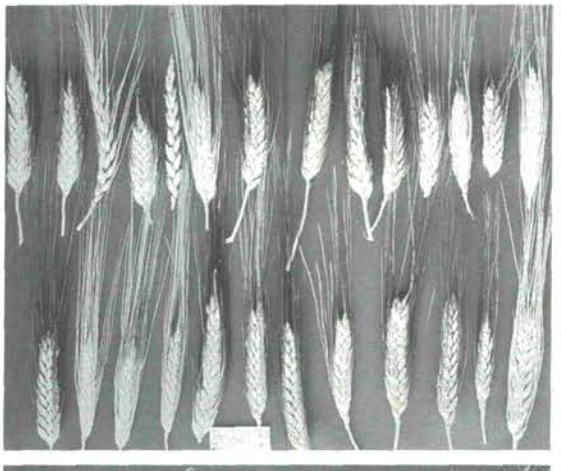

**FIGURE 5**
**Variability in a wheat field in Algeria**

**FIGURE 6**
**Variability in a wheat field in the Hoggar, Algeria**

The objective of the missions to Southern Africa was to survey and collect wild species of *Vigna*. The results of the first two missions have confirmed that Southern Africa is the most probable center of origin of *Vigna unguiculata* (L.) Walp (*see* Table 4).

**TABLE 4 Number of samples of different species of *Vigna* collected in southern Africa**

| | |
|---|---|
| *Vigna unguiculata* subsp. *unguiculata* cv-gr *unguiculata* (c)[1] | 153 |
| *V. unguiculata* subsp. *dekindtiana* (w)[1] | 11 |
| *V. unguiculata* subsp. *dekindtiana* var *protracta* (w) | 17 |
| *V. unguiculata* subsp. *dekindtiana* var. *pubescens* (w) | 3 |
| *V. unguiculata* subsp. *dekindtiana* var. *mensensis* (w) | 22 |
| *V. unguiculata* subsp. *tenuis* (w) | 5 |
| *V. unguiculata* subsp. *stenophylla* | 1 |
| Intermediate type between subsp. *stenophylla* and subsp. *dekindtiana* (w) | 20 |
| Probable new taxon (w) | 30 |
| *V. davyi* (w) | 2 |
| *V. decipiens* (w) | 2 |
| *V. longiloba* (w) | 1 |
| *V. luteola* (w) | 1 |
| *V. nervosa* (w) | 23 |
| *V. oblongifolia* var. *parviflora* (w) | 1 |
| *V. oblongifolia* var. *oblongifolia* (w) | 4 |
| *V. subterranea* (c) | 11 |
| *V. vexillata* var. *angustifolia* (w) | 10 |
| *V. vexillata* var. *vexillata* (w) | 13 |
| *V.* spp. (w) | 1 |
| Total | 331 |

1 (c) cultivated species; (w) wild species

As a general conclusion to the surveys, exploration and collection work carried out in Ethiopia and North Africa by the Germplasm Institute, we can say that the situation with regard to the genetic resources of several crops varies considerably from one country to another and is closely related to the migrations of peoples and to the development of agricultural research in general and of breeding work in particular. It goes without saying, however, that the overall situation is extremely serious, that genetic erosion is by now very advanced and that there is very little time left to collect these precious resources which nature and earlier civilizations have bestowed upon us and which it is our duty to save and hand down to future generations.

## DISTRIBUTION OF PLANT GERMPLASM TO AFRICAN COUNTRIES

In all, 5,374 accessions have been distributed by the Germplasm Institute (*see* Table 5). Most of them were requested by North African countries and Ethiopia, the same countries from which they were collected. Most of the accessions distributed were wheat, barley, faba bean and pea. The 2,376 other accessions were Gramineae, such as oats or *Aegilops*, and small

grain legumes, both cultivated species (for example, cowpea and lentil) and wild species (for example, vetches and *Medicago* ). Algeria and Egypt are among those countries that seem to have made greatest use of their own germplasm. Libya was especially interested in wild *Medicago* collected by Libyan collectors and by experts from FAO. In this particular case, Bari was involved only in providing storage facilities and some multiplication.

**TABLE 5 Number of accessions distributed to African countries from 1972 to 1988**

| Crop | Algeria | Egypt | Libya | Ethiopia | Morocco | Nigeria | Somalia | Tunisia | Total |
|---|---|---|---|---|---|---|---|---|---|
| Wheat | 891 | 205 | 94 | 349 | 55 | 50 | - | 117 | 1,761 |
| Barley | 110 | 78 | 80 | 143 | 92 | - | - | 52 | 555 |
| Maize | 21 | 82 | 11 | 11 | 15 | - | 28 | 5 | 173 |
| Faba bean | 77 | 92 | 7 | 16 | 9 | - | - | 36 | 237 |
| Pea | 71 | 19 | 6 | 95 | 1 | - | - | 17 | 209 |
| Chickpea | 9 | 15 | - | 25 | - | - | - | 14 | 63 |
| Others | 290 | 666 | 786 | 189 | 235 | 80 | - | 130 | 2,376 |
| Total | 1,469 | 1,157 | 984 | 828 | 407 | 130 | 28 | 371 | 5,374 |

## EVALUATION AND UTILIZATION

Most of the material collected in Africa has been multiplied and distributed both to African countries and elsewhere. Unfortunately, the Institute is not always able to provide much information on the value of the collections because of lack of feedback from the users. Only recently, as a result of greater collaboration and education, has feedback improved, and pathologists, geneticists and other specialists as well as breeders are requesting germplasm with the aim of finding new sources of variability for several useful traits.

The results of evaluation work carried out by the Institute and its collaboration with other institutions are reported in Chapter 2.1.

## CONCLUSION

More collaboration with other genebanks and international organizations may give plant breeders the chance to make greater use of germplasm collected in Africa and in other countries, but breeders must explain the purpose for which they need germplasm samples and be aware that only small samples may be available. Multiplication and regeneration of collections are far more expensive than collecting.

New genebanks based in Africa have begun to take care of plant genetic resources. We have two suggestions for them which would enhance their effectiveness. The first is that they should continue to collect, especially in remote areas where, because of host-parasite co-evolution, genetic material carrying new sources of resistance may be collected and used in breeding programs. The second is that they concentrate on wild species related to crops and, at least initially, on pre-breeding activities in collaboration with specialists. In both cases, collaboration within and among countries is essential if the goal is to preserve and utilize plant genetic resources.

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# PART 2

## Characterization, Evaluation and Conservation of Germplasm

# 2.1

# *Characterization and Evaluation of Plant Germplasm: A Problem of Organization and Collaboration*

P. PERRINO and L. M. MONTI

Large collections of various crop species already exist in several genebanks throughout the world. However, many of them have yet to be classified, characterized and evaluated, and thus are not being widely used by breeders.

## CONSTRAINTS TO CHARACTERIZATION AND EVALUATION

When new genebanks are established, they are likely, during the first stage, to concentrate on exploration and collection, which can last several years. In the second stage they may pay more attention to improving multiplication techniques and storage capacities; this, too, can last several years. During the third stage, emphasis may be placed on characterization, evaluation and documentation because distribution and utilization activities, which constitute the fourth stage, depend on a thorough knowledge of the value of the material in the collections.

Much time has been devoted to the question of how to measure the value of plant germplasm collections. To help genebanks and improve their effectiveness, international meetings have been organized, with the participation of well-known breeders and experts, to decide on the most relevant descriptor or descriptor states. The International Board for Plant Genetic Resources (IBPGR) has published more than 30 descriptor booklets and suggested three main categories of evaluation data (Erskine and Williams, 1988; Hawkes 1985): characterization of morphobotanical and agronomic characters of high hereditability; preliminary evaluation (special agronomic characters); and full or secondary evaluation (several useful characters).

Most of the genebanks have used such descriptors but mainly for morphological characters, and accordingly computer lists, catalogues, seminum indexes and so on have been

printed and circulated. Even so, it was felt that on a global scale breeders and more general users were not making sufficient use of the plant genetic resources maintained in genebanks.

The results of a recent survey (Williams, 1985) showed that the reason why breeders were not making more use of the data and materials from genebanks was that the important information required was not always available. According to Hawkes (1985), the information they sought was, in order of priority:

- pest and disease resistance data;
- stress tolerance and adaptation information;
- information on maturity;
- information on yield potential;
- plant height measurements;
- any rare or interesting data;
- some morphological data.

Although crop breeding has different objectives according to the species and to the knowledge of its genetic system (the higher the genetic knowledge, the more sophisticated the breeding approach), the priorities listed above indicate clearly the importance of information on biotic and abiotic stress resistance and on qualitative traits.

This sequence therefore represents an almost complete reversal of the stages of work currently followed by most genebanks. It seems, then, that the limited use of genetic resources from genebanks stems from lack of information. But what explanation can be given for the even more limited use of wild species? In a workshop on pre-breeding in relation to genebanks, which was organized by Eucarpia (1983) and held at Beograd-Zemun, this problem was discussed and it became clear that breeders are reluctant to extend the length of their programs by having resort to wild species and primitive forms as initial breeding material. In this case, characterization and evaluation would also include the transfer of useful genes of chromosomes or genomes or even cytoplasmic organelles from wild species into breeding lines (Blanco and Porceddu, 1983; Feldman, 1983).

The type of studies needed for extending evaluation to the exploitation of wild species can be carried out in centers where there are specialists; they do not have to be undertaken in genebanks, but should at least be conducted in close cooperation with them. In other words, characterization and evaluation cannot be the responsibility of genebanks alone.

## PURPOSES OF CHARACTERIZATION AND EVALUATION

Characterization and evaluation work in genebanks may have four main objectives:

- to study genetic variability of certain characters in relation to their geographical distribution in order to develop new and more adequate collecting strategies for further collection of useful germplasm in the same or similar areas (Bogyo et al., 1980);

- to study the genetic variability present in the collections, especially within samples, and develop the most appropriate techniques and strategies for maintaining the genetic integrity of such diversity (Scarascia Mugnozza and Porceddu, 1978); studies on genetic drift (Sergio et al., 1988), seedborne diseases (Gambogi et al., 1987) and the physiology of seed aging (Dell'Aquila et al., 1984; Dell'Aquila and Margiotta, 1986; Petruzzelli and Taranto, 1985) are the most appropriate;

- to widen genetic diversity of crops through intraspecific, interspecific and intergeneric hybridization and mutation (Pignone and Attolico, 1986); other genetic engineering techniques may also be of interest;

- to screen the collections for traits which, from time to time, are considered important for breeding programs aiming to improve agriculture in a given country, region or geographical area; this includes studies on seed quality, resistance to diseases and pests and adaptation to adverse soil conditions (some of these aspects are discussed below).

## THEORETICAL APPROACHES TO CHARACTERIZATION AND EVALUATION

Information data can be divided, for the sake of convenience, into two main categories, characterization and evaluation data; these, in turn, can be divided into subcategories according to the nature of characters and plants.

### Characterization

Characterization may be defined as the scoring of characters that can be easily detected and that have high hereditability. There are four main subcategories of characters: morphological, botanical, agronomic and chemical characters (Simpson and Withers, 1986). They can be recorded on plants or their products (for example, seeds) grown only in one environment. For this reason, characterization may begin during exploration and collection and continue in the laboratory before or after multiplication.

### Evaluation

Evaluation may be defined as the scoring of traits not easily detected, controlled by one or more genes and estimated to be important for breeding programs or for direct use, but usually having a strong genotypic environmental interaction. For this reason, a complete evaluation of the collection cannot take place in one environment, as use of the results of the evaluation would be limited to that environment. However, even in one environment, evaluation should be carried on at least for two or more years. Alternatively, in the case of evaluation for disease resistance, artificial inoculations with special isolates in glasshouses or growth chambers can justify the use of one environment and one life cycle of the plant.

During evaluation, the identification of useful traits should be as broad as possible because of the pleiotropic effects of a gene on other traits. A gene can interact differently with different genetic backgrounds and with different environments. In peas, for example, an investigation of some genes showed their wide range of pleiotropic effects in 14 morphophysiological

traits; interactions between alleles at one locus with the allelic state of the other two loci and with the environment were also found (Cardi et al., 1988).

The factors involved in the evaluation process may concern environmental stresses and seed quality. Three main subcategories can then be distinguished: biotic stresses; abiotic stresses; and seed and grain quality.

Biotic stresses include: tolerance, resistance and immunity to pests and diseases; grazing; intra- and interspecific competition; and intra- and interplant competition.

Abiotic stresses include: environmental adaptation; tolerance or resistance to frost, cold, heat and drought; and adverse soil conditions, such as high or low aluminum, sulfate, acidity and salinity.

At this juncture it necessary to make a general point regarding biotic and abiotic stresses. The most important trait of a crop plant is yield, but yield is a complex trait which results from several biochemical and metabolic processes, each of which is under genetic control. The use of yield as an evaluation index of germplasm of different plant species is therefore inefficient. Breeders' work is now based mainly on traits that in some way are related to yield itself and, as a result, germplasm screening should be carried out to find genetic variability for these traits (Monti, 1987). This is particularly true when abiotic stresses are considered, because the heritability of yield in stressed environments is very low (Johnson and Frey, 1967).

The seed and grain quality subcategory includes several characters which may also be used during characterization. Among these characters are protein content and quality; amino-acid composition; oil content and quality; acid composition; lysine content; antiphysiological substances (trypsin inhibitor activity); polyphenols (sulfur-amino acid deficiency); and protein and carbohydrate digestibility. This list is far from complete; it is given only as a general guideline, but what it does show is that in order to effectively evaluate germplasm collections for so many characters, genebanks need the assistance of specialists. Moreover, it is almost impossible to draw a sharp distinction between characterization and evaluation work and data. As most of the data which are relevant mainly to characterization work have already been published elsewhere, this paper will deal with some examples relevant mainly to evaluation work carried out by the Germplasm Institute, usually in collaboration with other centers.

## BIOTIC STRESSES

### Wheat

*Resistance to leaf and stem rusts in a wheat germplasm collection*

This study was conducted in collaboration with the Agriculture Canada Research Station, Winnipeg, Manitoba (Jedel et al., 1988). In 1985, 494 accessions of tetraploid and hexaploid wheats from Bari were planted in the rust nursery near Winnipeg. Spreader rows were inoculated with a composite of leaf rust races, *Puccinia recondita* Rob. ex Desm., prevalent in western Canada, and a mixture of 10 stem rust races, *P. graminis* Pers.

Most of the tetraploid accessions from all countries were highly resistant to leaf rust, while most of the hexaploid accessions from Algeria and Egypt were susceptible to or segregated

**TABLE 1** **Adult plant resistance in a field nursery to leaf rust and stem rust in *Triticum* accessions from the Germplasm Institute, Bari, Italy**

| Country of origin | Ploidy level [1] | Leaf rust No. accessions | | | Stem rust No. accessions | | |
|---|---|---|---|---|---|---|---|
| | | R [2] | M | S | R | M | S |
| Algeria | H | 3 | 2 | 39 | 1 | 1 | 41 |
| | C | 1 | 0 | 2 | 2 | 0 | 3 |
| | T | 20 | 0 | 2 | 11 | 1 | 8 |
| Egypt | H | 2 | 5 | 5 | 10 | 0 | 0 |
| | C | 2 | 0 | 0 | 4 | 0 | 0 |
| | T | 12 | 0 | 0 | 5 | 1 | 4 |
| Ethiopia | H | 12 | 2 | 14 | 9 | 4 | 12 |
| | C | 3 | 0 | 2 | 2 | 1 | 5 |
| | T | 24 | 1 | 1 | 13 | 3 | 8 |
| Greece | H | 13 | 9 | 17 | 16 | 2 | 15 |
| | C | 1 | 2 | 1 | 7 | 0 | 4 |
| | T | 43 | 3 | 1 | 29 | 1 | 8 |
| Italy | H | 9 | 2 | 16 | 5 | 12 | 5 |
| | C | 3 | 0 | 1 | 1 | 7 | 1 |
| | T | 57 | 1 | 0 | 26 | 18 | 10 |
| Spain | H | 64 | 26 | 96 | 44 | 2 | 137 |
| | C | 12 | 1 | 3 | 6 | 0 | 11 |
| | T | 31 | 1 | 0 | 25 | 1 | 5 |
| Tunisia | H | 1 | 0 | 0 | 1 | 0 | 0 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 |
| | T | 1 | 0 | 0 | 0 | 0 | 1 |

1 Ploidy levels: H, hexaploid; C, composite of hexaploids and tetraploids; T, tetraploid.
2 Reactions: R: resistant; M: mixture of susceptible and resistant plants; S: susceptible.

Source: Based on Jedel et al., 1988.

for leaf rust reaction (*see* Table 1). Sources of resistance to leaf rust were found in the accessions from Ethiopia, Greece, Italy and Spain. Resistance to stem rust was generally greater in the tetraploid than in the hexaploid accessions. The hexaploid accessions from Egypt all showed resistance to stem rust, while those from Algeria showed very little resistance. Sources of resistance to stem rust were also found in accessions from Ethiopia, Greece, Italy and Spain.

This study showed that extensive germplasm collections are required in order to ensure the presence of useful genetic resistance to leaf rust, and only 10 accessions were found to be resistant to all the races of stem rust used in this study (*see* Table 2 *overleaf*).

**TABLE 2 Number of accessions, heterozygous and homozygous, resistant to leaf and stem rust**

| | | Leaf rust (*P. recondita*) | | | Stem rust (*P. graminis*) | | |
|---|---|---|---|---|---|---|---|
| | | R[1] | M | S | R | M | S |
| Hexaploids | 337-317 | 104 | 46 | 187 | 86 | 21 | 210 |
| Composites | 34-54 | 22 | 3 | 9 | 22 | 8 | 24 |
| Tetraploids | 198-168 | 188 | 6 | 4 | 99 | 25 | 44 |
| Total | 494 | 314 | 55 | 200 | 207 | 54 | 278 |
| Homozygous for resistance to all tested races | | 15 | | | 10 | | |

1 R: resistant; M: mixture of R and S; S: susceptible.

Source: Based on Jedel et al., 1988.

*Resistance to rusts and powdery mildew in obsolete wheats*

In the past, cultivation of 'small farro', *Triticum monococcum* L., and 'farro', *T. dicoccum* Schübler (Perrino, 1982), was widely distributed in the Middle East. However, it is now difficult to determine the extent of the cultivation. While these species seemed to have disappeared in Italy, some cultivated fields were found in southern Italy (Perrino and Hammer, 1982, 1984), Yugoslavia, Armenia and India (Borojevic, 1956; Rao, 1963). There is substantial information on the genetic variability present in *T. monococcum* and *T. dicoccum* for resistance to diseases (Tomerlin et al., 1984; Vallega,1977).

The aim of the research, conducted in collaboration with the Cereal and Plant Pathology Institutes in Rome (Pasquini et al., 1988) was to identify suitable genotypes carrying resistance to several Italian isolates of leaf rust, stem rust and powdery mildew. Almost all the accessions tested were resistant to biotypes of *Puccinia recondita* and several accessions produced low infection types when inoculated with the isolates of powdery mildew and stem rust.

A lower level of resistance to rust and powdery mildew was observed in *T. dicoccum* than in *T. monococcum*. Nevertheless, many of the *T. dicoccum* accessions tested proved to be resistant to one or more biotypes of *P. graminis* (*see* Table 3).

**TABLE 3 Number of accessions of wheat resistant to mildew and rust**

| | | Mildew (*E. graminis*) | | | Leaf rust (*P. recondita*) | | | Stem rust (*P. graminis*) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | N[1] | 1B | 2B | 3B | 1B | 2B | 3B | 1B | 2B | 3B |
| *Triticum monococcum* | 12 | 2 | 2 | 2 | 1 | 2 | 9 | 0 | 1 | 4 |
| *Triticum dicoccum* | 33 | 10 | 4 | 0 | 8 | 3 | 1 | 4 | 1 | 1 |

1 N: number of accessions; B: one biotype; 2B: two biotypes; 3B: three biotypes.

Source: Based on Pasquini et al., 1988.

Results on the reaction to rusts indicated that a high number of *T. dicoccum* accessions showed moderate susceptibility to leaf rust. Nevertheless, the probable presence of major genes for resistance to different biotypes of *P. recondita* and *P. graminis* was observed in several strains.

## Faba bean

### *Broomrape tolerance*

Broomrape, *Orobanche crenata* Forsk., is one of the main problems in the cultivation of faba bean, *Vicia faba* L., in the Mediterranean region. Yield losses resulting from broomrape are well known in the semi-arid and dry-farming areas.

Populations of faba bean of different origins were tested for broomrape tolerance. Evaluation was carried out at Valenzano, Bari, in field plots which were naturally infested. During growth, eight plant host traits and three of the parasitic weed were recorded. It seems that the taller the host plants at flowering time, the higher the tolerance. The positive correlation between seed yield and number of spikes of broomrape suggested tolerance rather than resistance.

Among the 42 accessions analyzed, none was found to be as good as the cultivar 'locale di Castellana'. On the other hand, some variability was observed within accessions. Single tolerant plants within accessions were identified and will be used for further tests.

Climatic conditions may improve or reduce tolerance to broomrape by reducing or prolonging the life cycle of the crop. A simple delay in sowing time may improve or reduce tolerance. Despite this limitation, screening of germplasm for tolerance to broomrape in naturally infested fields can be a non-destructive and relatively inexpensive technique.

### *Resistance to rust*

The rust caused by *Uromyces viciae (fabae) fabae* occurs in most areas where faba bean is grown. It is common throughout the Mediterranean region and is considered one of the most serious diseases of faba bean in Egypt. Crop losses of up to 50% have been reported (Bernier and Conner, 1982). In collaboration with pathologists and the Italian Agency for Nuclear and Alternative Energy in Agriculture, a program for the evaluation of rust resistance in the collection of faba bean was started in 1987. Of the 123 accessions analyzed, 14 were found to be resistant, 46 medium-resistant and 63 susceptible (Polignano et al., 1988). Further studies will assess the kind of resistance.

## Chickpea

### *Resistance to Ascochyta blight*

Ascochyta blight, *Ascochyta rabiei*, can devastate crops throughout West Asia, North Africa, southern Europe, Pakistan and north-west India. Several resistant sources have been identified and some resistant cultivars have been released. Studies for identifying new

sources of resistance have been intensified as a result of the availability of germplasm and appropriate screening methods. Collaborative evaluation in several environments is essential to finding new variability, because different pathogenic agents are peculiar to each environment. This principle is particularly important in the study of the behavior of the germplasm collection in response to pests and diseases, since it allows the discovery of the genetic variability existing in the pathogenic agents. Great variability in *Ascochyta rabiei* that had not appeared previously (Porta Puglia et al., 1987) was found as a result of an analysis of a sample of chickpea germplasm in Italy (*see* Table 4).

**TABLE 4 Reaction of chickpea accessions to 19 Italian isolates of *Ascochyta rabiei***

| Material | Origin | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ILC 194 | USSR | I[1] | I | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| ILC 249 | India | R | I | S | S | S | S | - | - | - | - | - | - | S | S | S | S | - | - | - |
| ILC 484 | Turkey | R | R | S | S | I | I | S | S | S | S | S | S | S | S | S | S | S | S | S |
| NEC 138-2 | USSR | R | R | S | S | I | I | S | S | S | S | S | S | S | S | S | S | S | S | S |
| ICC 5127 | India | R | I | R | S | S | S | - | - | - | - | - | - | S | S | S | S | - | - | - |
| ILC 3279 | USSR | I | I | I | R | R | I | - | S | S | S | S | S | S | S | S | S | S | S | S |
| ILC 72 | Spain | R | R | R | R | R | R | I | S | S | S | S | S | S | S | S | S | S | S | S |
| ICC 3996 | Iran | R | R | R | R | R | I | - | - | - | - | - | - | S | S | S | S | - | - | - |
| ILC 482 | Turkey | R | R | R | R | I | R | S | - | - | - | S | I | S | S | S | S | S | S | S |
| ILC 202 | USSR | R | R | R | R | R | R | S | - | S | S | S | S | S | S | S | S | S | S | S |
| ILC 191 | USSR | R | R | R | R | R | R | R | R | R | R | I | I | S | S | S | S | I | S | S |
| ILC 200 | USSR | S | I | S | S | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S |
| ILC 1929 | Syria | I | I | S | S | S | S | - | - | - | - | - | - | S | S | S | S | - | - | - |
| ILC 182 | USSR | S | S | S | S | S | S | I | S | S | S | S | S | S | S | S | S | S | S | S |
| Principe | Italy | S | R | S | S | R | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| Calia | Italy | S | I | S | S | I | S | S | S | S | S | S | S | S | S | S | S | S | S | S |

1 R: resistant; I: intermedium; S: susceptible; - : not tested.

Source: Porta Puglia et al., 1987.

## Pea

### *Resistance to cyst nematode*

Infestations of pea cyst nematode, *Heterodera goettingiana*, occur worldwide and cause considerable yield losses. Growing resistant cultivars is the simplest, and probably the cheapest, way to prevent nematode damage and to avoid air and soil pollution. Unfortunately, no resistant cultivars are yet available. Investigations by Di Vito and Perrino (1978) have shown that some accessions of *Pisum abyssinicum* Brown, *P. arvense* L. and *P. elatius* Ster were moderately resistant (*see* Table 5). $F_2$ segregation data showed that the resistance results from recessive genes and some moderately resistant plants have been selected. An increase in ascorbic free radical reductase (AFR) and ascorbic acid (AA) has been observed in resistant lines of *P. abyssinicum* (Zacheo et al., 1981). Changes in peroxidase and superoxide disumtase (SOD) were also reported (Zacheo et al., 1986).

**TABLE 5 Reaction of *Pisum* species to infestations of *Heterodera goettingiana***

| Host | Number of lines tested | Range of infestation (% to control) | Resistant | Susceptible |
|---|---|---|---|---|
| *Pisum abyssinicum* | 14[1] | 6-66 | 5 | 9 |
| *P. arvense* | 91 | 15-194 | 0 | 91 |
| *P. elatius* | 2 | 12-46 | 1 | 1 |
| *P. sativum* | 5 | 28-91 | 0 | 5 |
| *P. sativum* (control) | 1 | 100[2] | 0 | 1 |

1 Inoculum level 6,000 egg and juveniles/plant.
2 100 = 810 females and cysts/plant.

Source: Di Vitto and Perrino, 1978.

## ABIOTIC STRESSES

### Wheat

Durum wheat is one of the most important staple food crops in West Asia and North Africa, the area covered by the mandate of the International Center for Agricultural Research in the Dry Areas (ICARDA). About 80% of durum wheat in developing countries is grown in this region. Despite its importance, progress in increasing its potential yield and nutritional qualities has been slow. There is a need for greater effort in the genetic improvement of this crop and in widening its adaptation to the diverse agroclimatic environments that prevail in the region (Srivastava et al., 1988).

West Asia, which is the center of diversity for durum wheat and cultivated landraces, together with natural populations of its ancestors, is a region where this extremely valuable genetic resource can still be found. A systematic evaluation of durum wheat germplasm for adaptation to adverse conditions such as drought, temperature extremes and soil salinity, as well as resistance to disease and pests, is the objective of a special project started at ICARDA in 1985 in collaboration with the Germplasm Institute and the University of Tuscia in Viterbo, Italy.

So far, approximately 10,000 accessions have been screened for 28 agromorphological traits at three locations in Syria and one in Tunisia. In Syria, most of the evaluation was done at Tel Hadya, ICARDA's main experimental station, which has relatively moderate rainfall with favorable soil characteristics. The second site in Syria was Breda, which has low rainfall and moderately fertile soils, and the third was Hegla, which is a saline and drought-affected site.

The evaluation process is in its third year and has reached a stage at which a number of lines have been identified as possessing positive attributes for various economically important characteristics, including good food-processing qualities, tolerance to low rainfall conditions and resistance to some diseases, and these lines are now available to breeders for further tests. Some 200 lines out of 10,000 were selected for continued evaluation in Ethiopia, Kenya, India, Pakistan, Turkey, Tunisia and Italy. These countries will meet again later this year to review the work carried out by all concerned and make plans for the 1988-89 season.

## Pea

### *Drought tolerance*

Twenty *Pisum* germplasm lines belonging to different species were analyzed in water-stressed as well as well-watered conditions; the lines were chosen because of a drastic modification in their canopy structure or because of their varying geographical origins. A high genotypic variation was found and four genotypes of *P. sativum* were identified which showed small differences between stressed and non-stressed conditions; these four genotypes and one control were analyzed for several parameters under stressed and non-stressed conditions; the genotypes showed little variation for stomatal frequency and size, while differences were found for percentage of senescent leaves, dry-matter accumulation and $CO_2$ Exchange Rate (CER) values. As only CER values showed a close correlation with seed yield per plant under water stress, CER seems a trait worth analyzing in germplasm collections for the identification of drought-resistant types (Leone et al., 1987).

### *Cold tolerance*

Studies have been carried out on pea germplasm to find variability in reaction to cold stress. A protocol to screen genotypes in a growth chamber at -6°C at seedling stage was followed, and some genotypes were identified and used in a cross-breeding program. In 1987 an unusual cold snap (-6°C) permitted the screening of the breeding material in the field. A scale from 1 to 5 was used, 5 being the most tolerant of cold: of the 874 $F_3$-$F_6$ progenies from crosses, 43 showed a score of 5, many of them deriving from genotypes selected for cold tolerance at the seedling stage in artificial conditions (*see* Table 6).

**TABLE 6** **Number of pea families showing differences in cold tolerance in the field, where 1 = least tolerant and 5 = most tolerant**

| Families | Total No. | Cold tolerance score | | | | |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 |
| $F_5$-$F_6$ | 376 | 142 | 118 | 76 | 29 | 11 |
| $F_3$ | 498 | 73 | 163 | 140 | 90 | 32 |

Source: Torre Lama, 1987.

## SEED AND GRAIN QUALITY

Seed storage proteins of cereals and legumes have recently been the object of intensive biochemical, genetic and molecular studies, not only because of their impact on quality but also because of the possibility of using them as a tool to trace evolutionary pathways. As for many other traits, useful variation for seed storage proteins can be observed in genebank collections

of wild relatives and landraces. With this objective in mind, a few years ago studies on the variation of storage protein components in *Triticum*, *Phaseolus*, *Vicia* and *Vigna* collections at the Germplasm Institute were started, in collaboration with the University of Tuscia.

The results of this work show that there is high genetic variability in *Triticum* and *Vicia* and lower variability in *Phaseolus* and *Vigna*, but that in all of them there are interesting geographical distributions and surprising differences (Lafiandra et al., 1988).

## CONCLUSION

Because genebanks have, for the most part, been established to preserve plant genetic resources, their major efforts have been geared toward maintaining the viability of genetic stocks collected or obtained through exchange activity. Characterization and evaluation are very important activities, especially if the objective is to study new strategies for collecting genetic variability and for avoiding loss of genetic diversity during regeneration and conservation. When the objective of evaluation is pre-breeding or germplasm enhancement, the collaboration of breeders, geneticists, agronomists, pathologists, specialized institutions and international organizations is essential.

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# 2.2

# *Evaluation of Cowpea Germplasm for Insect Pest Resistance*

L. E. N. JACKAI and S. R. SINGH

The success of any host plant resistance program is a direct function of the diversity of germplasm available for evaluation and the chances of the occurrence of resistance in these genotypes (Ortman and Peters, 1980). The range of local genotypes available for evaluation is expected to be greatest in the crop's center of origin or domestication, and this range usually provides the best opportunity for identifying resistance to the pests and diseases known to attack/infest the crop in that particular region. When it does not, large amounts of germplasm can be introduced from elsewhere to widen the genetic base. In either case, local germplasm should not be overlooked since it is most likely to provide the most adaptable genotypes (Buddenhagen and de Ponti, 1982). The identification of useful levels of pest and disease resistance precedes hybridization, selection and testing; it is the basic challenge and most important hurdle to overcome in the development of pest-resistant varieties.

If, as is commonly said, the quality of (the identified) resistance is only as good as the method used for its identification, then, obviously, enough time and effort must be put into developing an appropriate and reliable methodology for use in the resistance identification and evaluation process of germplasm and, subsequently, in segregating breeding lines.

## CONCEPTS UNDERLYING GERMPLASM EVALUATION

### Target pests

It is necessary first to determine the key pest problems and their biology and to understand adequately their behavior. In the case of cowpea, *Vigna* species, there is a complex of key insect pests. The most important are: the cowpea aphid, *Aphis craccivora* Koch; flower bud thrips (FTh), *Megalurothrips sjostedti* Tryb.; the legume pod borer (MPB), *Maruca testulalis*, Geyer; and a range of pod-sucking bug (PSB) species, of which the most important is

*Clavigralla tomentosicollis* Stal. In storage, the cowpea beetle, *Callosobruchus maculatus* F., is the most widespread pest in West and Central Africa. These pests appear on the crop in an overlapping sequence and infest foliage, flower buds, flowers, pods and seeds.

Despite their designated status as key pests, some of them cause more damage than others and some pose more difficult research problems than others. In setting research priorities, these and other factors (such as manpower availability, time frame and other practicable control options) must be taken into account.

In the present program of cowpea germplasm enhancement at the International Institute of Tropical Agriculture (IITA), the post-flowering pests are given the highest priority. These include the MPB, PSBs and FTh.

## Screening techniques

In the past, several methods have been used to evaluate germplasm for pest resistance. The nature of the pest problem and our knowledge of its biology, as well as the damage caused, determine, among other things, the type of technique to be used — whether it is best to conduct the evaluation under natural field populations/infestations or use laboratory or screen cage assays. If field screening is to be conducted, the location and season are of critical importance. Locations with severe pest infestations are often used, but these have their limitations. Locations with low pest infestation are inappropriate for resistance evaluation.

The range of methods available for cowpea germplasm evaluation for resistance was recently compiled by Jackai and Singh (1988) and is available in French and English. As a general rule, the 'funnel and sieve' approach is adopted in screening large quantities of germplasm. It involves a systematic evaluation that increases in intensity as the number of entries diminishes. This enables the investigator to channel selected entries into various field trials or other tests for more intensive screening.

Field screening is used for most of the major field pests of cowpea at the initial stages of germplasm evaluation. However, because of the overlapping nature of these pests, it is often necessary to design different trials for each pest species or to use selective insecticides at different growth stages to eliminate those other pests that might mask the presence of resistance to the target pests. For example, to evaluate germplasm for PSB resistance, FTh and MPB, which infest the crop earlier, must be eliminated, or no pods will set and the effect of PSB infestation (normally on pods) cannot be measured.

Following field tests, laboratory and screenhouse testing is used to screen further for resistance to the MPB and PSBs using recently developed assays (IITA, 1987). The evaluation of aphids is conducted mainly in the screenhouse (Singh and Jackai, 1985), but confirmatory tests are generally conducted in the field; screening for storage pest resistance is conducted solely in the laboratory, using seeds or pods.

The process of screening methodology development and improvements is greatly enhanced by the availability or identification of a good resistance source. Quite often, resistance sources are first identified under field conditions. It is, therefore, no surprise that most host plant resistance screening programs begin with field testing. Some programs are sustained entirely on field evaluations where pest populations are consistent, uniform and predictably severe (such locations are often referred to as 'hot spots'), or simply because of a lack of other techniques. The results of germplasm evaluation for resistance to insect pests over the years show that a wide range of resistance sources are available against many pests (*see* Table 1).

**TABLE 1 Cowpea varieties/cultivars with resistance to different members of the insect pest spectrum**

| Insect (common name/species) | Resistance source | Advanced breeding line |
|---|---|---|
| Leafhoppers, *Empoasca* spp. | TVu Nos. 59, 123, 662, 1190 (VITA-3) | IT83S-742-11, -742-13, -742-14, IT83S-747-4 |
| Aphids, *Aphis craccivora*, Koch | TVu Nos. 36, 408-P2, 410, 801, 2896, 3000 | IT81D-1020 IT82E-1-108 |
| Beanfly, *Ophiomyia phaseoli*, Tryon. | TVu Nos. 3192, 1433 Farv 13, IT81D-1205-174 | IT81D-1205-174 IT82D-644 |
| Flower bud thrips, *Megalurothrips sjostedti*, Tryb. | TVu Nos.1509, 2870 TVx 3236 | IT82D-716 IT82D-713 IT84S-2246-4 |
| Legume pod borer, *Maruca testulalis* Geyer | TVu 946 Kamboinse Local | * [1] |
| Pod-sucking bugs, *Clavigralla tomentosicollis*, Stal. | TVu Nos. 1, 1890 | * |
| Cowpea storage weevil, *Callosobruchus maculatus* (L.) | TVu Nos. 2027, 11952 11953 (seeds), 625 (seeds), 4200 (pod wall) | IT81D-1007 IT81D-1137 IT81D-1157 IT84S-2246-4 |

1 *Still in early generations.*

The aim of this paper is not to provide details of the measurement of cowpea resistance to all key pests but to describe IITA's approach to germplasm evaluation using a few examples as illustration. The interested reader is referred to the review of the subject by Jackai and Singh (1988) and to the more general appraisal by Davis (1985).

In evaluations for resistance, the design of the specific method used is generally dictated by both the biology and behavior of the target pest, as well as by the phenology and physiology of the crop in question. For example, resistance to foliage feeders generally lends itself to more straightforward and simpler methodology than does resistance to internal feeders.

## Type and level of resistance

Usually, when the subject of resistance is discussed, it is obvious that the desire is often to obtain sources or varieties with high levels of antibiosis to insect pests. Many breeding programs are geared toward this goal, and any results short of that mark are generally

considered unacceptable. However, antibiosis is not necessarily always the most desirable mechanism of resistance.

There are three major types of resistance: antixenosis; antibiosis; and tolerance (Painter, 1951; Kogan and Ortman, 1978). Each is controlled by different genetic characters which are interrelated in their expression. These mechanisms are not mutually exclusive. The desirable level of resistance is determined by, *inter alia*, the intended use — whether as a solitary tactic or in conjunction with other methods in an integrated pest management (IPM) setting — and the amount of investment in time, materials and manpower required to develop the resistance fully. This, in turn, dictates the method to be used, which must be determined on an individual crop/pest basis.

The type of resistance is not usually of primary importance in resistance screening. Once resistance is identified, it can be characterized as to type and used in the breeding program. Specific methodologies are, however, available for use in the identification of the different types of resistance (Davis, 1985). Therefore, if a specific type of resistance mechanism is desired, the appropriate method can be identified *a priori* and used for its identification.

Resistance screening in the IITA program, as in most programs, begins with locally cultivated landraces and breeding lines. In the event that inadequate levels of resistance are not available in these materials, or in order to increase the resistance base, exotic germplasm is screened. Finally, closely related species and other wild relatives are evaluated for possible sources of resistance, especially to those pests for which useful levels of resistance cannot be identified in the cultivated genotypes. This generally makes hybridization difficult, but modern biotechnology techniques can be relied upon to break fertilization barriers.

## CASE HISTORIES

We shall examine here specific examples selected from the wide range of insect pest problems affecting cowpea. For the sake of brevity, we have selected the following pests: a seedling pest, *Aphis craccivora*, for which screening is conducted almost entirely in the screenhouse; a pest of the reproductive phase of the crop, *Maruca testulalis*, in which a combination of field, laboratory and screenhouse procedures are used; and a post-harvest pest, *Callosobruchus maculatus*, for which only laboratory procedures are used. The evaluation of cowpea resistance to the other pests on the crop is fully described by Jackai and Singh (1988).

### Cowpea aphid

The cowpea aphid, *Aphis craccivora*, usually attacks the plant during the early seedling stage. Germplasm evaluation for resistance to the cowpea aphid is conducted mainly in screenhouse cages. Wooden trays are filled with vermiculite or sterilized soil to a depth of about 8-12 cm. Test genotypes, as well as checks, are planted in single rows. The germination trays are placed in saran mesh cages. Plants are infested when they are 10 days old with five 4th-instar aphids per plant. These insects feed on the young seedlings and within 10-15 days those entries which are susceptible begin to die off. Plants in each row are scored for vigor using a scale of 1 to 5, where 1 indicates maximum vigor (no plant mortality) and 5 indicates dead or dying plants. If the investigator is interested in variation within accessions, the same scoring

procedure is carried out within each row, on a plant-to-plant basis. Scoring can be done more than once.

Generally, scores of 1 and 2 indicate high resistance; > 2 < 3 indicates moderate/low resistance; scores of 4 and 5 suggest low and high susceptibility, respectively. Several hundred lines can be evaluated per month using this method.

Resistant lines are retested and selections from these are evaluated in replicated field trials. Field trials are sometimes also used as a preliminary method or, where natural field infestations are considered adequate, as the only method. This process generally uses aphid incidence (that is, percentage of infested plants per row in single-row plots, or in two center rows where larger plots are used) at 10, 20 and 30 days after seedling emergence. Severity of infestation can be measured using the rating scale (1 to 9) proposed by Litsinger et al. (1977) and adopted by Irwin (1980) for use on soybean. The number and size of aphid colonies are the main descriptors used in this scale (*see* Table 2).

**TABLE 2 Screening procedure and rating scale for cowpea resistance to aphids**

| | |
|---|---|
| I[1] | a) Count number of plants in 2 center rows.<br>b) Determine number of plants infested by aphids in the same 2 rows.<br>c) Calculate percentage infestation for each test entry/cultivar. |
| II[2] | Assess 30 stands per plot, 5 stands per row at fixed intervals (e.g., every 5th stand) to avoid bias.<br>Record the level of infestation using the following:<br>0) No infestation<br>1) 1-4 aphids: a few individual aphids<br>3) 5-20 aphids: few isolated colonies<br>5) 21-100 aphids: several small isolated colonies<br>7) 100-500 aphids: large isolated colonies<br>9) >500 aphids: large continuous colonies.<br>Calculate the mean score for each entry/cultivar. |

1 I Gives an indication of aphid incidence, an important parameter because of virus (CAMV) transmission.
2 II Provides information on the severity of infestation.

Source: Based partly on Litsinger et al., 1977.

Over the years, several germplasm accessions have been identified with high levels of resistance to the cowpea aphid. These are shown in Table 1, along with a list of elite genotypes that have been developed with aphid resistance. This has been one of our most successful efforts in the use of germplasm sources to develop pest-resistant varieties.

### Legume pod borer

The legume pod borer (MPB), *Maruca testulalis*, infests several plant parts, but causes the greatest damage to the fruiting structures (the flowers and pods). More than 8,000 accessions have been evaluated, mainly by means of the field screening procedures described by Jackai (1982).

Unreplicated single-row plots were planted at Mokwa in Niger State, Nigeria, where MPB infestation is heavy. An early-maturing susceptible spreader was planted 2-3 weeks before the test material in two rows so that each row of test material had a spreader row running perpendicular to one of its ends. In addition, a known (susceptible and/or resistant) check was planted once in every 20 entries (50 entries in material > 1,000 entries). These checks provide a means of assessing pest distribution as well as the relative performance of test entries. The effect of MPB infestation and subsequent damage is often masked or confounded by earlier- and later-occurring pests. For this reason, material which is to be evaluated for resistance to MPB is often sprayed with 200-250 gai/ha of monocrotophos (as Nuvacron 40 EC) at intervals of 10-14 days, starting at flower bud formation to eliminate Fth and PSBs. These spray applications are continued only if these pests are present at damaging levels. MPB populations show no significant reductions under monocrotophos sprays (Jackai, 1983). Because of the often large number of accessions evaluated, initial measurements are restricted to pod load (PL) and pod damage (PD). PL measures the degree of successful podding, while PD measures the level of feeding damage on whatever pods are formed. These measurements are made using a rating scale of 1-9, with the desirable levels of PL and PD occurring on opposing ends of the scale. For example, a perfect score for PL is 9, while that for PD is 1 (*see* Table 3). Measurements are best taken when the pods are still green but fully mature.

**TABLE 3 Visual rating scale for *Maruca testulalis* damage to cowpea**

| Score: pod load [1] (PL) | Score: % pod damage (PD) |
|---|---|
| 1 = Most peduncles (≥ 75%) bare (i.e. no pods) | 1 = 0 - 10 |
| | 2 = 11 - 20 |
| | 3 = 21 - 30 |
| 3 = Up to 60% peduncles bare | 4 = 31 - 40 |
| 5 = Up to 30% peduncles bare | 5 = 41 - 50 |
| | 6 = 51 - 60 |
| 7 = Up to 15% peduncles bare | 7 = 61 - 70 |
| 9 = Occasional bare peduncles | 8 = 71 - 80 |
| | 9 = 81 - 100 |

Calculation Pod Evaluation Index ($I_{pe}$) as: $I_{pe}$ = PL/PD where higher values of $I_{pe}$ indicate better levels of resistance (with PL ≥ 5 and PD ≤ 5).

1 Pod load is based on the ratio of the number of peduncles without pods: peduncles with pods (i.e., degree of successful podding).

Because of differences in the maturity of germplasm accessions, the test materials should be arranged in maturity groups and scored when about 50% of the entries are mature. Subsequently, entries with PL scores of 5 or more and PD scores of 5 or less are moved into the next screening stage — our experience has shown that this will not exceed 20-25 for every 1,000 entries screened in the first stage in most locations; however, the number is generally much lower at the Mokwa site). This is done in 2-row replicated plots with protected and unprotected (but sprayed with monocrotophos) treatments. At this stage, measurements

might include assessment of flower infestation/damage, depending on the number of entries involved.

Until 1986, only a few accessions, out of the several thousand screened, were considered worth giving a second selection challenge. In 1987, we screened about 2,500 accessions at Ibadan, where the natural infestation is lower and more representative of most locations where the crop is grown. Ninety-two accessions were selected for further testing, and are currently being screened in the MPB Preliminary Trial at Ibadan and Mokwa, with two dates of planting at the latter location. We believe that the use of Ibadan as a test location provides sufficient selection pressure to enable the identification of moderate levels of resistance. Although it was usually impossible to obtain such levels under the very high pest pressure at Mokwa, this location has been retained as a test site for the identification of high levels of resistance, but only after initial testing at Ibadan. At a later stage, field screening at Ibadan involves the use of artificial infestations with MPB larvae to augment natural infestation.

Bioassays have also been developed for use in the laboratory and screenhouse. Since these require more careful evaluation, they are used only after genotypes have reached the Advanced-2 Trial stage, the same stage at which artificial infestations are made. These assays use a specific number of insects of a defined developmental stage. A brief description of the laboratory assay is reported in IITA (1987) and a more detailed account of both laboratory and screenhouse procedures is given in IITA (1988). These methods require the back-up of an insect-rearing laboratory, which may not be readily available to many national research programs. However, a small culture maintained on the natural food of the insect might suffice for a small testing program.

From the field, laboratory and screenhouse tests conducted over a two-year period, four lines — MRx 2-84S, MRx 10-85S, MRx 66-85S, MRx 67-85 — have shown moderate levels of resistance at Ibadan. None of these, however, is a germplasm accession.

### Cowpea storage beetle

The cowpea storage beetle, *Callosobruchus maculatus*, is probably the most important post-harvest cowpea pest in West and Central Africa. In Southern Africa, a different but equally devastating species, *C. rhodesianus*, is known to occur. In Asia, *C. chinensis* is the predominant species. All species cause similar damage to stored cowpea.

Although a field-to-storage pest, the cowpea storage beetle is important only in stored grain, and thus germplasm evaluation for resistance to this pest is conducted on seeds, using laboratory techniques. At IITA this involves putting 20-40 seeds of each accession to be screened in small plastic boxes (5 x 5 x 2 cm). It is important to ensure that any prior infestation of the seeds is eliminated. Two pairs of day-old adults are introduced into each box and allowed to oviposit for 24 hours. The boxes with infested seeds are left at 28°C and 70-80% relative humidity; other ambient conditions may be used as long as they are monitored for consistency. Five days after infestation, the eggs are counted, and about 20 days later, or whenever adult emergence commences, the emerging insects are counted daily and removed from each container. This procedure continues until there is no more emergence from the susceptible check.

The percentage of adult emergence is then determined, and a suitability index (Growth Index, GI) (Howe, 1971) is calculated: GI = log S/T, where S = % adult yield and T = mean development time. Using the GI as well as the oviposition counts, the level of resistance of

each test accession is determined. Several hundred genotypes can be screened per month using this technique.

Out of 10,000 germplasm accessions only one, TVu 2027, was identified as having a moderate level of resistance to this pest (Singh, 1980). This single accession has been the source of bruchid resistance in all improved cowpea varieties from IITA (Singh and Jackai, 1985). Two other accessions, TVu 11952 and 11953, from Borno State in Nigeria, with similar levels of resistance to that of TVu 2027, have been identified recently.

Pod wall resistance is important in areas where cowpea is stored in pods. There is some evidence that $F_1$ adults emerging from field infestation of pods cause only minimal damage (Owusu, 1987), but no estimates are available for long-term effects. Most of the initial damage in storage is thought to be caused by *Bruchidius atrolineatus* (Owusu, 1987). In the past, we have screened for cowpea pod resistance to the storage beetle by confining 6-12 pods of the test line with 3-6 pairs of newly emerged adults in a paper bag for 24 hours. The insects are then removed and the same information collected as for seeds. TVu 4200 and 625 have been shown to have pod resistance. Using different techniques, Owusu (1987) at Ibadan and Kitch et al. (unpubl.) at Purdue University, USA, have identified other sources of pod resistance. A number of these are wild cowpea relatives.

In addition to screening cultivated cowpea accessions, we have initiated a systematic program to evaluate wild cowpea relatives. This became necessary in order to identify higher levels of resistance to the more difficult post-flowering pests. A number of accessions of *Vigna vexillata* have already been identified which have good levels of resistance to FTh, MPB, PSBs and bruchids. Although no successful cross has yet been accomplished between *V. vexillata* and *V. unguiculata* (IITA, 1987), these lines have enabled us to design the highly sensitive screening procedures which are now being used. As more germplasm is collected from both cultivated cowpea and its wild relatives, we are optimistic that better and increased diversity of resistance sources will be found for even the most intractable cowpea pest problems.

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# 2.3

# In Vitro *Conservation and Distribution of Root and Tuber Crop Germplasm*

S. Y. C. Ng

Cassava, yams, sweet potato and cocoyams are major tropical root and tuber crops in many parts of the tropical and subtropical Africa, where they are widely grown and consumed as staples and, to some extent, used as livestock feed and industrial raw materials. The plant germplasm of these crops provides an important repository of genetic source materials for future plant breeding. Preservation of such materials and their safe transfer across national boundaries has played a major role in the agricultural development of many countries.

Root and tubers crops are vegetatively propagated by cuttings, corms or tubers. The seeds of these crops usually have a high degree of heterozygosity and some do not produce flowers or viable seeds. They are maintained as living collections in the field, but maintaining germplasm in the field presents problems. It is not only costly in terms of the labor, land and space required but may also lead to the loss of valuable genetic materials as a result of damage by diseases and pests, both in the field and in storage.

The development of *in vitro* techniques and the ability to regenerate plants from these root crops have made *in vitro* conservation an alternative to maintaining germplasm in the field. This conservation technique is a valuable tool for conservation of germplasm that is difficult to maintain by the seed storage method (Ruredzo and Hanson, 1988). A recent review indicated that a total of 49 plant species have been successfully conserved by *in vitro* reduced growth storage methods (Ng and Ng, 1990).

Meristem-tip culture, since it was first used by Morel and Martin (1952) to produce virus-free dahlias, has been widely applied to obtain virus-free clones from a wide range of economically important crops (Quak, 1977; Walkey, 1980). This technique has greatly facilitated the international distribution of vegetative materials, as shipment in this form circumvents most quarantine restrictions.

This paper describes the *in vitro* methods that can be used or are being used in germplasm conservation and distribution. The achievements of applying some of these methods is summarized.

## EXPLANT MATERIALS AND REGENERATION MODE

The success of the *in vitro* technique in germplasm conservation depends on the choice of explant materials and the availability of a reliable plant regeneration protocol.

The explant materials and the mode of plant regeneration should be able to maintain the highest degree of genetic stability. It is generally agreed that meristems/shoot-tips are the most suitable explant materials for both *in vitro* conservation and disease elimination. One of the important features of using meristem-tip culture is that the regenerated plants usually retain the genetic integrity of the parent plants. This is probably because of the more uniformly diploid nature of the meristematic cells (Murashige, 1974). The non-adventitious plant regeneration mode is most preferred, since callus can induce genetic abnormalities in the regenerants (Scowcroft, 1984).

## *IN VITRO* TECHNIQUE AND GERMPLASM CONSERVATION

The tissue culture methods which have been reported for crop germplasm storage, with particular reference to root and tuber crops, are: storage under normal growth conditions; storage under reduced growth conditions; and cryopreservation.

### Normal growth storage

Under normal culture medium and incubation conditions, the *in vitro* plantlets can be kept for a short period, about six months. This method can be used for temporary storage of some germplasm collections, especially those used for international distribution.

### Reduced growth storage

Reduced growth storage is based on the manipulation of culture conditions to allow the culture to remain viable but with a very slow growth rate. The main advantages of this method are that culture deterioration can be detected visually and therefore losses of viability can be avoided; the space requirement is smaller than that for field cultivation; the propagation potential of the cultures can be very high; the problems of genetic erosion can be avoided; and the technique can also be used to maintain pathogen-tested genetic materials. The disadvantage is that plantlets need to be subcultured at regular intervals and that the reduced growth selection pressure might favor certain mutant development.

Reduced growth storage can be achieved by the reduced incubation temperature, the manipulation of culture media, and the combination of the former two methods (Ng and Ng, 1990).

A summary of the use of reduced growth storage methods for root crop germplasm storage is shown in Table 1 (*overleaf*). The explant materials are meristem-tip or nodal cuttings. After surface disinfection, the explant materials are inoculated to culture media under aseptic conditions. The cultures are then incubated in a culture room (with normal incubation conditions) and cultures that have been established are then transferred to a lower incubation temperature for storage, if desired. The pre-growth of cultures at normal incubation

conditions before transfer to reduced incubation temperature has been found to be very important in the conservation of root crops (Ng and Hahn, 1985). Cultures are checked periodically and subcultured to fresh culture media when required. Theoretically, a culture can be subcultured indefinitely.

This method has been applied to conserve germplasm of several root crops species by a number of agricultural research centers, including the AVRDC for sweet potato, the Centro Internacional de Agricultura Tropical (CIAT) for cassava, the Centro Internacional de la Papa (CIP) for potato and sweet potato, and IITA for cassava, yams, sweet potato and cocoyams.

*Reduced incubation temperature*

The reduction of incubation temperature has shown to be very effective in prolonging the subculturing cycle by reducing the growth rate of the explant. Plant tissue cultures are usually grown at a temperature of 25°-30°C. Temperatures below 25°C tend to reduce the growth rate. However, the recommended temperature regimes differ from crop to crop; some crops are more cold tolerant than others, and their cultures can be maintained at very low temperatures.

Henshaw and O'Hara (1983) reported that meristem-derived potato plants can be kept for 1 year at a storage temperature of 6-12°C. CIAT (1981) reported that reducing incubation temperature alone increased the transfer period of cassava clones from 18 to 24 months; however, temperatures below 20°C resulted in culture deterioration in some genotypes. Kartha (1981) also reported that cassava plantlets can be stored at 20°C with low light intensity for 1 year. Ng and Hahn (1985) demonstrated that by reducing incubation temperature to 18°C and 22°C, yam germplasm could be kept for 1.5-2 years without culture media modification. Alan (1979) found that temperatures below 18°C were detrimental to sweet potato plantlets; however, temperatures of about 23°C could prolong the culture period for over 1 year. Ng (unpubl.) also found that a storage temperature of 20°C increased the transfer interval of cocoyams to more than 1 year. Zandvoort and Staritsky (1986) reported that cocoyams can be stored for 3 years at 9°C.

*Manipulation of culture media*

Reducing the concentration of ions, increasing or lowering sucrose concentration, adding osmotica (mannitol or sorbitol) and growth inhibitors, and increasing culture media volume were found to be effective in prolonging plantlet storage life, as shown in Table 1. In some cases, the addition of growth inhibitors (such as ABA) do not significantly reduce the growth rate or increase the storage life of the plantlets (Zandvoort and Staritsky, 1986). Sealing culture vessels with parafilm strip or tape can minimize evaporation and avoid desiccation of the culture media.

The addition of mannitol (3-6%) to sucrose can significantly reduce the explant growth rate. This phenomenon was observed in sweet potato and cassava. In the case of yams, growth reductions of 13% and 57% were observed when the culture medium contained a combination of 3% each of sucrose and mannitol, and 5% sucrose and 3% mannitol respectively (Ng, unpubl.). Growth reduction was also observed in sweet potato when cultured on media that

**TABLE 1** **Summary of the application of reduced growth storage in maintaining root crop germplasm**

| Species | Explant | Culture type | Storage method | Storage duration | Reference |
|---|---|---|---|---|---|
| **A. Reduced incubation temperature:** | | | | | |
| *Colocasia esculenta* | Buds or meristem | Plantlet | Stored at 9°C | 3 years | Zandvoort & Staritsky, 1986 |
| *C. esculenta* | Buds or meristem | Plantlet | Stored at 18-22°C (day/night) | 1 year | Ng, 1986 |
| *Dioscorea alata* | Node | Plantlet | Stored at 18-22°C(day/night) | 1-2 years | Ng & Hahn, 1985 |
| *D. rotundata* | Node | Plantlet | Stored at 18-22°C (day/night) | 1-2 years | Ng & Hahn, 1985 |
| *Ipomoea batatas* | Node | Plantlet | On MS media with 3% sucrose at 22°C | 55 weeks | Alan, 1979 |
| *Manihot esculenta* | Node | Plantlet | Stored at 20°C | 18-24 months | CIAT, 1981 |
| *M. esculenta* | Node | Plantlet | Liquid media with filter paper bridge incubated at 20°C, 16 h photoperiod with 100-200 lux light intensity | 1 year | Kartha, 1981 |
| *Solanum* spp. | Node | Shoot | On MS medium with 3% sucrose stored at 6°C | 12-24 months | Westcott et al., 1977 |
| *S. tuberosum* | Node | Plantlet | Incubate at reduced temperature ranged from 6 to10°C with a mean survival rate of 61% at 6°C and 83% at 10°C day/6°C night | 1 year | Henshaw et al., 1980 |
| *Xanthosoma brasiliense* | Buds or meristem | Plantlet | Stored at 6°C under dark conditions | > 2 months | Staritsky, 1980 |

**TABLE 1 (contd)**

| Species | Explant | Culture type | Storage method | Storage duration | Reference |
|---|---|---|---|---|---|
| *Xanthosoma* spp. | - | Plantlet | Stored at 13°C | | Staritsky, 1980 |
| *X. sagittifolium* | - | Plantlet | Stored at 18-22°C (day/night) | 1 year | Ng, 1986 |
| **B. Manipulation of culture media:** | | | | | |
| *I. batatas* | Node | Plantlet | On Heller's medium + 3% sucrose, MS or Nitsch's medium with 1% sucrose | 89 weeks | Alan, 1979 |
| *I. batatas* | Node | Plantlet | MS media with 1μM each of BAP and NAA and 5% each of sucrose and mannitol, incubated at 26°C, 16 h photoperiod with 4,000 lux light intensity | Observed growth reduction within 3 months | Kartha, 1981 |
| *M. esculenta* | Node | Plantlet | In large culture vessel, with culture media contained low osmotic concentration and activated charcoal | 2 years | Roca et al., 1983 |
| *X. sagittifolium* | Corm pieces | Plantlet | White's media incubated at 29°C | 1 year | Acheampong & Henshaw, 1984 |
| **C. Combination of A and B:** | | | | | |
| *I. batatas* | Node | Plantlet | MS with 3% sucrose and 3% mannitol stored at 18-22°C (day/night) | 1-2 years | Ng & Hahn, 1985 |
| *Solanum* spp. | Nodal | Plantlet | MS + 2% sucrose + 50mg N-dimethly-succinamic acid. Pre-growth at 20-22°C, 16 h photoperiod at 4,000 lux light intensity, then stored at 10°C, 16 h photoperiod at 2,000 lux light intensity | 2 years | Mix, 1982 |
| *Solanum* spp. | Node | Plantlet | On MS medium with 4% mannitol and stored at 8°C | 2-3 years | Espinoza et al., 1984 |

contained 5% each of sucrose and mannitol (Kartha, 1981). The use of larger culture vessels can prolong the storage life of cocoyams (Acheampong and Henshaw, 1984) and cassava germplasm (Roca et al., 1984). A low salt culture medium can effectively extend sweet potato plantlet storage life (Alan 1979) and cocoyam plantlet storage life (Acheampong et al. 1984). Reduction of the sucrose supply in the medium can also extend the storage life in sweet potato (Alan, 1979) and cassava (Roca et al., 1984).

*Reduced incubation temperature and manipulation of culture media*

A combination of the reduced incubation temperature and manipulation of culture media methods has also given satisfactory results in the conservation of crop germplasm. However, storage requirements, such as temperature, sucrose concentration, inhibitors, osmotica and subculture interval, must be carefully determined for each crop. Sweet potato has been stored for 1-2 years with the addition of mannitol and a lower incubation temperature (Ng and Hahn,1985). It was also reported that potato plantlets can be stored for 2 years at 10°C in a lower sucrose culture medium that contained n-dimethyl-succinamic acid (Mix, 1982).

## Cryopreservation (freeze preservation)

The principle underlying cryopreservation of plant materials involves maintaining the cultures at a non-cell division stage and inactive metabolism. This approach, which can be used for long-term conservation, can be achieved by storing the cultures in liquid nitrogen. Attempts have been made to cryopreserve cassava, sweet potato and cocoyams. However, most progress is being made with cassava (IBPGR, 1986). Cassava has been found to be very sensitive to cryoprotectants (Kartha and Gamborg, 1978).

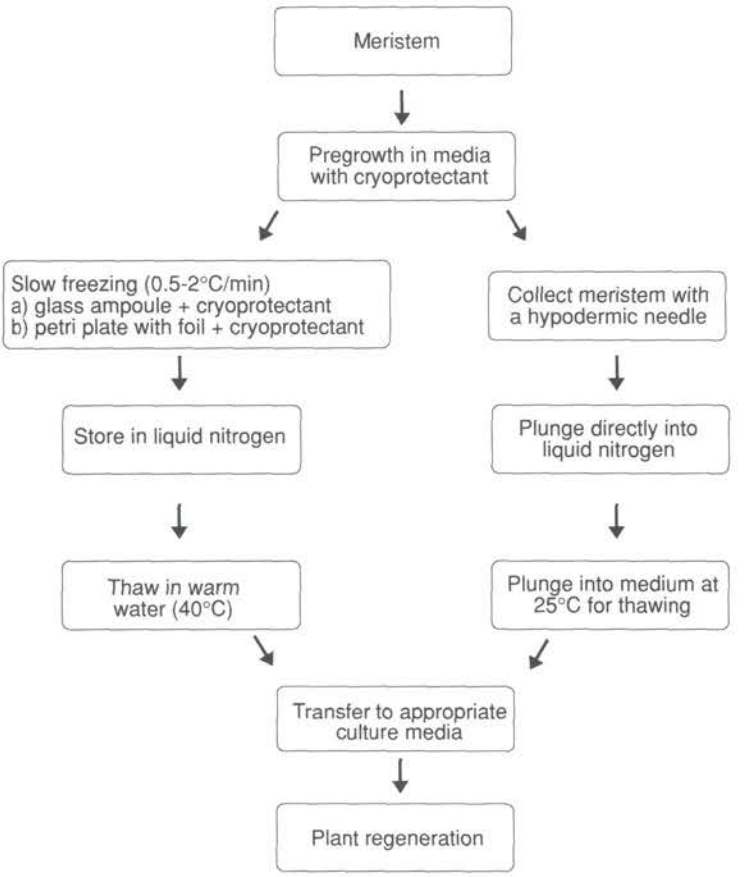
Cryoprotectants are usually added to the culture media to minimize freeze damage. Commonly used chemicals are dimethyl sulfoxide (DMSO) and glycerol. Most of the cryoprotectants exhibit varying degrees of cytotoxicity at higher concentrations. Kartha (1985) reported that DMSO at 2% and 5% had no cytotoxicity effect on cassava and sweet potato meristems, respectively. However, at 5% DMSO cytotoxicity occurred in cassava and at 7.5% in sweet potato.

The procedure for cryopreservation of explants is represented in Figure 1. Three types of methods have been used to freeze the cultures before storage in liquid nitrogen — fast freezing (at several hundred °C/min.), slow freezing (at 0.5-2°C/min.) and droplet freezing. The explants (meristems) are raised in appropriate liquid culture media that contains cryoprotectant for 1-2 days and then treated directly with cryoprotectants or treated with a gradual increase in the concentration of cryoprotectant in the medium, followed by cryoprotectants alone.

For fast freezing, the cryoprotected meristem is collected from the solution with a hypodermic needle and placed directly in liquid nitrogen. For slow freezing, the cryoprotected specimen is dispensed into sterile ampoules and cooled at rates varying from 0.5 to 2.0°C/min down to -40°C in a programmable freezing machine. The ampoules are then stored in liquid nitrogen (-196°C). The droplet freezing method was developed by Kartha et al. (1982) for freezing cassava meristems. It is essentially the same as that of slow freezing, but instead of freezing meristems in glass ampoules, the cryoprotected meristem is frozen with

a small quantity of cryoprotectant solution on aluminum foil in a petri dish. The frozen samples are then stored in liquid nitrogen. For regeneration, the samples are removed from the liquid nitrogen and thawed in warm water (+40°C). After washing with several changes of sterile distilled water, the meristems are placed on a standard culture media.

**FIGURE 1 Procedure for cryopreservation**




The cassava plantlet regeneration rate was very low after thawing, only 10% (Kartha et al., 1982). However, plant regeneration rate was increased to 30% by preculturing meristems, prior to freezing, in a liquid medium containing 9% sucrose and gradually increasing the sorbitol concentration from 0 to 1M over a 24-hour period (Kartha, 1988). The regenerated plants were field tested. Agronomic and morphological characters and esterase isoenzyme patterns were shown to be stable. Bajaj (1983) was able to regenerate cassava plants from excised meristems that had been cryopreserved for 3 years in liquid nitrogen. It was reported that meristems treated with a combination of 5% each of sucrose, glycerol and DMSO as

cryoprotectant has increased the survival rate to 26% (Bajaj, 1983). However, in order for the technique to be employed on a routine basis, more research needs to be carried out on increasing the recovery rate. Using ultra-rapid freezing methods, several genotypes of potato were tested for cryopreservation (Henshaw et al., 1985). Depending on the genotype and culture medium, the recovery rate ranged from 0% to 60%. Preliminary studies on the cryopreservation of cocoyam shoot-tips have been unsuccessful. However, microscopic observations showed that no cell damage occurred immediately after freezing (Zandvoort and Staritsky, 1986).

Despite the advances that are being made in cryopreservation, only a few plant species — apple, potato, pea, chickpea, strawberry and cassava — have been successfully cryopreserved to date (Kartha, 1985). The major difficulty is the regeneration of plants after freezing.

## *IN VITRO* METHODS AND GERMPLASM DISTRIBUTION

The primary aim of disease elimination is to produce virus-free stocks for international distribution. Quarantine inspection and the issue of phytosanitary certificates is the last stage of this process. Research institutions such as IITA are aiming at distributing improved germplasm to national programs. They are also interested in obtaining new germplasm from elsewhere.

The important factors that contribute to the success of *in vitro* methods in disease elimination are the protocol for plant regeneration from meristem and reliable virus indexing methods. The *in vitro* methods that are used for disease elimination are meristem culture, thermotherapy and meristem culture, and chemotherapy and meristem culture. Details of these methods have been described by Ng et al. (1987). Several agricultural research institutions are currently using such methods in the exchange of clonal germplasm materials of their mandated root crops.

Tissue culture materials are generally accepted by quarantine authorities worldwide as the safest means for the movement of vegetative materials. Kahn (1986) stated that tissue cultures represent plant materials of significantly improved health status which should enjoy a more liberal entry status.

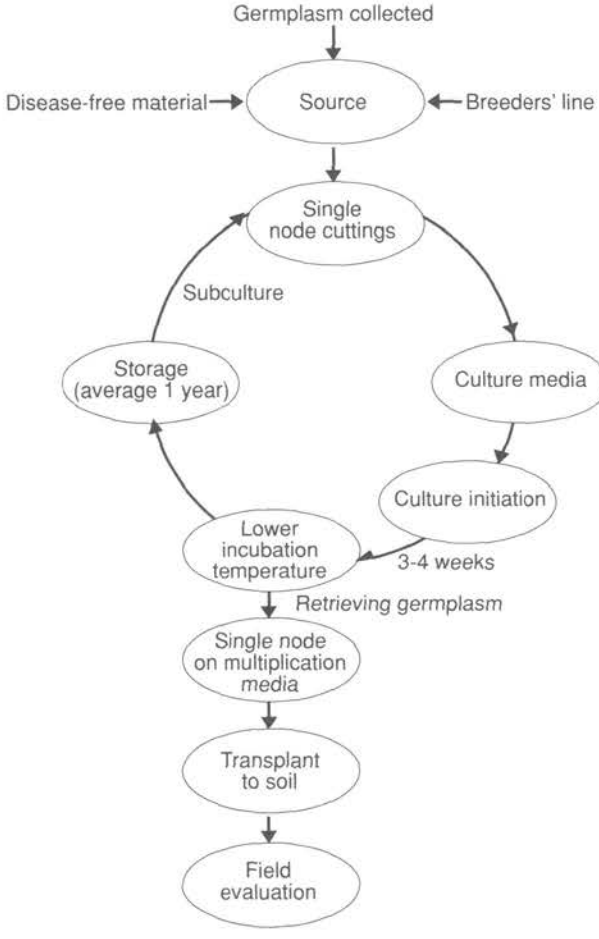
The advantages of using tissue culture materials in germplasm exchange are:

- they provide opportunities for the easy movement of clonal materials across national boundaries;
- the aseptic conditions of tissue culture materials considerably reduce the risk of introducing pests, such as insects or mites, as well as the risk of fungal and bacterial contamination;
- indexing the plant materials for the presence of certain viruses can provide assurance of the absence of such pathogens;
- once pathogens have been eliminated, such materials can be maintained *in vitro*, thus avoiding the risk of accidental reinfection.

## APPLYING TISSUE CULTURE METHODS TO THE CONSERVAITON AND DISTRIBUTION OF ROOT CROP GERMPLASM

The methods used by IITA for germplasm conservation of root crops (cassava, sweet potato, yams and cocoyam) are *in vitro* reduced growth storage and field cultivation (*see* Figure 2). The disinfected explants (meristems or node cuttings) are first placed in a culture medium, as described by Ng (1983) and Ng and Hahn (1985). The cultures are then placed in an incubation room with a controlled temperature of 26-30°C, a 4,000 lux light intensity and a 12-hour photoperiod for culture initiation. About 3-4 weeks later, those nodes that have developed into plantlets are transferred to a lower incubation temperature (18-22°C), a 3,000 lux light intensity and a 12-hour photoperiod. Under these conditions, the plantlets can be stored from 8 to 24 months, depending on the genotype and species involved. The cultures are inspected periodically and those that show deterioration are subcultured to a fresh culture

**FIGURE 2** ***In vitro*** **germplasm conservation at IITA**



medium. For the past several years, IITA has used this system for conserving its sweet potato, yam and cocoyam collections.

At present, IITA holds more than 1,500 accessions of sweet potato, 150 of cocoyams, 300 of yams and 100 of *Manihot* species (10 species, including cassava) in its *in vitro* collections. In 1988, another 600 accessions of yam germplasm were transferred to the *in vitro* collections.

IITA applies meristem-tip culture techniques to eliminate viruses from its mandated root and tuber crops (Ng and Hahn, 1985). The regenerated plants are tested intensively to ensure absence of all known viruses. Virus-tested, certified plants are then multiplied *in vitro* and prepared for international distribution. Plantlets are grown in transparent containers with sterile culture medium which does not contain charcoal, in order to facilitate the detection of fungal or bacterial contamination. For distribution to national programs, these containers are either hand-carried or transported by airfreight.

To date, virus-tested cassava has been distributed to more than 30 countries in Africa and virus-tested sweet potato materials have been sent to more than 60 countries in various parts of the world. Clonal germplasm is being used by the recipient countries and institutions in their breeding programs. Selected varieties that perform well under local environmental conditions may also be distributed directly to farmers in the country.

Recently, several varieties of white yams were certified by the Plant Quarantine Service, Nigeria, and will be ready for distribution by early next year 1989. The characteristics of the induced *in vitro* microtuber formation (Ng, 1988) will be used as a means of international distribution, as has been done for potato.

Genetic stability is one of the main concerns in germplasm conservation and distribution. Randomly selected germplasm accessions were multiplied and morphological and agronomic characters were evaluated under field conditions to monitor changes. Isoenzyme electrophoresis can be used to screen the entire germplasm. IITA is planning to develop this technique to evaluate its *in vitro* germplasm collections.

Field evaluation of several accessions of sweet potato and cassava that were maintained *in vitro* for 6-7 years revealed that morphological characters remained stable (IITA, 1986; IITA, 1988).

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# 3.1

# *Yam Germplasm Diversity, Uses and Prospects for Crop Improvement in Africa*

O. O. Okoli

Yam, *Dioscorea* species, is an important source of food energy for humans in the humid and subhumid tropics of West, Central and East Africa in particular, and in other tropical areas of South-East Asia, the Caribbean, Oceania and parts of India, Japan and China. While only landraces are currently in cultivation, there exists great diversity in food and agronomic characteristics of the crop where improvements are needed. Although propagated vegetatively, hybridization occurs freely in nature and the seeds produced are viable. The possibilities of improving the agronomic characteristics and food qualities of yam are good, but germplasm must be collected, evaluated, maintained and used for creating source populations for genetic improvement. The need is urgent, as forests where these yams and their wild relatives grow are fast disappearing in most developing countries of the humid and subhumid tropics. Also needed are investigations on methods of storing yam germplasm to reduce costs and risks of loss, which are high. Regional cooperation with international funding for storage and maintenance of germplasm is a viable and cost-effective proposition.

Edible yams are important food crops in the economy of Africa, particularly in sub-Saharan Africa, which in the 1975-84 period produced 21,855,000 metric tons, accounting for 95.6% of the total world output of the crop (Gebremeskel and Oyewole, 1987). Yams grow as far north as parts of Chad, Mali and Mauritania in the Sahel (latitude 20°N), and as far south as South Africa (latitude 20°S), but most of the crop is grown in West, Central and East Africa. As much as 1.71% of the total arable land was allotted to yams annually in the 1975-84 period in sub-Saharan Africa, where the crop contributed an average of 113 calories per capita per day in the diet of the people (Gebremeskel and Oyewole, 1987).

The importance of yams in sub-Saharan Africa is increasing. Annual growth in hectarage cultivated, total production and yield per hectare for the period 1965-84 were 1.05%, 2.24% and 1.19%, respectively. Although more yams are being produced annually in the region, fewer are available for consumption. An annual decline of 0.42% in the 1965-84 period for average per capita supply of yams was reported. This could be partly a result of the rapid

increase in population in the region, put at 2.91% per annum during the 1965-84 period (Gebremeskel and Oyewole, 1987). The need to improve crop productivity to meet increasing demands is thus essential.

Yams of any appreciable economic value come from the tropical species of the genus *Dioscorea*. Some of the better varieties of edible yams are now cultivated in countries other than those where they originated. In general, they have migrated westward. Asiatic species have passed to Africa and thence to America. African types have also been transferred to America, but there has been virtually no corresponding transfer of American species to the Old World (Coursey, 1967). Human migration and international trade have been the main channels of distribution.

The cultivation of a number of food yams originated in South-East Asia, probably in Burma or Thailand. Four distinct centers of origin have been proposed for yams. One is a location on the fringe of the West African forest belt, either within the savanna areas or possibly in the 'Dahomey gap' where savanna conditions penetrate southwards to the sea; *D. cayenensis, D. rotundata* and *D. dumetorum* originated here. There may have been a subsidiary center nearer to or in the Congo basin (Coursey, 1967). Thus the most common species of yams now grown in West Africa — *D. rotundata* and *D. cayenensis* — are believed to have originated there.

## GERMPLASM COLLECTIONS IN AFRICA

Germplasm collection and conservation have been reported by 11 institutions in eight African countries (Lawrence et al., 1986). Collections made largely within Africa have been of edible cultigens and some wild relatives. These have been variously described as indigenous primitive cultivars or indigenous wild accessions. Côte d'Ivoire and Nigeria report accessions from Puerto Rico; in most other countries accessions comprise local collections and intra-African exchanges of materials. Most countries also report IBPGR support for their collections. Institutes holding yam germplasm are given in Table 1.

The aim of collecting, evaluating and preserving germplasm is to reduce genetic erosion, produce source populations for crop improvement and preserve the materials for future use. Yam germplasm is held mainly in vegetative form. This means that the materials must be planted in the field every year or season to rejuvenate them, a process that is not only costly but also increases the risk of loss by diseases, pests, drought or other stresses.

Some species of cultivated yams, notably *D. rotundata, D. dumetorum, D. alata* and *D. bulbifera*, produce viable seeds. There is, therefore, the possibility of preserving the genes of these cultivars in seed form once the factors affecting how long true seeds of yam remain viable are known.

Germplasm holding *in vitro* has been reported from the International Institute of Tropical Agriculture (IITA) (S.Y. Ng, pers. comm.). It is known that there are clonal differences in response to this method of preservation. Although the extent of induced variation arising from callus transfers is unknown, this method of holding yam germplasm is, for now, the best, as it saves on the cost of field planting and considerably reduces the risk of loss. Because of the sophistication needed in handling the materials and the relatively expensive equipment required, at least initially, this method is recommended for the conservation of yam germplasm on a regional basis.

**TABLE 1 Germplasm of major edible yams held in institutions of Africa**

| Yam species | No. collections | Institution/country |
|---|---|---|
| *D. abyssinica* | 5 | IDESSA, Côte d'Ivoire |
| | 18 | FAST, Côte d'Ivoire |
| *D. bulbifera* | 1 | IDESSA |
| | 1 | Bouaké |
| | 2 | Ghana |
| | 15 | IITA |
| | 6 | NRCRI |
| | 2 | Togo |
| | 37 | FAST |
| *D. dumetorum* | 2 | IDESSA |
| *D. esculenta* | 8 | Abidjan |
| | 3 | Ghana |
| | 4 | IITA |
| | 1 | FAST |
| *D. hirtiflora* | 3 | Abidjan |
| | 46 | FAST |
| *D. rotundata* | 24 | Benin |
| | 103 | Ghana |
| | 218 | IITA |
| | 350 | NRCRI |
| | 41 | Togo |
| *D. cayenensis* | 38 | Benin |
| | 250 | Burkina Faso |
| | 289 | IDESSA |
| | 8 | Ghana |
| | 753 | FAST |
| *D. alata* | 27 | Benin |
| | 6 | Burkina Faso |
| | 90 | IDESSA |
| | 41 | Ghana |
| | 213 | IITA |
| | 30 | NRCRI |
| | 7 | Togo |
| | 324 | FAST |
| *D. praehensilis* | 10 | Bouaké |
| | 4 | Ghana |
| | 105 | FAST |
| *D. togoensis* | 5 | Bouaké |
| *D. mangenotiana* | 2 | Bouaké |
| | 22 | FAST |
| *D. minutiflora* | 7 | Bouaké |
| | 37 | FAST |
| Unspecified | 89 | Benin |
| | 4 | Burkina Faso |
| | 5 | Uganda |
| Wild | 74 | Benin |
| | 86 | IITA |
| *D. composita* | 2 | Republic of South Africa |
| *D. floribunda* | 2 | Republic of South Africa |
| *D. spiculifora* | 1 | Republic of South Africa |
| *D. cetinifolia* | 2 | Republic of South Africa |
| *D. burkilliana* | 12 | FAST |
| *D. preussii* | 2 | FAST |

Source: Based on Lawrence et al., 1986.

## DIVERSITY OF YAMS

### Morphology, physiology and anatomy

Genotypes of yam exhibit wide variation in their morphological and anatomical characteristics. While some clones of *D. rotundata* cv Ekpe may have between 3,000 and 4,000 leaves on a plant at full foliage (18 weeks after planting), some clones of *D. rotundata* cv Nwopoko may have as few as 200 leaves for an equivalent yield. While Nwopoko leaves are palmate, glossy and dark green in color, Ekpe leaves are septate, drab and light yellowish-green. It appears that leaf density has affected cultural practices or has developed in response to them. Thus, while Ekpe is grown in Nigeria, where staking to hold up the leaves is the common practice, Nwopoko is grown in areas where stakes are scarce and the practice non-existent; instead, the vines are encouraged to crawl around the large mounds.

Several shapes of tubers have been described (Okoli et al., 1984), ranging from rotund with a shape index (SI) of less than 1, to cylindrical with an SI greater than 3. This factor has implications for harvesting yams. While the short rounded tubers are easy to harvest and may even be suited to mechanical harvesting, the long tubers are more difficult to harvest and are prone to damage, leading to problems in storage.

Although yams have no pre-formed eyes, as in potato, and any healthy surface of the yam tuber can be stimulated to differentiate and grow into roots and shoots (Okoli et al., 1982), different clones respond in different ways to the minisett propagation technique. In a study at Umudike, Nigeria, cultivars of *D. alata* responded most readily to the technique, while cultivars of *D. dumetorum* least readily. This may suggest differences in the anatomy of the cultivars, differences which can be exploited for the improvement of the crop.

Yams are dioecious, male and female flowers being borne on different plants. Few clones, however, bear both male and female flowers. Whether this variation is an indication of varieties or whether male and female forms of the same variety exist has not been reported. There are, however, differences among clones in the period from planting to flowering. Okoli et al. (1984) showed variations of up to 70 days among 300 clones studied at Umudike. Based on these results, classification according to maturity regimes was proposed. Clones maturing within 200 days of planting were classified as early-maturing; between 240 and 270 days as medium-maturing; and longer than 270 days as late-maturing (*see* Table 2). Variations also exist in regularity and profuseness of flowering, especially in the clones, in fruit set and in viability of seeds formed in the capsules. Studies to elucidate the occurrence and pattern of these physiological phenomena will benefit breeding procedures for yams.

Yam growth has been described as a continuum (Okoli, 1980), yet tuber dormancy — a period of no vegetative growth — exists for most edible clones in cultivation. The dormancy period varies in the different clones, ranging from 25 to 50 days (Okoli, 1984), as shown in Table 2; knowledge of its duration is useful in planning the storage (Hahn et al., 1987) and planting date of particular clones, especially in areas with irregular rainfall patterns.

### Response to stresses

Yams also vary in their responses to diseases, pests, weed competition, drought and nutrient stresses. In a study to evaluate the tolerance of *D. alata* clones to anthracnose disease caused by *Colletotrichum* species, UM 680 and Ominelu clones were reported to be highly tolerant

**TABLE 2 Growth characteristics of cultivars of three *Dioscorea* species**

| Cultivar | Inflorescence | | Maturity | Tuber dormancy |
|---|---|---|---|---|
| | Type | Days to flowering | | |
| *D. rotundata* cv Nwopoko | N | - | medium | medium |
| *D. rotundata* cv Obiaoturugo | P | 110-120 (late) | late | medium |
| *D. rotundata* cv Abi | S | 50-70 (early) | early | short |
| *D. rotundata* cv Okwocha | S | 110-120 | medium | medium |
| *D. rotundata* cv Ukom | N | - | medium | long |
| *D. rotundata* cv Ekpe | S | 50-70 (early) | early | short |
| *D. alata* cv Ominelu | S | 90-120 (medium) | late | long |
| *D. alata* cv UM 680 | N | 90-120 (medium) | late | long |
| *D. dumetorum* cv Ona | P & S | 120-140 (late) | late | medium |

(NRCRI, 1981). These clones also maintained their tolerance at Ibadan — a forest savanna transition location in Nigeria. Similarly, Cercospora leaf spot, tuber rot and nematodes appear to affect clones differently.

Crickets, *Gryllidae* species, beetles and termites, as well as various rodents and birds (especially partridges), eat yam tubers in the field. It appears that partridges prefer Nwopoko and Obiaoturugo cultivars to Abi cultivars (Okafor, pers. comm.), which are all *D. rotundata* cultivars, and that they very seldom eat the Ominelu cultivar of *D. alata*. Unfortunately, this is also the order in which yam species are preferred by humans.

The Abi cultivar of *D. rotundata* is often planted on sandy acid soils in parts of south-east Nigeria. Other clones planted on such soils give uneconomic yields. This suggests that the Abi cultivar is more tolerant of acid, low-nutrient soils than other *D. rotundata* clones. This superior ecological adaptability can be exploited for yam improvement.

## Biochemical qualities

Variations exist in the protein content, amino-acid profile, phenol content, color, starch types and dry matter content of various clones of yams. These variations, in turn, affect the storability, cooking time, flavor, and eating and pounding qualities of the various clones (*see* Table 3 *overleaf*), and must be taken into account in production of yams for different purposes. Thus, for pounded yam flakes, yams of *D. rotundata* cv Ekpe appears best (Hahn, pers. comm.), while *D. alata* cv Ominelu is best for bread flour composites (Alozie, pers. comm.). Similarly, *D. rotundata* cv Pona, commonly grown in Ghana, is excellent for baking.

## Yield

Yield is the most important economic index of a crop. In the case of yam, yield depends to a large extent on the sizes (weights) of seed planted (Miege, 1957; Onwueme, 1972). Equivalent seed sizes give different yields in different clones of yams (Okoli et al., 1988b). The components of yam yields are the average numbers of tubers per stand and average tuber

**TABLE 3 Indices of quality in *Dioscorea* species**

| Cultivar | Shape index of tubers[1] | Storability[2] | Flesh color | Cooking time (min.)[3] | Eating quality[4] | Pounding quality[5] |
|---|---|---|---|---|---|---|
| *D. rotundata* cv Nwopoko | 1-2 | good | white | 6-7 | 1 | good |
| *D. rotundata* cv Obiaoturugo | 1-2 | fair | milk-white | 6-7 | 1 | good |
| *D. rotundata* cv Abi | 1, 2, 3 | good | yellow | 7-7 | 2 | fair |
| *D. rotundata* cv Okwocha | 1-2 | poor | white | 6-7 | 1 | good |
| *D. rotundata* cv Ukom | 1 | good | milk-white | 6-7 | 1 | good |
| *D. rotundata* cv Ekpe | 2-3 | good | white | 6-7 | 2 | good |
| *D. alata* cv Ominelu | 2-3 | good | brownish | 8-10 | 4 | poor |
| *D. alata* UM 680 | 1-2 | good | purplish-white | 8-10 | 3 | poor |
| *D. dumetorum* cv Ona | 1 | good | yellow | 30-3 | 2 | poor |

1 Shape index = length of tuber/girth of tuber = an index of eye appeal of tuber. 1-2 = good, less than 1 or 2-3 = fair; greater than 3 = poor.
2 Storability = measure of how long whole tuber can keep without deteriorating. Less than 90 days = poor; 91-190 days = fair; greater than 150 days = good.
3 Cooking time = time taken to cook a 1-cm slice taken from the middle of the tuber in boiling water.
4 Eating quality is a subjective score of how tasty a boiled piece of the yam is to a subject. 1 = friable and tasty; 4 = bland or even bitter.
5 Pounding quality is a measure of how well the dough draws after being pounded for two minutes, using a mechanical yam pounder. Good = dough draws; fair = dough holds together but does not draw; poor = dough does not hold together.

weight (a function of tuber volume and dry-matter content). Cultivars of yams vary in each of these components. Okoli (1988a) determined these components for nine clones from three species of yams (*see* Table 4). While *D. esculenta* clones have an average of 10.6 tubers/stand, *D. rotundata* cv Nwopoko has 1.27. One *D. dumetorum* cultivar has an average of 3.43 tubers per stand, although the tubers are clustered around the head. Table 4 shows dry-matter content of clones of yams. Dry-matter content influences the weight of a given volume of yam and hence the average tuber weight of any clone. Average tuber weight is dependent on the size of seed planted, and thus no general figures can be given. This information may be used in breeding manipulations for increased yield in desired clones of yams.

## PROSPECTS FOR YAM IMPROVEMENT

In an article on diversity in Bendel State of Nigeria, Nwabuikwu (1988) wrote: 'Heterogeneity, the fact of being different by whatever criterion, is often a spur, a road map to further

**TABLE 4 Yield characteristics of cultivars of three *Dioscorea* species**

| Cultivar | Yield (mt/ha) | Yield return | Harvest index (%) | DM (%) | No. tubers /plant |
|---|---|---|---|---|---|
| *D. rotundata* cv Nwopoko | 6,902.1 | 3.16 | 75.2 | 30-39 | 1.27 |
| *D. rotundata* cv Obiaoturugo | 6,946.8 | 3.16 | 77.8 | 30-35 | 1.12 |
| *D. rotundata* cv Abi | 6,553.9 | 3.02 | 65.1 | 28.35 | 1.25 |
| *D. rotundata* cv Okwuacha | 7,509.3 | 3.34 | 83.1 | 30-37 | 1.55 |
| *D. rotundata* cv Ukom | 7,920.0 | 2.42 | - | 30-38 | 1.16 |
| *D. rotundata* cv Ekpe | 5,277.0 | 2.40 | 75.6 | 32-39 | 1.70 |
| *D. alata* cv Ominelu | 13,464.9 | 6.18 | 41.5 | 26-32 | 2.06 |
| *D. alata* cv UM 680 | 12,661.3 | 5.81 | 76.0 | 27-32 | 1.38 |
| *D. dumetorum* cv Ona | 9,295.1 | 4.15 | 81.9 | 28-35 | 3.43 |

progress. Most of man's achievements have been forged on the anvil of diversity.' Prospects for yam improvement are, therefore, very good, as diversity exists in most characters where improvement is needed.

In the genetic improvement of a crop, the breeder attempts to manipulate several characteristics he/she needs in order to develop selection indices with weightings for the economic value and heritability of each trait and the genetic correlations between traits. To date, there is no information on the genetic control of traits in yams. For many species, the natural breeding system is not known. Hybridization occurs freely in nature in some edible yams, but attempts at hand pollination have not been successful. Also, some clones are not known to flower and until they can be induced to flower, and set viable seeds, their potential remains untapped.

In spite of these difficulties, standard breeding procedures are relevant to yams once the optimum clones and source are determined. These procedures include selection and progeny testing, seedling establishment, recurrent selection and clonal propagation of specific combinations. Because yams are vegetatively propagated, there is the advantage that desirable diversity, assembled at a high cost in hybrid varieties of seed-propagated crops, can be fixed without danger of loss, as vegetative propagation permits retention of superior and uniform materials in monoclonal plantings.

## Recorded improvements

Because of the difficulty of hybridization, together with that of obtaining sufficient planting materials for the evaluation of clones, little exists in the literature on improved varieties of yams. However, landraces have been selected and recommended for cultivation in Nigeria. These include the Nwopoko, Obiaoturugo, Abi, Ekpe cultivars of *D. rotundata* and the UM 680 cultivar of *D. alata*. Yield trials of progenies of *D. rotundata* and *D. alata* have reached an advanced stage in the field at IITA (Hahn, pers. comm.).

Since hybridization in yams occurs freely in nature, suggestions must be made for population improvements based on needs. Populations for earliness in maturity should be maintained for ecological zones with short rainy seasons and early season yam production,

as should populations for good eating/pounding quality combined with long dormancy and good storability in *D. rotundata*. Populations for high protein content and good flavor would appear necessary in *D. alata*, where there seems to be wide variation in these desirable characteristics (*see* Tables 5 and 6).

**TABLE 5 Values of crude protein and water content of Nigerian species of yams**

| *Diocorea* spp. | No. samples | Crude protein (%) | Average (%) | Water content (%) |
|---|---|---|---|---|
| *D. rotundata* | 19 | 3.2 - 13.9 | 8.18 | 40.5 - 71.0 |
| *D. alata* | 20 | 3.8 - 10.3 | 6.2 | 48.3 - 78.0 |
| *D. dumetorum* | 17 | 4.9 - 14.0 | 8.69 | 66.7 - 78.0 |
| *D. cayenensis* | 1 | 4.0 | 4.0 | - |
| *D. bulbifera* | 2 | 7.9 - 9.6 | - | - |
| *D. esculenta* | 22 | 5.5 - 5.9 | - | - |

Source: Based on Baquar and Oke, 1976.

**TABLE 6 Protein content of yams**

| *Dioscorea* spp. | No. clones | Countries of origin | Crude protein range | % dry weight mean |
|---|---|---|---|---|
| *D. alata* | 28 | India, Puerto Rico, Trinidad, Barbados, Philippines | 6.28 - 11.22 | 8.33 |
| *D. esculenta* | 6 | India, Puerto Rico, Trinidad | 7.84 - 13.41 | |
| *D. rotundata* | 1 | Côte d'Ivoire | 7.28 | 7.28 |
| *D. bulbifera* | 1 | India | 10.94 | 10.94 |
| *D. bulbifera* | 1 | Puerto Rico | 11.06 | 11.06 |
| *D. trifida* | 1 | Puerto Rico | 7.38 | 7.38 |

Source: Martin and Thompson, 1971.

## CONCLUSION

In the forests of the humid tropics, cultivated yams and their wild relatives grow in large numbers. This is especially so in the case of the *D. rotundata-cayenensis* complex and probably for *D. dumetorum*, all of which are believed to have their center of diversity in tropical West Africa.

Along with other yams that have been introduced, and their spontaneous variants, these yams face the threat of genetic erosion as the forests give way to highways, development projects and urban sprawl. Even where germplasm is held in field genebanks by institutions, an annual loss of over 10% has been reported. A call is therefore made for urgent and intensive collection of cultivated yams and their wild relatives throughout the world, but especially in Africa. These collections should be held in three to five regional centers where they may be

characterized and stored. There should also be intensive investigations on yam germplasm storage techniques. To further reduce loss, regional cooperation is needed in germplasm maintenance, whereby duplicate samples are held in three to five different centers in different forms — field genebanks, *in vitro* cultures and, for seed-producing clones, as seeds. National plant quarantine services, the African Phytosanitary Organization and international funding agencies should work together to facilitate the exchange of materials and to make these regional germplasm banks take effect without delay.

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# 3.2

# *New Trends for Yam Improvement in the* Dioscorea cayenensis-rotundata *Complex*

P. HAMON and B. TOURE

The genetic laboratory of the Faculté de Sciences et Techniques of the University of Abidjan, Côte d'Ivoire, has been involved in the national research program on yams since 1974. The first studies were on the behavior of some traditional varieties in two ecological conditions and a comparison of different types of vegetative propagation in *Dioscorea alata* cv Brazo Fuerte. Most of the national yam collection (*see* Table 1 *overleaf*) was collected by three missions in Côte d'Ivoire, supported by the International Board for Plant Genetic Resources (IBPGR) (Hamon and Ahoussou, 1988). Two main research priorities evolved: collection and evaluation of genetic resources for yam improvement; and *in vitro* culture. This paper presents the main results of the former; details on the latter are provided in Hamon (1987).

## CULTIVATED YAMS OF WEST AFRICA

The use of morphological descriptors enables us to describe 20 varietal groups (Hamon et al., 1986) (*see* Table 2 *overleaf*). Two of them are not represented in Côte d'Ivoire, but come from the Republic of Benin.

The results of clustering analysis using morphological characters are shown in Figure 1 (*overleaf*). The two main clusters differ according to the number of varietal groups that they contain. The main characteristics of the two clusters are:

| | |
|---|---|
| *Cluster 1* | 18 varietal groups; color of tuber flesh (white, cream, white and purplish, white, cream and purplish); one or two harvests; many branching; vegetative cycle 10 months or less |
| *Cluster 2* | 2 varietal groups; color of tuber flesh (yellow, pale yellow, purple, white); one harvest; minimal branching; vegetative cycle more than 10 months |

**TABLE 1 Total number of samples of yams collected in Côte d'Ivoire**

| Species | Total sample |
|---|---|
| **Cultivated species** | |
| *D. alata* | 324 |
| *D. bulbifera* | 16 |
| *D. cayenensis-rotundata* | 753 |
| *D. dumetorum* | 5 |
| *D. esculenta* | 1 |
| **Wild species** | |
| *D. bulbifera* | 21 |
| *D. burkilliana* | 12 |
| *D. dumetorum* | 6 |
| *D. hirtiflora* | 46 |
| *D. mangenotiana* | 22 |
| *D. minutiflora* | 37 |
| *D. praehensilis* | 105 |
| *D. preussii* | 2 |
| *D. abyssinica* (seeds) | 18 |
| Total | 1,368 |

**FIGURE 1 Clustering analysis using morphological characters with cultivated yams (showing number of varietal groups included in each sub-cluster)**

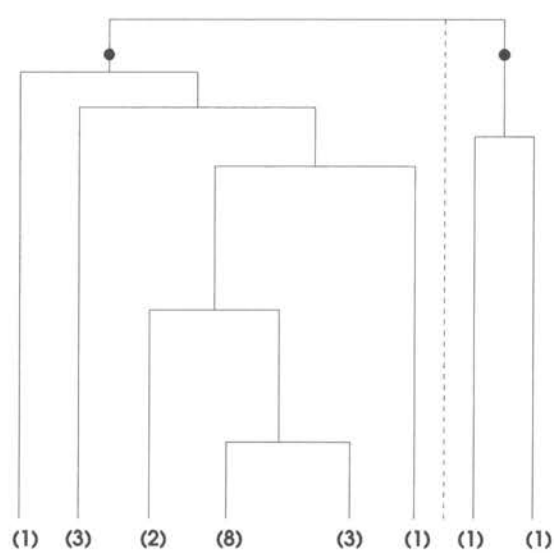


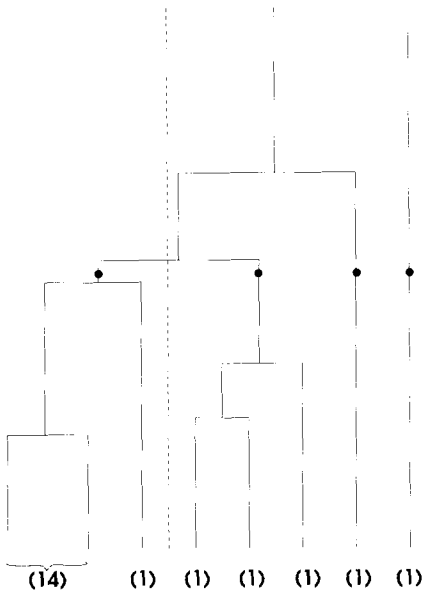
**TABLE 2** **Varietal groups of *Dioscorea cayenensis-rotunda* complex and some of their peculiarities**

| Name of varietal group | Variability status | Flowering sex | Ecological habitat |
|---|---|---|---|
| Afoubessou | monomorphe | female | forest |
| Baniakpa | monomorphe | male | |
| Cocoassie | polymorphe<br>3 tuber flesh color | male + female | forest |
| Frou | 2 leaves shape | female | boundary forest<br>- savanna |
| Gnan | monomorphe | male | forest - savanna |
| Kangba | 4 tubers shape<br>7 tuber flesh color | male + monoic | forest - savanna |
| Kpokpokpokpo | 2 leaves shape<br>3 tubers shape<br>2 tuber flesh color | | forest |
| Kponan | 4 leaves shape<br>3 tuber flesh color<br>3 tubers shape | male + female | savanna |
| Krandoupou | monomorphe | male + female | forest |
| Krengle | 5 tubers shape<br>2 tuber flesh color | female + male | forest - savanna |
| Kroukroupa | 2 tubers shape | male | savanna |
| Lokpa | polymorphe | female + male | forest - savanna |
| Nandokaka | monomorphe | male | savanna |
| Sammancou | monomorphe | male | forest |
| Sopere | polymorphe | female + male | forest - savanna |
| Soussou | monomorphe | male | |
| Vinvan | monomorphe | male | savanna |
| Waraga | 2 tubers shape | male | savanna |
| Yaobadou | monomorphe | male | forest - savanna |
| Zrezrou | monomorphe | male | forest |

Enzymatic polymorphism was studied using starch gel electrophoresis (Hamon and Touré, 1982). Five enzymatic systems were analyzed: four dehydrogenase (malate dehydrogenase [MDH], isocitrate dehydrogenase [ICD], 6-phosphogluconate dehydrogenase [PGD], shikimate dehydrogenase [SKDH]); and one isomerase (phosphoglucoisomerase [PGi]).

The combination of the type of pattern obtained for each enzymatic system and each accession give an 'enzymatic formula' (EF). On 454 accessions, 62 EF are noted. These EFs permit characterization of 12 varietal groups and, sometimes, the identification of morphological types (leaf or tuber types) within varietal groups. These enzymatic characters are interesting for two reasons: they are independent of environmental conditions; and they permit the identification of duplicates in this type of collection (in addition to morphological traits). This point is important to consider in the conservation of collections. The results of clustering analysis using enzymatic characters are shown in Figure 2 (*overleaf*).

**FIGURE 2 Clustering analysis using enzymatic characters with cultivated yams (showing number of varietal groups included in each sub-cluster)**




We have four major clusters, one of which includes 15 varietal groups. All 15 varietal groups are characterized by a vegetative cycle shorter than 10 months. Furthermore, this cluster differs from the other three in that it lacks slow migrating bands for ICD and PGD.

## WILD YAMS IN WEST AFRICA

The wild yams of West Africa can be divided into three physiological groups:

- the perennial species, in which plant and tuber are perennial (*D. burkilliana, D. minutiflora* and *D. smilacifolia*);
- the semi-perennial species, with annual plant but perennial tuber (only one species in West Africa, *D. mangenotiana*);
- the annual species, in which plant and tuber are annually renewed (most of wild species are of this type, for example *D. abyssinica, D. hirtiflora* and *D. praehensilis*)

We have studied six wild species: *D. burkilliana, D. minutiflora, D. mangenotiana, D. abyssinica, D. hirtiflora* and *D. praehensilis*. For all these species, enzymatic polymorphism was studied by starch gel electrophoresis. The enzymatic systems cited above for cultivated yams were analyzed.

The results indicate that for ICD and PGD some patterns show slow-migrating bands. According to the presence or absence of these slow-migrating bands, we can constitute three groups: species in which slow-migrating bands for ICD and PGD are always present; species in which slow-migrating bands for ICD are always present and for PGD are sometimes present; and species in which these slow-migrating bands for ICD and PGD are absent. If we compare the three physiological groups with the three enzymatic groups, we note that there is considerable overlapping between the groups (*see* Table 3).

Therefore, wild perennial species can be identified by the presence of slow-migrating bands for ICD and PGD. On the contrary, wild annual species can be identified by the exclusive presence of fast-migrating bands for ICD and PGD. An intermediate situation is observed for the semi-perennial species, *D. mangenotiana*.

**TABLE 3 Comparison between physiological and enzymatic subdivisions**

| | Perennial | Semi-perennial | Annual |
|---|---|---|---|
| Slow migrating bands for ICD and PGD | *D. burkilliana* | - | - |
| Slow migrating bands for ICD | - | *D. mangenotiana* | - |
| Fast migrating bands for ICD and PGD | - | - | *D. abyssinica, D. hirtiflora, D. praehensilis* |

## RELATIONSHIPS BETWEEN CULTIVATED AND WILD YAMS

Because selection has a significant effect on morphological characters during domestication, we have chosen enzymatic characters, which are neutral characters, for the study of the relationships between cultivated and wild yams.

The results of clustering analysis for cultivated and wild yams indicate that two major clusters exist. Both clusters include cultivated and wild yams. Annual wild species are associated with 15 varietal groups, while perennial and semi-perennial wild species are associated with five varietal groups. When we consider morphological characters and ecological criteria for the 15 varietal groups, we must assume that the species *D. abyssinica* and *D. praehensilis* are related to these cultivated yams. On the other hand, within the five varietal groups, the vernacular nomenclature and morphological traits indicate that one or two are closely related to *D. mangenotiana* (Hamon, 1987). The other three appear to be hybrids between perennial and annual wild species, because of enzymatic and morphologic characters. One of them seems more closely related to *D. burkilliana* than *D. minutiflora* because of the tuber morphology and the color of tuber flesh.

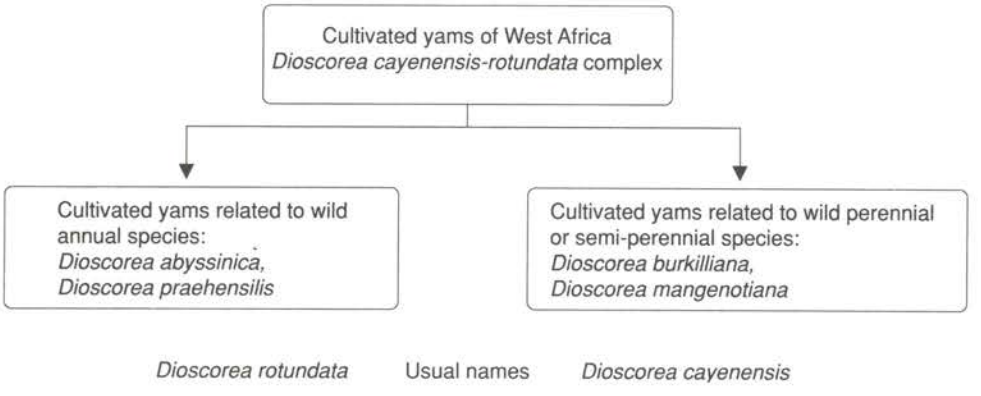
It seems, therefore, that at least four wild species could be related to cultivated yams originating in West Africa.

## CONCLUSION

West African cultivated yams constitute a complex in which morphological types can be well defined. Only traditional varieties exist on the farms, and because a morphological variability

is observed, we call them varietal groups. Wild perennial, semi-perennial and annual species are related to cultivated yams, as shown in Figure 3.

**FIGURE 3 Genetic organization of West African cultivated yams**




We can consider that *D. cayenensis, D. rotundata* or *D. cayenensis-rotundata* have the status of cultivated species if, and only if, we can achieve hybridization and produce viable seeds within each cluster or between the two clusters. On the contrary, if reproductive barriers exist, these specific names indicate whether the cultivated yams are related to wild annual species or to wild perennial and semi-perennial species.

A better knowledge of the structure of the cultivated complex is important for yam improvement. Indeed, if we look at our collection, we note that four of the five varietal groups related to wild perennial species bear flowers, and especially male flowers. One of them includes one monoic clone. Of the 15 groups related to wild annual species, six include male and female clones, one bears only female flowers, and eight only male flowers.

Thus, two types of hybridizations must be considered. One involves hybridizations between cultivated yams. In this case, we must try crosses between male and female clones of the same varietal group, but also between varietal groups of the same cluster and, finally, between the two clusters. The other type of hybridization — between cultivated yams and their wild relatives — is, in our opinion, is very important, but has been neglected.

Wild species of many crops are commonly used for crop improvement. They must also be used for yam improvement. There is, however, another good reason to use wild species — that the progeny of hybridizations between wild and cultivated species may include clones bearing both male and female flowers. This progeny can be used subsequently in backcrosses with the cultivated parental clone or in crosses with other cultivated clones.

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# 3.3

# *Cassava Germplasm Strategies for Africa*

A. C. ALLEM and S. K. HAHN

Cassava, *Manihot esculenta* Crantz (Euphorbiaceae), is indigenous to South America and is a major staple of 500 to 800 million low-income families in the neotropics and paleotropics.

Conservative estimates date the domestication of cassava at about 8000 BC. Over the past 40 years, many botanical and anthropological studies have tried to find the geographical and botanical origins of the crop, but little progress has been made beyond speculation. It is now known that cassava does grow wild over parts of the American neotropics. An initial account of the geography of wild cassava populations in South America is provided in Allem (1987). A comprehensive review of the geography, ancestry and updated taxonomy of cassava is provided in Allem (1990).

Tropical Africa has been losing natural resources dramatically as the population continues to grow. The early depletion of forests and savannas for fuelwood, logging and shifting cultivation (Aubréville, 1947), a trend which has since intensified (FAO, 1981, 1982; Myers, 1982), makes cassava conspicuous in subsistence agriculture (Hahn and Keyser, 1985; Hahn et al., 1987) as the staple of much of tropical Africa in the foreseeable future (for example, Nigeria, Zaire and the Central African Republic rely so heavily on this single crop to feed their rural populations that any abrupt fall in productivity might lead to social upheaval). This situation warrants a search for and selection of germplasm less dependent on high inputs for sustained productivity.

Cassava can be grown anywhere. The results of regional performance trials indicated that the crop has basic requirements, as other crops, for normal growth, and showed genotype x environment interaction. Adaptation is thus a determinant feature of the relationship between plants and abiotic and biotic stresses.

It has long been believed that genetic information passes without change through vegetative planting material. However, a question has been raised by the recent International Board for Plant Genetic Resources (IBPGR)/Centro Internacional de Agricultura Tropical (CIAT) pilot project (1987-89) as a result of electrophoresis — namely, whether the genetic stability of a genotype can hold when moved from *in vitro* conditions back to field conditions (Chavez et al., 1987).

In the context of the agroclimatic conditions of tropical Africa, there are some core areas where cassava may prosper, such as the densely populated wet coastal belt, the region with moist, closed broad-leaved forests, the savanna expanses and the sub-Sahelian areas. The types of the germplasm needed for each of these ecological zones are distinct. They are outlined briefly here in terms of what the main supplier (South America) has to offer.

## GENETIC EROSION

Those involved in the collection of plant genetic resources have become increasingly familiar over the past two decades with genetic erosion. In the specific problem faced by agriculture, the phenomenon appears in the discontinuance of local primitive cultivars ('indigenous landraces') in favor of a few improved commercial lineages of superior productivity. Such dynamic changes result in a gradual decrease of the genetic basis of crops. The gradual replacement of the primitive landraces of a crop by its improved commercial forms is already a reality for a number of crops, particularly grains, and a similar trend is emerging for some root and tuber crops. The implementation of such a policy is regarded as sound, given the population growth rates, but it is worth recalling that in its wake follows a local reduction of the genetic variability of the crop.

Specific data about the extent of genetic erosion threatening cassava worldwide do not exist. Informal reports from the peasants of north-east Brazil show that, in general, the adoption of any new variety always depends on productivity as the decisive factor (Allem, 1985). The same situation has been observed in Nigeria. The authors believe, however, that the amount of genetic erosion affecting cassava on a regional basis is minimal, especially in comparison with grains. This assumption is based on the evidence that farmers continuously maintain their old cultivars for special use and taste, and take the varieties with them when they move to distant places. In addition, 'tradition drift' is particularly effective in the adoption and spread of cassava as a staple (Albuquerque, 1969; Harris 1971; Moran 1975).

The importance of cassava for aboriginal people stems from the ecological factors favoring its establishment and cultural traits. South American Indians, for example, have shown marked interest in certain vegetative characters, and select varieties according to prevailing cultural conditioning (Boster, 1984, 1985; Chernela, 1986). Productivity is not the most important character of the root crop for such autochthonous groups. This set of social relations emphasizes the overall properties of the species and, as such, indirectly militates against the progression of genetic erosion.

A second factor fostering variation in cassava in both South America and tropical Africa is that small farmers allow seedlings from open-pollinated seeds to establish as adults in their fields and, if a plant shows desired traits, the farmers preserve, select and begin cultivating it. A third factor is that most commercial firms concentrate their research on grains, pulses, forage crops and other cash crops, whereas cassava, like many other subsistence crops, has had little commercial attention. Thus, existing local cultivars may change more slowly.

Finally, one must consider the precautionary measures taken by both Amerindians and Africans. Before a new variety is allowed to displace old time-tested types, it must prove its higher output. On both continents, farmers commonly plant mixtures of distinct varieties, with one or two generally occupying the greater part of the planted area. This practice illustrates the prudence of the peasantry, associating successful resistance against pests, diseases and sudden environmental changes with genetic variability.

It is known that *Solanum tuberosum* subsp. *andigena*, traditionally grown throughout the Andean zone, is being gradually replaced by improved commercial varieties (Ochoa, 1975, 1984). But potato can hardly be called a subsistence crop. The complete replacement of primitive varieties of a crop species in favor of improved alienigenous strains in centers of genetic diversity of the crop — a phenomenon termed 'genetic wipe-out' (Harlan 1972) — does not seem to apply to cassava. There is no tangible evidence that cassava is undergoing serious genetic erosion in its centers of origin — the American neotropics — although Gulick et al. (1983) have expressed some concern on the subject. Clearly, a sociological survey is needed.

## CASSAVA GERMPLASM COLLECTIONS

Major American, African and Asian collections of germplasm of cassava have been updated during the 1980s (*see* Table 1 *below*; Tables 2 and 3 *overleaf*). A detailed list of Brazil's major collections is given in Table 4 (*overleaf*). It is fairly safe to assume that the numbers of

**TABLE 1 Major American collections of cassava (only inventories over 100 accessions)**

| Institute | Country | Germplasm |
|---|---|---|
| Centro Internacional de Agricultura Tropical (CIAT) | Colombia | 4,250 cvs [1]<br>3,080 cvs [2] |
| Centro Agronomico Tropical de Investigacion y Ensenanza (CATIE) | Costa Rica | 251 cvs |
| Estacion de Santo Domingo | Cuba | 251 cvs |
| Facultad deAgronomia | Guatemala | 199 cvs |
| Instituto Nacional de Investigaciones Agricolas (INIA) | Mexico | 157 cvs |
| Instituto Agropecuario Nacional (IAN) | Paraguay | 145 cvs |
| Instituto Nacional de Investigacion y Pesquisa Agricola (INIPA) | | |
| Iquitos | Peru | 134 cvs |
| Lima | Peru | 294 cvs |
| Tarapoto | Peru | 214 cvs |
| Universidad Nacional Pedro Ruiz Gallo (UNPRG) | Peru | 167 cvs |
| Universidad Central de Venezuela (UCV) | Venezuela | 212 cvs |

1 living collection.
2 *in vitro* true seeds.

Source: IBPGR, 1986.

**TABLE 2 Major African collections of cassava (only inventories over 100 accessions)**

| Institute | Country | Germplasm |
|---|---|---|
| Station Agronomique de Loudima | Congo | 121 cvs |
| Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) | Côte d'Ivoire | 105 cvs |
| Makoka Agricultural Research Station | Malawi | 291 cvs |
| International Institute of Tropical Agriculture (IITA) | Nigeria | 2,000 cvs[1] |
| National Root Crops Research Institute (NRCRI) | Nigeria | 777 cvs |
| Serere Agricultural Research Station | Uganda | 100 cvs |
| Institut National d'Etudes et de Recherches Agricoles (INERA) | Zaire | 250 cvs |

1 living collection.
2 *in vitro* true seeds.

Source: IBPGR, 1986

Some new data emerged during this workshop. Malawi's representative mentioned that out of an original collection of 510 cvs, it was now down to 260. Congo's representative stated that, because of collections made as from 1985, its collection now consisted of 1,250 accessions.

**TABLE 3 Major Asian collections of cassava (only inventories over 100 accessions)**

| Institute | Country | Germplasm |
|---|---|---|
| Central Tuber Crops Research Institute (CTRI) | India | 1,327 cvs |
| Sukarami Agricultural Research Institute for Food Crops (SARIF) | Indonesia | 954 cvs |
| Philippine Root Crop Research and Training Centre (PRCRTC) | Philippines | 117 cvs |
| Central Agricultural Research Institute (CARI) | Sri Lanka | 107 cvs |
| Department of Agriculture | Thailand | 272 cvs |
| Kasetsart University | Thailand | 100 cvs |

Source: IBPGR, 1986.

**TABLE 4 Brazil's major collections of cassava germplasm (as of May 1987)**

| Institute | Municipality and State | Details of Germplasm |
|---|---|---|
| UEPAE manaus | Manaus, Amazonas | Clones; 129 accessions |
| UEPAE Belém | Belém Pará | Clones; 160 accessions |
| UEPAE Boa Vista | Boa Vista, Roraima | Clones; 70 accessions |
| UEPAE Teresina | Teresina, Piauí | Clones; 57 accessions |
| CPA Cerrados | Brasília, Distrito Federal | Clones; 159 accessions[1] |
| CNP Mandioca e Fruticultura | Cruz das Almas, Bahia | Seedlings; 924 accessions |
| CPATSA | Petrolina, Pernambuco | Clones; 61 accessions |
| CENARGEN | Brasília, Distrito Federal | 650 accessions[1]<br>*Manihot silvestre*[2]: 200 accessions/20 species |
| IPAGRO | Taquarí, Rio Grande do Sul | Clones; 239 accessions |
| IAC | Campinas, Sao Paulo | Clones; 311 accessions |
| IPA | Serra Talhada, Pernambuco | Clones; 140 accessions |
| IAPAR | Londrina, Paraná | Clones; 453 accessions |
| PESAGRO | Rio de Janeiro, Rio de Janeiro | Clones; 75 accessions |
| EMEPA | João Pessoa, Paraíba | Clones; 27 accessions |
| EMAPA | São Luis, Maranhão | Clones; 121 accessions |
| EPACE | Fortaleza, Ceará | Clones; 17 accessions |
| EPEAL | Maceiló, Alagoas | Clones; 125 accessions |
| EPABA | Salvador, Bahia | Clones; 57 accessions |
| EMCAPA | Linhares, Espírito Santo | Clones; 130 accessions |
| EMPAER | Campo Grande, Mato Grosso do Sul | Clones; 28 accessions |
| EMGOPA | Goiania, Goias | Clones; 64 accessions |
| EMPARN | Natal, Rio Grande do Norte | Clones; 16 accessions |

1 *in vitro* true seeds.

2 living collections.

Source: Allem, A., and Goedert, C. Management of Brazil's genetic resources of cassava. (unpubl.)

accessions in these collections do not represent the number of distinct varieties; the inventory is considerably smaller if repetition is taken into account. It is common to give the same genetic stock different popular names (Rogers and Appan, 1970). During an expedition to north-east Brazil, for example, one variety was found to be called by up to six different names as the team moved from one municipality into another (*see* Table 5 *overleaf*). The same holds true in Africa. For example, the improved clone TMS 30572, developed by the International Institute of Tropical Agriculture (IITA), was released by the Government of Nigeria throughout the country, and available information indicates that it already has several different popular names; in one place it is called 'IITA's cassava', in another it is known by the names of the farmers or villages who shared the sticks with their neighbors.

As a rough rule of thumb, the larger the sample, the more difficult it is to distinguish individuals from each other. Ciferri (1938) managed to identify only four major groups of cultivars within the crop, while Rogers and Fleming (1973), who carried out far wider sampling on a regional basis, recognized 19 distinct groups of cultivars. Inevitably, there is always overlapping and superimposition of models in such a study, but the chances of this

**TABLE 5 Local names for varieties of cassava in north-east Brazil and their synonyms**

| Collector's No. | Variety | Synonym |
|---|---|---|
| Allem 3141 | I água-morna (*but see* III) | morna-água (Allem 3118); água-fria |
| 3135 | II milagrosa | praiana (Allem 3147) |
| 3162 | III maniva-do-céu | água-morna; pão-de-chile; roça-do-céu; franco-rabelo |
| 3172 | IV boinha-branca | boinha-rasteira |
| 3196 | V manivainha (*but see* VII) | cambadinha |
| 3201 | VI rosa (*but see* IX, X *and* XV) | pé de-pombo; rosinha (Allem 3283); cravo (Allem 3296) P; rosada (Allem 3205) P; pau-ferro (Allem 3185); macaxeira-rosa |
| 3206 | VII cambadinha (*but see* XI) | cabocla-branca |
| 3216 | VIII macaxeira-preta | bahia; bahia-preta (Allem 3290) P; preta (Allem 3322) P; pretinha (Allem 3372) |
| 3227 | IX canelinha (*but see* X) | macaxeira-rosa |
| 3245 | X macaxeira-rosa | boinha (Allem 3264); rosinha (Allem 3292) |
| 3256 | XI landi | cabocla-branca P; cambadinha P |
| 3262 | XII oiovérde (but see XIV) | verdinha (Allem 3262) P |
| 3269 | XII cedinha | macaxeira-pão; estrangeira (Allem 3282) |
| 3273 | XIV nove-folhas | oioverde (Allem 3262); verdinha (Allem 3284) |
| 3274 | XV pé-de-pombo | num-tem-roxa |
| 3286 | XVI passarinha | enrica-homem (Allem 3288) |
| 3328 | XVII oiroxo | mela-porco; also tapicuru or oipreto (municipality of Caém, Bahia) |
| 3349 | XVIII purnunça | periquito (Allem 3360) |
| 3355 | XIX cidade | euclides-da-cunha (Allem 3356) |

are reduced when samples are smaller (for example, local collections), where extreme morphs are less likely to meet their peers.

## GENETIC RESOURCES OF WILD SPECIES OF *MANIHOT*

Despite its economic importance, *Manihot* still remains a poorly known genus. Although the study by Rogers and Appan (1973) has led to a better understanding of the taxonomy of the genus, a conservative estimate points at a persistant level of inflation in the order of 30% for the genus as a whole.

The use of wild species in genetic improvement programs may help improve this situation. Africa and Asia have a long-established tradition of regular use of related species in a number of interspecific crosses. Such programs started in the 1930s in Dutch-occupied Java (Indonesia) and Tanganyika (now Tanzania) (Nichols 1947; Magoon et al., 1970; Hahn et al.,

1973, 1979, 1987; Hahn, 1984). Hybrids between *M. esculenta* and *M. glaziovii* showing resistance to cassava mosaic virus (CMV) were first obtained in Tanzania (Nichols, 1947; Jennings, 1957; Hahn et al., 1973, 1980). *M. glaziovii* seems to have been introduced into Africa in the early 1930s, where it is known as 'sierra rubber' or 'rubber tree', and was initially used as a source of rubber (for example, in Nigeria) and later as a shade tree in cocoa plantations. A fair amount of introgression from *M. glaziovii* into cassava seems to be occurring under natural conditions. The evidence for this is the existence of a number of intermediate forms which are spreading naturally over parts of Africa; they are particularly noticeable in the forests of Nigeria. People call such natural hybrids 'tree-cassava'. Jones (1959) was probably the first to draw attention to natural hybridization taking place between these two species in Africa. Tree cassava is often used as a leafy vegetable, a shade tree or a fencing tree in Africa.

Since the 1950s, there has been a renewed interest in increasing the crude protein content of the roots of *M. esculenta*. To that end, crosses were carried out between cassava and *M. melanobasis* (= *M. saxicola*), a native of Suriname known for its higher crude protein content (Bolhuis, 1953, 1967; Jennings, 1959, 1963). It is now known that *M. melanobasis* is cassava in the wild state (Allem, 1990). The African germplasm of *M. melanobasis* seems to have descended from an original collection made by Lanjouw (1939) at the Surinamese sierra of Voltzberg. The $F_1$ hybrid generation showed an increase in the protein content of the roots, but subsequent clonal generations did not retain the trait. Such results tended to confirm cassava as a caloric food crop.

Wild species should be tested primarily for resistance to pests and diseases and for low cyanide and special starch quality characters, since it is unlikely that any one of them may significantly contribute to the increase in productivity which has already been attained with *M. esculenta* (Hahn et al., 1973). Wild stocks of *M. flabellifolia* and *M. peruviana* (both conspecific with cassava) may reveal untapped genes, particularly the latter, which is pubescent at all levels. It is known that hairy material makes oviposition difficult, for the adult insect is prevented from reaching the stem and leaf blade because of the hairs. The character is important in combatting such pests as the cassava mealybug (CMB) and green spider mite (CGM).

Current stocks of wild species of cassava are low. The Centro Internacional de Agricultura Tropical (CIAT) in Colombia stores some 1,000 seeds belonging to a dozen species. IITA preserves over 30 species. CENARGEN in Brazil stores the seeds of some 15 species. The amount of genetic variability, as measured by the size and range of the samples, is quite restricted. In addition, CENARGEN holds a small field genebank in Brasilia, from which small quantities of open-pollinated seeds are distributed to interested institutes (*see* Table 6 *overleaf*).

Roots of an edible wild species are occasionally consumed in north-east Brazil in times of famine. There is confusion as to whether related species are edible or not. Some people emphatically dismiss 'maniçoba' (*M. caerulescens*) as inedible. *M. glaziovii*, known as 'borracha' (rubber), is certainly not edible. It seems that *M. epruinosa* is the most commonly used species, but it appears that distinct races of it are serving distinctly specific purposes. In the municipality of Wenceslau Guimarães in the State of Bahia, for example, one race, known as 'mandioca-sete-anos' (seven-year cassava), takes seven years before roots of up to 2 m long and 20-30 cm wide are harvested. Similar races (that is, low trees) are reported to be used as shade trees in southern Bahia, as well as near the coast where the name 'mandioca-sete-anos' is also used.

**TABLE 6 Living collection of *Manihot* held at CENARGEN (as of April, 1987)**

| Species | Growth form | Remarks |
|---|---|---|
| *M. alutacea* | shrub | |
| *M. anomala* | shrub | pubescent species |
| *M. caerulescens* | shrub to tree | pubescent species |
| *M. carthaginensis* | shrub to tree | |
| *M. cecropiaefolia* | shrub | |
| *M. epruinosa* | shrub to tree | |
| *M. flabellifolia* | shrub | cassava's conspecific form |
| *M. peruviana* | shrub | cassava's conspecific form |
| *M. grahamii* | shrub | |
| *M. hilariana* | grass | |
| *M. irwinii* | shrub | |
| *M. michaelis* | tree | Mexican species |
| *M. mossamedensis* | shrub | pubescent species |
| *M. orbicularis* | grass | |
| *M. pentaphylla* | shrub | |
| *M. pilosa* | shrub to tree | pubescent species |
| *M. quinquepartita* | shrub | |
| *M. sagittato-partita* | grass | |
| *M. tripartita* | shrub | pubescent species |
| *M. websterae* | shrub | Mexican species |
| *Manihot* spp. | shrub | native to Bahia State; new to science; compound leaves |

## AFRICA'S EXOTIC GERMPLASM NEEDS

The authors believe that any strategy of assembling germplasm, either through introduction or collection, should take into consideration the specific local needs of recipient countries — that is, to match as far as possible these needs with genotypes which respond satisfactorily to the local situations. Adaptation should be the key factor in any planning for the introduction and utilization of new germplasm.

Tropical Africa's needs for the exotic germplasm of cassava may be met to some extent from the South American continent, where there are similar physical environments and ecozones. We emphasize the importance of South American conditions because the African and Asian stocks originated from that continent. Although some divergence will have taken place in Asian and African stocks over the past three centuries, it seems clear from our work, as well as from earlier studies (Hahn et al., 1973, 1979; Patino and Hershey, 1981; Hershey, 1987), that South America is the major source of variation of cassava. Our comparative approach of ecogeographical conditions of Africa and America is based on the four main types of agroclimatic ecological zones which are suited to the cultivation of cassava in the paleotropics: Africa's Atlantic coastal belt; its tropical rain forests; its extensive savannas; and the arid zones. But, as Figures 1, 2, 3 and 4 show, Africa's true agroecology is more complex.

**FIGURE 1 Agroecological zones of West and Central Africa**

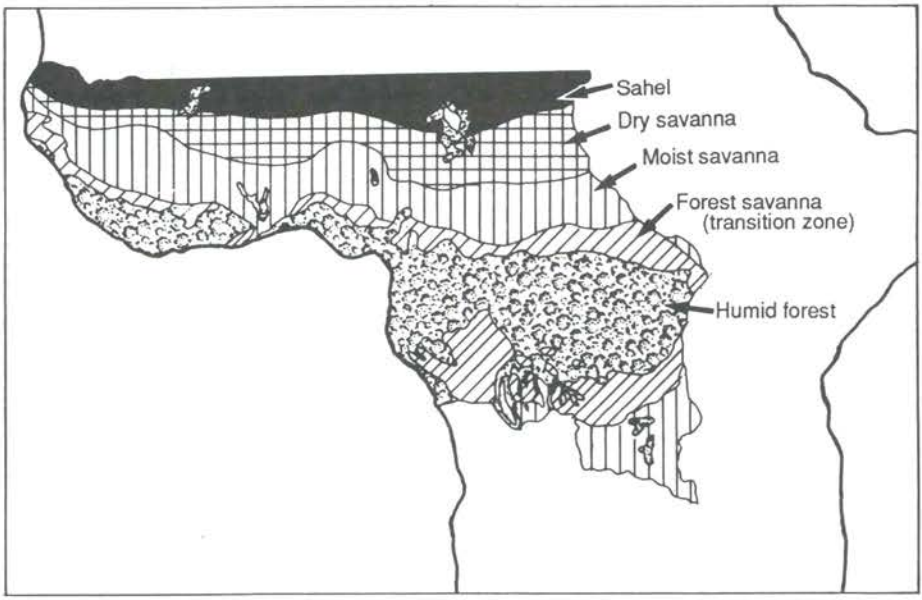
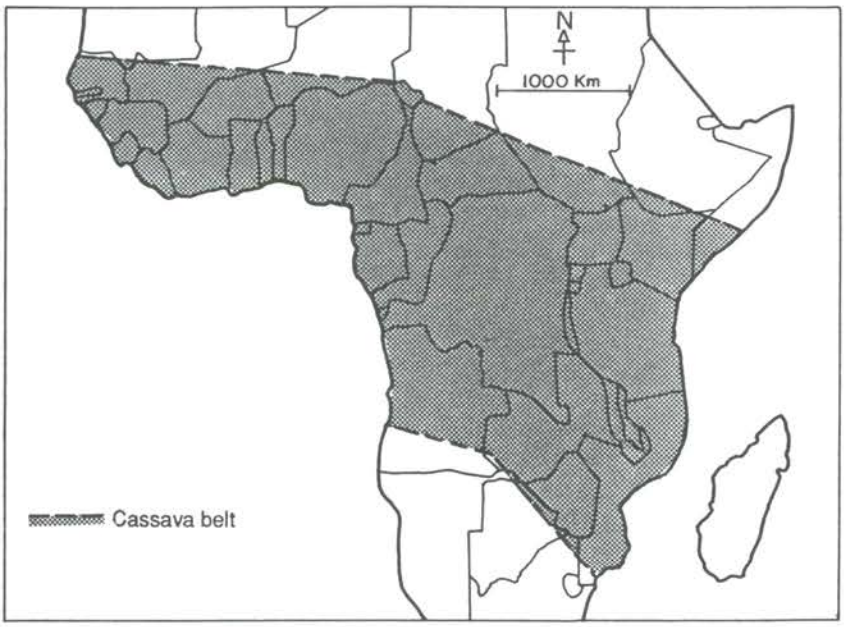


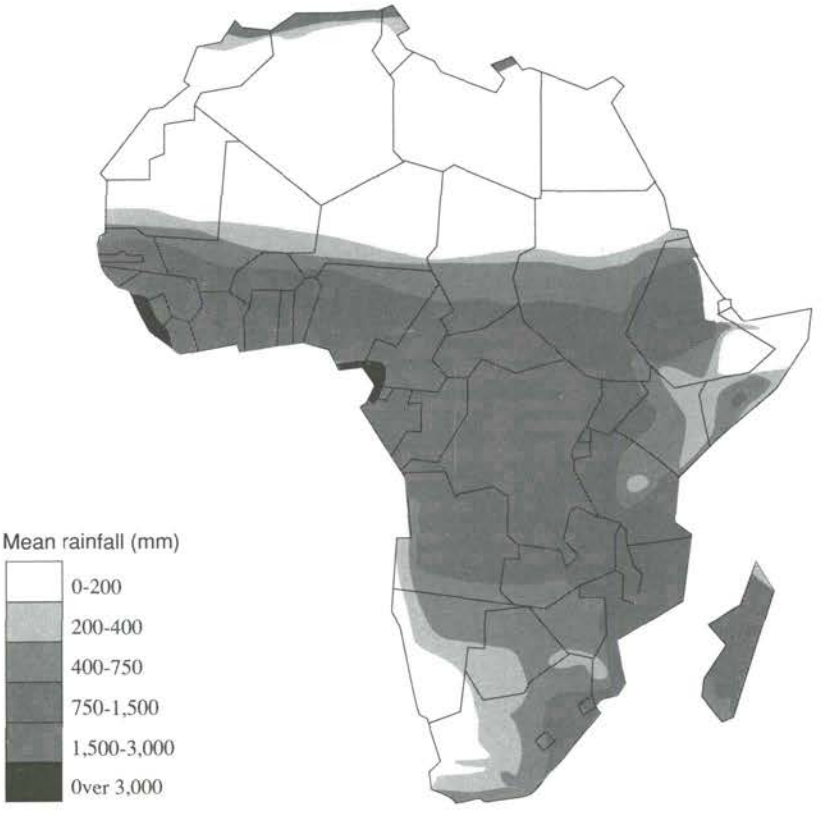

**FIGURE 2 The cassava belt in Africa**




Note how the cassava belt distribution roughly matches the agroecological zones shown in Figure 1.

Source: IITA, 1988.

**FIGURE 3 The annual rainfall distribution in Africa**




Source: Based on Harrison, 1987.

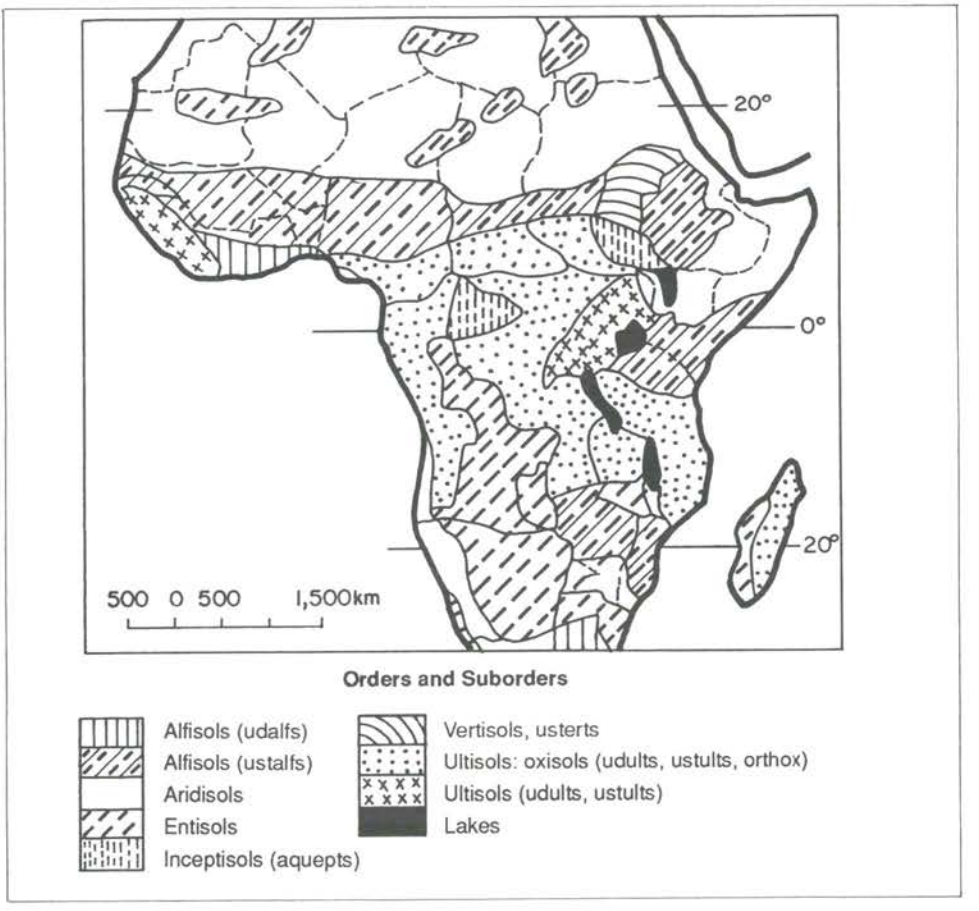
Each of the agroecological zones will be examined in relation to similar zones in South America, with particular emphasis on Brazil, which alone can meet most African needs for new trials, thanks to the diversity of its edaphic-climatic environments (*see* Figures 5 and 6).

## The coastal belt

The tropical coastal belt of South America houses a fair number of varieties long adapted to such strict ecological requirements. The South Pacific coast includes Peru, Ecuador and Colombia as potential donors. The South Atlantic coast ranges from Venezuela to Guyana, Suriname, French Guyana, and south to encompass Brazil, which alone has over 3,000 km of tropical coastline; cassava is found along most of it, in the backyards of local dwellers.

In a number of South American countries, within a range of 20-30 km from the coast there are extensive belts of wet tropical forests in which cassava is cultivated. The climate of both physiographic regions, the seashore and the coastal forest, is humid with heavy rainfall concentrated between May and August (autumn and winter), but distinct types of soils have

**FIGURE 4 Soil types in tropical Africa**




Source: R. Lal, 1987.

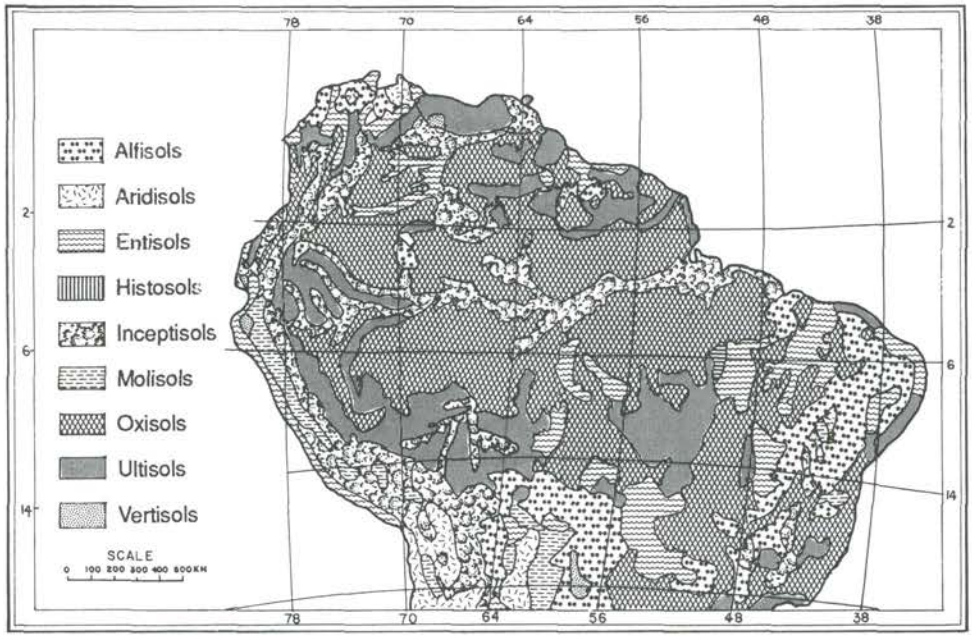
given rise to distinct types of vegetation. Brazil's Atlantic coastal forest is a fine example of this, stretching from the state of Pernambuco, in the far north, to the state of Santa Catarina, in the far south. This exuberant forest has virtually been wiped out (less than 5% of the virgin forest remains). In the north-east states such as Pernambuco, Alagoas and Sergipe this belt is called 'zona-da-mata' (forest zone), while the name given to the more arid zone is 'agreste'.

The agroecosystems of both physiographic regions are distinct, and cassava germplasm of such areas might prove to be of interest for field trials in similar environments of the African north Atlantic coast (for example, Guinea, Côte d'Ivoire, Ghana, Nigeria and Cameroon, all of which have an extensive Atlantic coastline).

### The tropical rainforest zone

South America contains more than one-third of the remaining tropical wet forests on in the world. Cassava is intensively cultivated by both Indians and white settlers in Bolivia, Peru,

**FIGURE 5 Soil types in northern South America**




Note that oxisols, ultisols and alfisols predominate in much of tropical Brazil

Source: Based on Sanchez and Isbell, 1979.

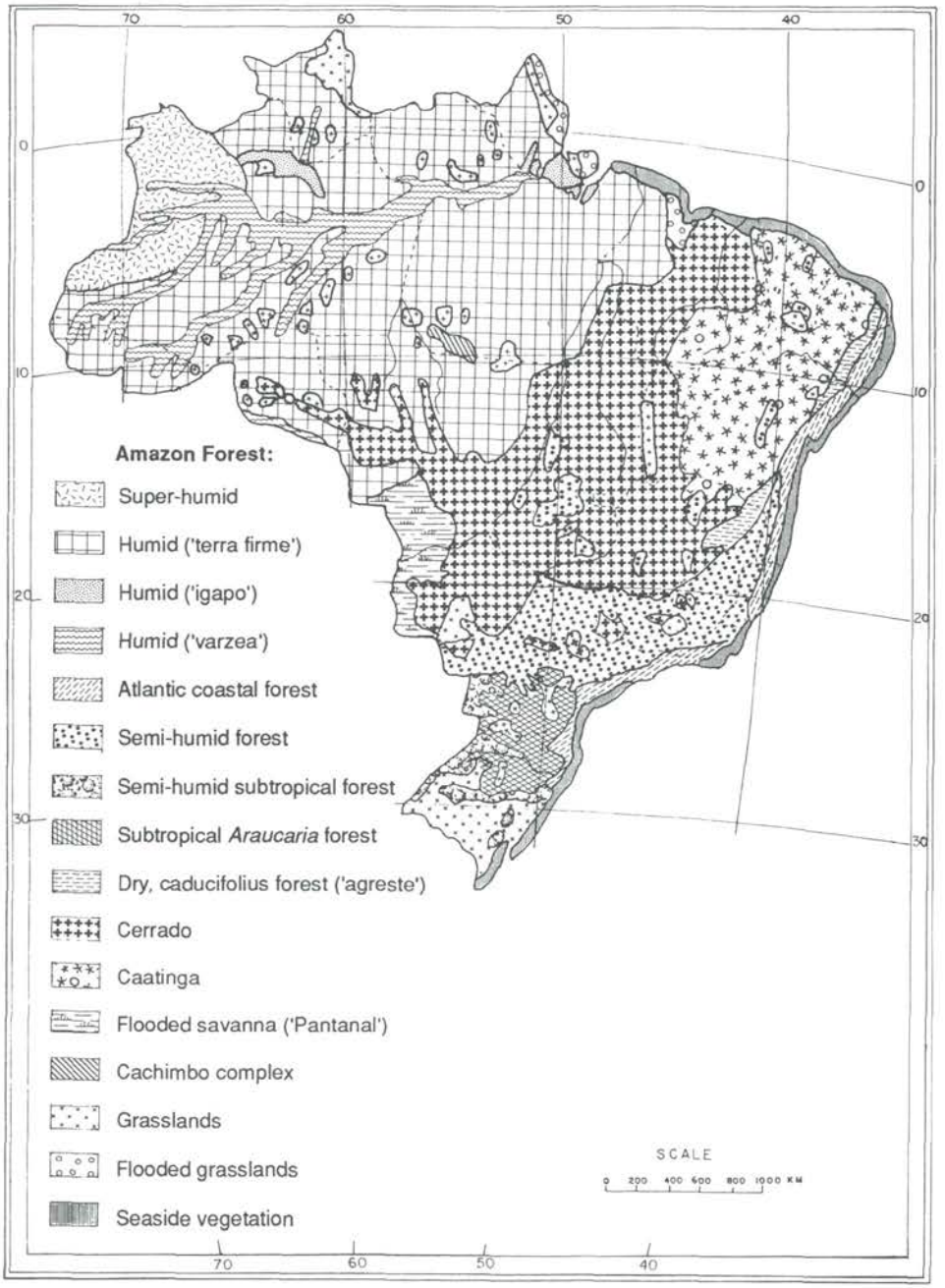
Colombia, Venezuela and the Brazilian Amazon. The ecology of the region parallels that of closed forests of West and Central Africa.

Of particular interest to African breeders may be the varieties confined mainly to Indian lands. High-cyanide yellow-flesh root varieties of cassava are particularly appreciated by Indians of the Amazon. These varieties are less common in the urban centers of the Amazon, whose populations prefer white-flesh cassava. It is worth recording that high-cyanide cassava provides the bulk of the cassava processed in the Amazon region, being adapted to conditions of low fertility and high acidity (oxisols and ultisols).

Preferences vary from area to area. The people of Central America, western South America and most of southern Brazil show a marked preference for low-cyanide varieties of cassava. By contrast, 90% of the plantations in the Amazon and north-east Brazil are composed of high-cyanide cultivars ('mandioca'). The distribution of cassava in Africa follows a similar cultural pattern. In West and Central Africa, high-cyanide varieties of cassava prevail. The roots have to be processed (fermented) to reduce the HCN content before consumption. In East Africa low-cyanide varieties prevail.

The importance of varieties of yellow-flesh cassava in northern Amazonia also stems from the fact that a number of regional dishes depend on a juice extracted from the roots of yellow cultivars; the juice is called 'tucupi' and is used in much favored dishes, such as 'pato-no-tucupi' and 'tacacá'. In Africa, yellow-flesh root cultivars are also valued. IITA has produced a few cultivars with yellow flesh roots. They are high in carotene content and thus improve

**FIGURE 6 Vegetation types in Brazil**




Note that the Amazon forest and the Cerrado (found mainly on oxisols, *see* Figure 5) and the Caatinga (found mainly on alfisols, *see* Figure 5) make up two-thirds of the country's vegetation. Cassava grows as far south as 32°S.

Source: Adapted from *Encyclopedia Mirador*.

the condition of people suffering from night blindness, which is caused by vitamin A deficiency and is common in the Sahelian Sudan zone. In Nigeria, palm oil is added during the processing of white-flesh cassava tubers into 'gari', making the product yellowish and at the same time increasing its carotene content and market value. In Ghana, plantain is added to the regional dish 'fufu' to improve palatability; when the cooked cassava tubers are pounded, the mixture becomes yellowish because of the plantain.

The 'mandiocabas' (so called because of their unusually high water content, about 90%) are very low-cyanide varieties found only in theAmazon. They were first domesticated by Indians, and from their voluminous roots, which are poor in starch content, a porridge called 'maniquera' is made (Albuquerque, 1969; Albuquerque and Cardoso, 1980). Unlike other varieties of cassava, successive agamic multiplications do not alter the starch content in the roots of mandiocabas, which are genetically predisposed for a high water content.

Another genetic resource of interest to Africa is the group of sweet riverine cassava varieties cultivated along the river banks and estuaries of the Amazon. These varieties are popular because of their earliness. Fertile tilled plains are reported to yield above 10 t/ha within 6 months (Albuquerque and Cardoso, 1980) whereas the usual biological cycle takes 10–24 months; the same authors also report that varieties planted during the dry spell in the region (mainly from late May to late October), along estuaries of the upper Amazon, yield up to 35 t/ha when harvested after 4 months of growth.

In summary, the Amazon offers Africa real possibilities for experimentation with new exotic germplasm of cassava. A coordinated effort with local agencies may prompt the collection of representative samples, particularly of the varieties grown on Indian lands.

### The savannas

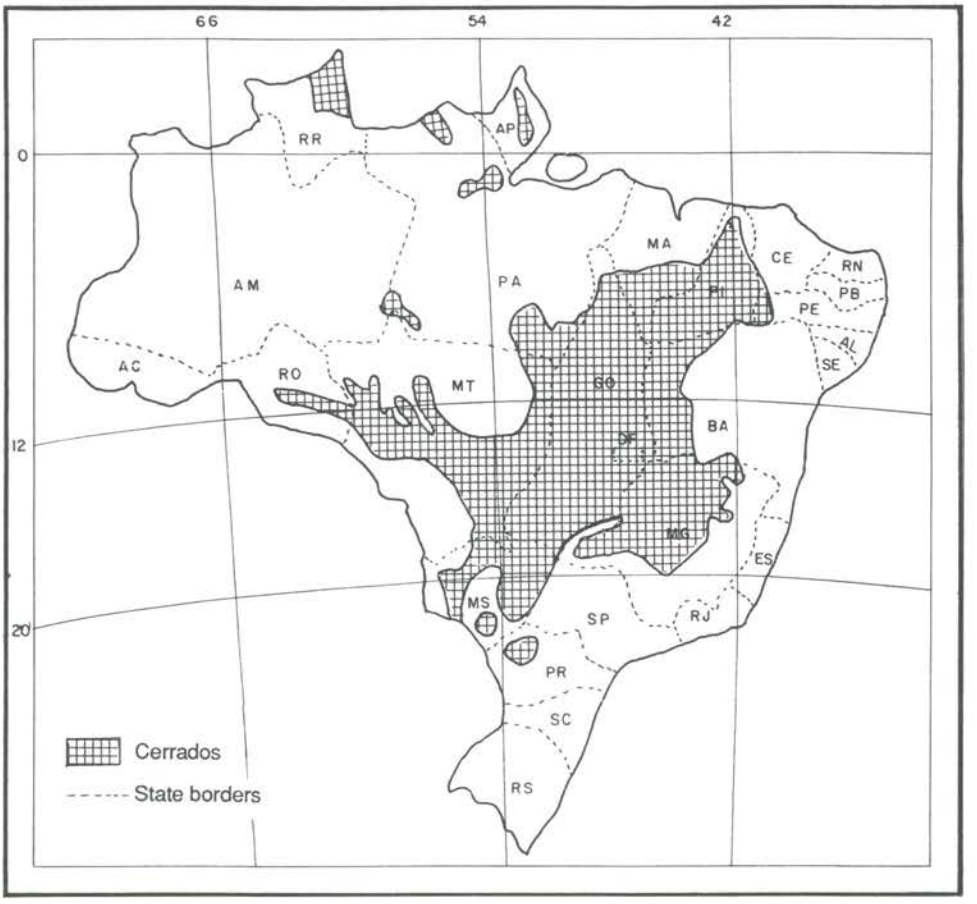
Savannas cover large parts of the African and South American continents. About 20% of the vegetational cover of both continents is composed of this sort of vegetation. In fact, the term is more often associated with the landscape than with floristic composition. In Africa, for example, the name savanna is variously used to mean the xerophilous vegetation (for example, in parts of south-west Nigeria) that correlates physionomically with the Brazilian Cerrado. In other places (such as Tanzania and Kenya), savannas mean grassland with thorny *Acacia* species dispersed amid grasses, similar physionomically to the flooded savannas of South America and to drier parts of north-east Brazil characterized by a vegetation known as 'Caatinga'. Elsewhere in Africa (for example, Niger, Chad, Sudan and Ethiopia) savannas may mean semi-desert areas with scarce vegetation cover.

South America has two basic types of savanna: flooded and non-flooded. Flooded savannas (known by such names as 'Chaco', 'low Llanos' and 'Pantanal mato-grossense') and non-flooded savannas (such as 'Cerrado' and 'high Llanos') have as common characteristics a similar plant landscape, low soil fertility and two extreme seasons, but differ substantially in floristic composition, topography and taxonomy of soils.

The Chaco, low Llanos and Pantanal have a dry season lasting 5-6 months and a rainfall of 1,000-1,500 mm (similar climatic conditions are found in Roraima, Guyana and Suriname). The main economic activity of all these flooded savannas is cattle raising; there is little crop cultivation.

By contrast, non-flooded savannas are intensively cultivated and cassava ranks as a staple in most areas of central Brazil in which this type of vegetation predominates (*see* Figure 7).

**FIGURE 7 Cerrado vegetation in Brazil**




Source: Azevedo and Caser, 1980.

Since the Cerrado vegetation is confined mainly to oxisols, it is clear that varieties of cassava have evolved by adapting to such soils, and this must be taken into account regarding introductions into Africa. Much of East Africa and some of Southern Africa can benefit from this sort of acid-tolerant germplasm, because parts of these areas match physionomically certain parts of Brazil's Cerrado. Although cassava is more important in West and and Central Africa, its presence in East Africa is by no means negligible. In Malawi, it is second only to maize as a staple (Membozanga, pers. comm.). In Uganda, 60% of the population eats the local staples, plantains and cooking bananas, but these foods are often accompanied by a popular mixture consisting of two-thirds dried cassava and one-third sorghum and finger millet (Hahn, 1984; Ssekimpi, pers. comm.). In addition, the savanna areas of Kenya, Tanzania, Zambia and parts of Angola all closely resemble the physical conditions of this Brazilian type of vegetation. In all these African countries cassava is grown to some extent (Jones, 1959).

## Arid zones

A collection of cassava germplasm suited to dry regions will be needed to relieve the famine which repeatedly afflicts areas with very low rainfall. Tropical Africa includes many such areas — parts of Mali, Niger, Chad, Central African Republic, Sudan, Ethiopia and Somalia).

South America has some physical environments which are very similar to the arid parts of Africa, and these environments could be exploited to meet some of African needs. The Peruvian coastal desert of the Casma valley, for example, is reported to grow five different varieties of low-cyanide cassava which mature in 9 months in an almost rainless region (Ugent et al., 1986). Such a unique germplasm could be of interest to African regions where the mean annual rainfall is below 300 mm and where the dry season lasts at least 7 months. The Sahelian zone as a whole might benefit from such arid-zone American germplasm.

The Paraguayan Chaco is also a good place for collection. It covers 60% of the country and becomes increasingly arid and rainless as one proceeds westward.

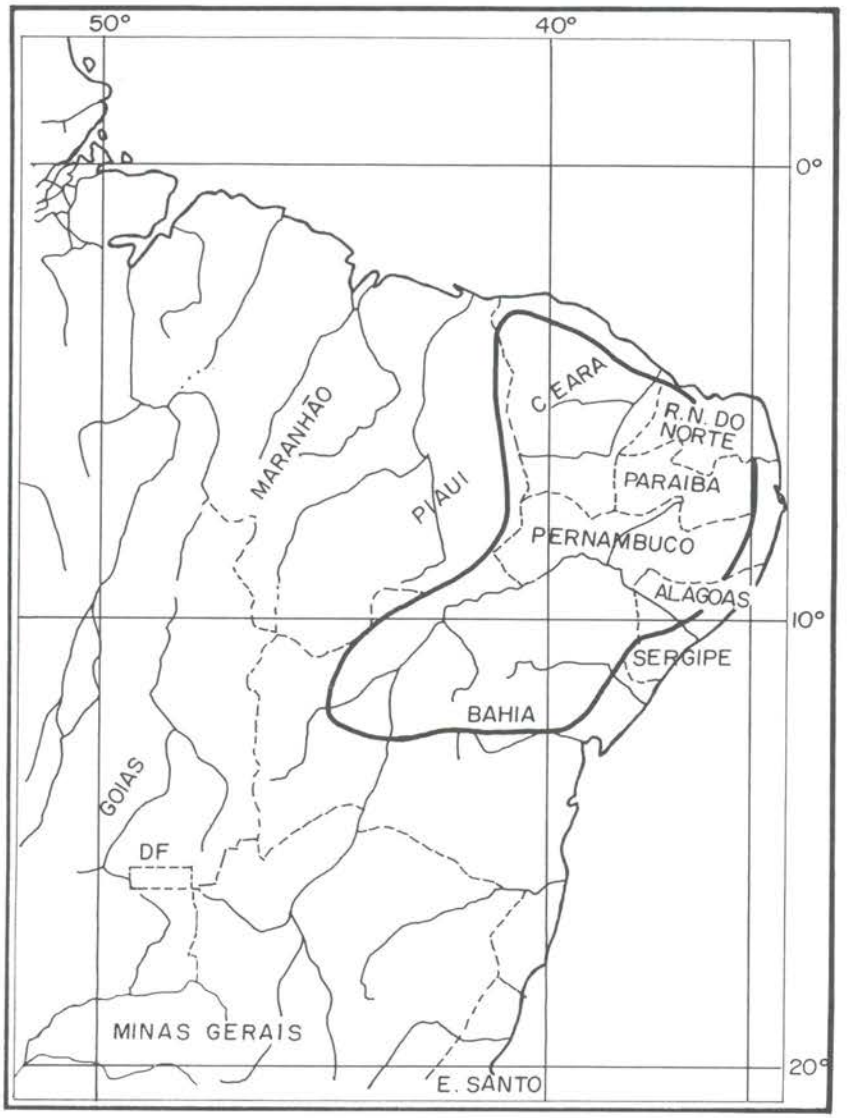
The xerophytic, thorny and dry Caatinga vegetation which covers much of north-east Brazil, is a climatic climax vegetation, the depletion of soil water determining its formation (Reis, 1971). The landscape and conditions of many arid parts of Africa (for example, countries in the Sahelian zones) resemble Brazil's Caatinga areas. This similarity increases as one progresses southwards; in the southern cone of Africa there are large areas covered with thorny bushes and low whitish grasses amid desert scenery, closely resembling the characteristics of the physiographic region of the Caatinga known as 'sertão', where thorny species of the legume genus *Mimosa* prevail. Cassava is a staple in Brazil's highly populated Caatinga zone because it is a drought-resistant crop. This is understandable in a region where 'farinha-de-mesa' (flour) is of paramount importance in reserve stocks because of unpredictable rains.

Many of Brazil's north-east states are covered with fertile alkaline alfisols and are suitable for cultivation if irrigation is available and land management techniques are practised to overcome various the side effects of irrigation, such as the release of salts. It is possible that cultivars growing in such climatic conditions will be well suited to the arid areas of tropical Africa.

Figure 8 shows Brazil's Caatinga zone, while Figure 9 (*overleaf*) shows the typical sequence of the main physiographic regions of north-east Brazil as reflected in the State of Pernambuco. Of interest to African semi-arid and arid zones is the germplasm from the sertão zones (250-500 mm) and, in particular, from the so-called 'semi-árido' (0-250 mm) (*see* Table 7 *overleaf*).

It would be relevant to study the levels of air humidity prevailing throughout the year in the target arid zones from which cassava germplasm is to be collected. Research is gradually revealing cassava's physiological mechanisms and it is now known that low levels of atmospheric humidity may inhibit the species' deposition of starch in the roots, irrespective of the water status of both soil and plant. IITA (1977) produced the first report on lower productivity of cassava as levels of humidity plunged below a certain threshold; more reports showing similar fndings began to emerge (Albuquerque and Cardoso, 1980; IITA, 1982; Hahn, 1984), and specific physiological research has now established the scientific basis of the relationship between productivity and humidity levels (El-Sharkawy and Cock, 1984; Cock et al., 1985). There is evidence that 45-50% humidity is the minimum threshold for photosynthesis to take place; below this level, the plant closes its stomata whether or not soil water is available.

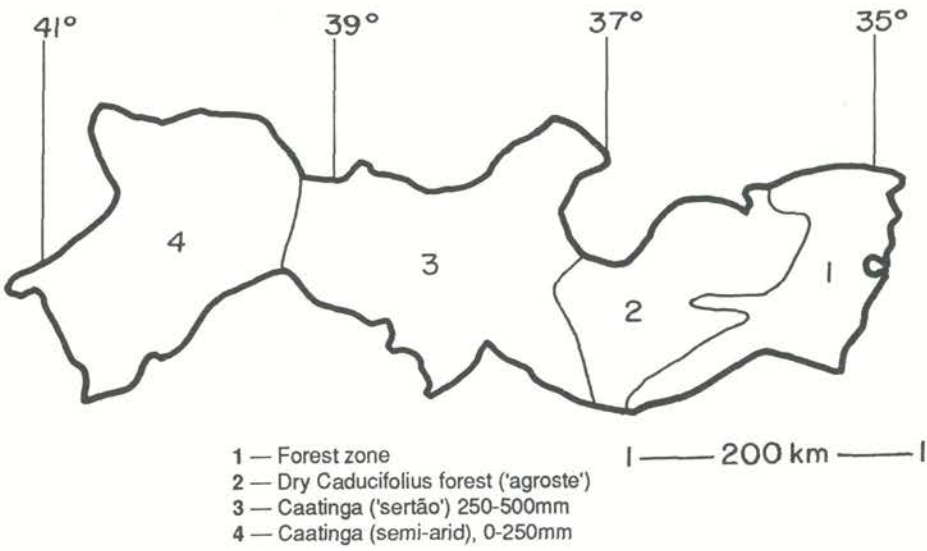
**FIGURE 8** **Rough delimitation of the Caatinga vegetation areas in the States of north-east Brazil**




## FUTURE STRATEGIES

This study has attempted to correlate some physiographic regions of South America and Africa with special reference to cassava germplasm collection and utilization. But clearly such an objective is inhibited by inadequate data. More ambitious future attempts will also have to consider more temperate latitudes farther south. For example, Southern African countries such as Zimbabwe, Botswana, Swaziland, Angola and Mozambique, where

**FIGURE 9 Physiographic regions of the State of Pernambuco in Brazil**




cassava grows as far as 25°S, correspond well to Brazil's temperate areas, where cassava is also a staple and is grown as far south as 32°S.

It is a common view that tropical agricultural research is obsessed with high productivity figures. However, the breathtaking figures reported from the field trials carried out by agricultural institutions have never been reproduced on a large scale in actual farming conditions, simply because the testing sites were seldom respresentative of the major cassava-growing areas. Cassava yields of up to 64 t/ha are recorded in the literature; a recent source has reported yields as high as 90 t/ha. These figures are far from the normal situation in Africa (average 6 t/ha) or in Asia and South America (average 9-11 t/ha). Investigators should observe the principle that for distinct agroecological zones there must be a supply of distinctly adapted genotypes (Stifel, 1988).

Research on cassava is responding well to calls for this sort of action. The 1980s have seen an increasing amount of work redefining cassava research strategies. For instance, research showed clear differences in the perfromance of a number of cassava genotypes tested under different field conditions (Hahn and Chukwuma, 1984). These results suggested the presence of genotype x environment interactions and opened the way toward site-specific breeding approaches. Whereas, initially, cassava researchers attempted to develop cultivars that would adapt well to a wide range of environments, they gradually began to realize that although cassava will grow in many types environmentals its varieties are, to a large extent, site-specific. Lozano et al. (1980) highlighted the abiotic factors inhibiting productivity, while Lozano et al. (1984) and Bellotti et al. (1987) stressed the biotic factors responsible. More recently, Cock (1987) has grouped cultivars according to 'macro-spatial stability' (only a few cultivars, showing stable performance over a wide range of ecological conditions) and

**TABLE 7** **Partial inventory of semi-arid municipalities for seven States of Brazil's north-east Caatinga vegetation area, where the annual rainfall ranges from 0 to 500 mm**

| | |
|---|---|
| **(0-250mm)** | |
| Pernambuco | Bélem de São Francisco, Floresta, Salgueiro, Pertrolãndia, Juazeirinho, Taperoá, Desterro, S.J. do Cariri, Prata, Panamirim, Ouricuri, Petrolina, Santa Maria de Boa Vista, Cabrobró |
| Paraiba | Picui, Pedra Lavada, Cubati, Soledade, São Mamede, Santa Luzia, |
| R.G. do Norte | Acu, São Miguel, Currais Novos, Carnaúbas dos Dantas, Parelhos, Caicó |
| **(250-500mm)** | |
| Pernambuco | Caruaru, Alpinho, S. Caetano, S. Bento de Una, Araripina, Bodocó |
| Alagoas | Piranhas, São José de Tapeua, Pão de Açucar, Jaeari dos Homens |
| Sergipe | Poço Redondo, Porto da Folha, Monte Alegre de Sergipe, Garam |
| **(0-500mm)** | |
| Bahia | Casa nova, Central, Chirrocho, Centro de Ouro, Glória, Ipupiara, Irece, Juazeiro, Jaguarari, Mauá, Santo Sé, Curaça, Xique-Xique, Queimadas, Santa Cruz, Valente, Itiuba, Conceição do Coité |
| Paraiba | São Mamede, Santa Luzia, Juazeirinho, Sumé, Serra Branca, Congo, Monteiro, S. Sebastião do Umbuzeiro |
| R.G. do Norte | S. João do Sabugi, Ouro Branco, Jardim do Seridó, Cruzeta, Acari, Currais Novos, S. Vicente, Campo Redondo, Cel. Ezequiel, Lages Pintadas, S. Tomé, Sitio Novo, Tangará, Santa Cruz, Cerro Corá, Lages, Angicos, Pedro Aurelino, Afonso Bezerra, S. Rafael |
| Ceara | Independência, Antonino do Norte, Potengi, Araripe, Campos Sales, Ainaba |

Source: Wania Fukuda, EMBRAPA-CNPMF, October 1987.

'micro-spatial stability' (many cultivars, showing stable performance under narrower ecological conditions).

Clearly, a far better understanding of the relationship between cassava's performance and the surrounding biological-chemical-physical environments is emerging, but there are still some noticeable gaps in the literature. A major one is apparent in a study by Lozano et al. (1980) in which six broad agroecological zones are associated with the growing of cassava, but where the minimum annual rainfall distribution starts in the range of 700 mm. Yet in many parts of Africa cassava is grown in areas with an annual rainfall of 80-300 mm (Jones, 1959). In Brazil's Caatinga area, all the cassava is grown in the range 0-500 mm annual rainfall range, as shown in Table 7.

Table 7 will be useful for any planning exercise leading to the transference of drought-tolerant germplasm into Africa. To begin with, much of the Caatinga region is composed of alfisols which are often very rich in sodium. Planners must take this salinity into account. Similarly, atmospheric humidity will have to be taken into account in comparative studies

dealing with sub-Saharan African and Brazil's north-east semi-arid regions, as the evidence suggests that this factor has a greater effect on root formation than the availability of groundwater. Brazil's Cerrado vegetation area is a potential donor of germplasm of cassava for East Africa and, unlike the Caatinga area, is relatively well-mapped agroecologically; 22 micro-agroecological zones have already been identified (Azevedo and Caser, 1980).

There is a growing consensus that stable productivity in cassava depends on a number of factors acting synergistically: abiotic elements (soils, temperature, annual rainfall distribution, evapotranspiration, atmospheric relative humidity, photoperiod, day length and latitude); biotic elements (diseases, pests and nematodes); and management (cultural practices, post-harvest technologies, alley cropping, and monocropping versus intercropping).

Our final point concerns reproductive biology, which is still a no man's land in cassava research. As knowledge of the crop on inceases, so new prospects emerge, and apomixis might turn out to be a factor whose potential could be exploited. There are indications that functional apomixis may exist within cassava itself (IITA, 1988), possibly of the pseudogamous type (the pollen is needed to form the endosperm). If further investigation confirms the preliminary results, then a whole new breeding strategy might be added to the existing ones, for apomixis provides an alternative path for the quick incorporation of desirable traits from one genotype to another.

## RECOMMENDATIONS

1. The evaluation of Africa's cassava germplasm needs should first aim at the screening and survey of its considerable range of variation on a regional basis. African national cassava programs should be encouraged to promote thorough collecting missions to explore their countries' agroecological zones.

2. Exotic cassava germplasm should tentatively be sought in South America, and the target areas selected must correspond to specific agroclimatic conditions in Africa. Soil taxonomy, seasons, atmospheric humidity and adaptive norms are, *inter alia*, all parameters to be considered prior to such an endeavor.

3. There should be a match between the agrobioclimatic characters of places of autochthonous germplasm and intended places of introduction. However, as the environmental limits of cassava are broad and the crop has an innate capacity to adapt in a moderate span of time, this match should be close but not necessaily the closest attainable.

4. Productivity criteria should come second to those of adaptation, where the crop has not yet been introduced or where it has not thrived.

5. A good deal of cassava germplasm has been stored over the past 20 years, and the time has come for specific, rather than random, collections.

6. With reference to disease and pest resistance, it has long been known that the degree of resistance is strongly correlated to prevailing local climatic factors. Thus, genotypes selected for resistance in one site may fail badly in similar conditions elsewhere because distinct local strains and races of pathogens interact with the environmental conditions. Therefore, the search for resistance should be site-specific in scope.

7. High priority should be given to collecting the following germplasm in South America for transfer to African regions:

   - coastal cultivars, particularly along the South Atlantic coast;
   - riverine cultivars in Amazonia, because of their earliness;
   - cultivars with yellow-flesh root on Indian lands throughout the Amazon;
   - drought-tolerant cultivars from Brazil's north-east region and, if feasible, from parts of the Peruvian coast and the Paraguayan Chaco;
   - wild populations of cassava in the neotropics. Such material forms the original stock from which *M. esculenta* evolved and, given its high genetic compatibility with the cultigen, it must be part any regional or global collection. *M. flabelifolia* is fairly widespread in America and collecting its germplasm is thus easier. *M. peruviana* is more restricted in distribution but it is relatively well mapped, and assembling its germplasm should pose no difficulty. The pubescent *M. peruviana* might confer useful genes against such pests as CMB andCGM. The two related *Manihot* species are of top priority.

8. The potential of the related *Manihot* species have not been well exploited, and warrants further investigation. Arboreal species of section *Glaziovianae* which have verifiably contributed useful genes to the cultigen have wide genetic variation and thus need attention. Other species, particularly herbaceous taxa and subshrubs, are low priority and should be studied in more detail taxonomically. The conservation of such germplasm in a field genebank is problematic, given its strict ecological requirements. Whenever possible, such species should be stored in the form of seeds, which retain greater genetic variability.

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# 3.4

# *Bananas in Africa: Diversity, Uses and Prospects for Improvement*

R. SWENNEN and D. VUYLSTEKE

Bananas, *Musa* species, are perennial giant herbs belonging to the family Musaceae (Zingiberales: Scitaminae) (Simmonds, 1966; Tomlinson, 1969). Most bananas are cultivated for their fleshy fruits, but some clones are planted for their edible corms or for fiber. Bananas are among the most important food crops of the tropical and subtropical world. Some 68 million tons of bananas are produced annually, of which only 7 million tons enter the world market (FAO, 1987). This demonstrates that the crop is far more important as a food crop for local consumption than it is as an export commodity.

The banana's center of origin is located in South-East Asia within an area bordered on the west by India and on the east by Samoa, Fiji and other South Pacific islands (Simmonds, 1966, 1976). High variability occurs, especially in India (Howes, 1928; Venkataramani, 1946; Bhaktavatsalu and Sathiamoorthy, 1979), Sri Lanka (Howes, 1928; Chandraratna and Nanayakkara, 1951), Thailand (Silayoi and Chomchalow, 1987; Silayoi, 1989), Viet Nam (Vakili, pers. comm.), Indonesia (Meijer, 1961), the Philippines (Allen, 1965; Valmayor, 1976; Valmayor et al., 1981; Pascua and Espino, 1987; Pascua, 1989) and Papua New Guinea (Simmonds, 1956, 1966; Argent, 1976; IBPGR, 1984).

It is believed that bananas were introduced into Africa by immigrants of Indo-Malayan origin and by Arab traders. There is much speculation on the ports of entry, but the areas of Zanzibar and Pemba (Tanzania) and Madagascar are the most likely candidates. From there, bananas were taken westward across the continent by African migrations (Simmonds, 1976). During the 16th and 17th centuries, slave traders took the banana to the New World. It is there, in Central and South America, that the export trade of dessert bananas flourishes today.

## DIVERSITY AND USES OF BANANAS IN AFRICA

All African bananas in which the fruit is consumed belong to the section *Eumusa* of the genus *Musa*. The genus *Ensete* is of importance only in Ethiopia, although it is also found in the drier

areas of Central and West Africa (Champion, 1967). *Ensete* represents the monocarpic non-suckering bananas with a distinctly swollen base. Its corms, not its seed-bearing fruits, are consumed. All African *Musa* that produce edible fruits can be grouped into three categories: plantains; highland beer and cooking bananas; and dessert bananas. Dessert bananas are distinguished by the sweet flavor of the fresh fruit at maturity, the result of the conversion of starch into sugar during ripening. In plantains, the fruits remain starchy at maturity, making them unpalatable when raw.

## Plantains

Among the bananas with starchy fruits at maturity, plantains form a well-defined group. They are of AAB genomic constitution as a result of natural interspecific hybridization between the two wild species *M. acuminata*, which provided genome A, and *M. balbisiana*, which provided genome B (Simmonds and Shepherd, 1955). The typical orange-yellow color of the compound tepal in the flowers and of the fruit pulp at ripeness are key characteristics used for determination. Fruits are slender and angular-pointed (Simmonds, 1966). Plantains fall into three major types, according to their degree of inflorescence degeneration: French; False Horn; and Horn (Tezenas du Montcel et al., 1983). Other features have recently been described (Swennen and Vuylsteke, 1987). With the aid of many descriptions and field studies (De Langhe, 1961, 1964; Tezenas du Montcel, 1979, 1983; Tezenas du Montcel et al., 1983), a determination key for 113 plantain cultivars was recently presented (Swennen, 1989). Of these, only one is not cultivated in Africa.

Plantains are cultivated from the lowlands of Guinea and Liberia to the central basin of Zaïre (Devos et al., 1978), where 50% of the world's plantains are grown (FAO, 1987) and where it is estimated that about 60 million people derive more than 25% of their carbohydrates from plantains (Wilson, 1987). Thus plantains are one of the most important carbohydrate sources throughout the lowland humid forest zones of Africa (Melin and Djomo, 1972; Flinn and Hoyoux, 1976; Fongeyn, 1976; Guillemot, 1976; Kabeya, 1976; Naku Mbumba, 1983; Wilson, 1983, 1987).

Plantains are cultivated mostly in backyards and in fields mixed with other food crops (Jurion and Henry, 1967; Karikari, 1972; Okigbo and Greenland, 1976). Attracted by high prices in the rapidly growing cities, many farmers are going into plantain monoculture. Pure plantain fields, however, are, managed like dessert banana plantations and manifest a rapid yield decline from the second production cycle. This is in sharp contrast with dessert banana fields, which remain productive for many years (Swennen, 1984; Wilson, 1987; Wilson et al., 1987). Several factors are believed to be responsible for the rapid yield decline, the most important ones being the reduced root ramification, inhibited sucker development and high mat (De Langhe et al., 1983; Swennen et al., 1986, 1988). The need for high levels of organic matter (Wilson et al., 1987) and the high susceptibility to nematodes (Sarah, 1987) and weevils (Mesquita et al., 1984; Mesquita and Caldas, 1986) are also contributing factors.

However, black Sigatoka leaf spot disease, caused by the fungus *Mycosphaerella fijiensis*, has recently invaded Central and West Africa (Frossard, 1980; Wilson and Buddenhagen, 1986) and now poses a major threat to food security in the plantain-growing regions. The disease attacks the leaves, reducing the yield by bewteen 30 and 50% (Stover, 1983). All plantain cultivars screened so far have been found to be susceptible (Hahn et al., in press) (*see* Table 1).

**TABLE 1 Levels of black Sigatoka resistance (BSR) in lowland bananas**

| Wild and edible aroids | | Dessert bananas | | Starchy bananas | |
|---|---|---|---|---|---|
| **Names** | **BSR** | **Names** | **BSR** | **Names** | **BSR** |
| ***AA genome*** | | ***AAA genome*** | | ***AAB plantains*** | |
| Calcutta 4 | +++[1] | Km 5 | ++ | 79 cvs | - |
| *M. acum.* type 3 | ++ | Dwarf Cavendish | - | | |
| *M. acum.* spp. | | Giant Cavendish | - | ***AA cooking banana*** | |
| *malaccensis* | ++ | Poyo | - | Pisang nangka | - |
| *M. tavoy* | ++ | Valery | - | | |
| *M. pahang* | ++ | Gros Michel | - | ***ABB cooking banana*** | |
| Pisang tongat | - | Green Red | - | Pelipita 2 | - |
| Pisang lilin | ++ | Red | - | Foulah | ++ |
| Muga | - | | | Bom | ++ |
| Wh-o-gu | - | ***AAAA genome*** | | Fougamou 1 | ++ |
| | | IC 2 | ++ | Gia Hui | - |
| ***BB genome*** | | | | Matavia | - |
| *M. balbisiana* | | ***AAB genome*** | | Maduranga | - |
| (1-63) | ++ | Pisang kelet | + | Bluggoe | - |
| | | Silk | - | Sabra | - |
| | | Pome | - | Monthan | - |
| | | | | Nzizi | + |
| | | ***ABB genome*** | | Simili Radjah | - |
| | | Ice cream | - | Cacamboa | - |

1 +++ resistant; ++ highly tolerant; + tolerant; - susceptible

### Highland beer and cooking bananas

In the highlands of East Africa (1,200-1,900 m above sea level), the annual per capita consumption of banana is about 300 kg (FAO, 1985), the highest consumption figure in the world. Depending on the cultivar, bananas are used for boiling and cooking or in beverage preparations. The former are called cooking bananas, the latter beer bananas (Sebasigari, 1987), but they are also known as 'mutika' and 'lujugira' (Shepherd, 1957). They are all AAA cultivars and are known only to the East African region (Shepherd, 1957). The highland cooking bananas should not be confused with the ABB cooking bananas cultivated in the lowlands and not yet of major importance in Africa.

These highland bananas are a dominant feature of the landscape in Rwanda, Burundi, eastern Zaïre, Uganda, western Tanzania and Kenya (Baker and Simmonds, 1951; Ddungu, 1987; Sebasigari, 1987). The number of clones is estimated at between 45 and 70 (Baker and Simmonds, 1952; Shepherd, 1957). The morphological characters of both beer and cooking bananas are quite similar and have been described (Sebasigari, 1987; Sebasigari, 1989). Yet, beer and cooking bananas can be identified only by means of color and taste tests. For example, in the case of a beer banana, the color of a green peeled banana's exudate is brown and the taste of its raw pulp and of the style of the male flowers is bitter (Sebasigari, 1987).

Given the high susceptibility of the most widespread cooking and beer bananas to black

Sigatoka (Vuylsteke and Swennen, 1988a) and the high consumption levels, black Sigatoka disease is the key production constraint in East Africa. Nematodes and the banana weevil, *Cosmopolites sordidus*, have always infested these bananas, but recently their virulence has increased abruptly (Bridge, 1988; Ddungu, 1988; Musabyimana, 1988; Sebasigari, 1988; Sebasigari and Stover, 1988). As a result of this development, the International Network for the Improvement of Banana and Plantain (INIBAP) organized a workshop during which research priorities were identified and recommendations for future research on nematodes and the banana weevil were formulated (INIBAP, 1988). At least some of the highland bananas also appear to be susceptible to the banana bunchy top virus (BBTV) (Sebasigari and Stover, 1988).

### Dessert bananas

Dessert bananas produce sweet fruits at maturity. They are cultivated almost throughout the humid and subhumid tropics of Africa. The varieties are, in descending order of importance, members of the Cavendish subgroup, Gros Michel, Pome or Prata, Red, Green Red, Km 5 or Yangambi, and Pisang Mas or Figue Sucrée. These cultivars are triploid AAA bananas, with the exception of Pisang Mas, which is an AA diploid, and Pome, which is an AAB triploid. They can be found growing in backyards and in fields near villages. Members of the Cavendish subgroup, such as Poyo and Giant Cavendish, are also cultivated on a large scale in plantations in Côte d'Ivoire and Cameroon, from where the bananas are exported to France, and in the Canaries and Somalia, which supply Spain and Italy, respectively. The Cavendish production in Morocco and South Africa is for the domestic market.

## CURRENT AND FUTURE RESEARCH ACTIVITIES

At present, black Sigatoka leaf spot disease is the overriding constraint to future plantain and banana production in Africa. It can be controlled by contact or systemic fungicides, but these chemicals are very expensive and are not readily available in Africa. Moreover, they present a health hazard if applied to bananas in backyards, where most bananas are cultivated. Therefore, the only feasible control method consists of the distribution of black Sigatoka-resistant bananas obtained through breeding.

The International Institute of Tropical Agriculture (IITA) has an advantage in this endeavor because it is located in the center of diversity of plantain. This region was invaded by black Sigatoka leaf spot disease a few years ago (Wilson and Buddenhagen, 1986). IITA plans to conduct research on improving plantains and other starchy bananas. The improvement of dessert bananas, however, will not be undertaken at IITA because other breeding programs (in Jamaica, Guadeloupe, Brazil and Honduras) have initiated such schemes. The improvement of the East African highland bananas is seriously handicapped by the lack of female fertile clones, and research efforts are continuing at IITA to determine female fertile clones.

IITA's plantain program is situated in Onne (near Port Harcourt, Rivers State, south-east Nigeria), where a collection of nearly 300 distinctly different accessions is maintained. The collection consists mainly of plantains, East African cooking and beer bananas, ABB cooking

bananas and wild diploids. Many accessions were received as *in vitro* propagules through the INIBAP Transit Center at the Catholic University, Leuven, Belgium. *In vitro* cultures have the advantage of being considerably less bulky and easier to handle than conventional propagules, but, more importantly, they are free of non-obscure pathogens. Because certain pathogens, such as BBTV, may pass undetected through *in vitro* culture and because a reliable indexing method is not yet readily available, all germplasm introductions pass through the INIBAP Transit Center. There, plants are screened for a period of at least 6 months for symptoms of such economically important diseases as BBTV, black Sigatoka and Fusarium wilt. This intermediate quarantine in a banana-neutral country occurs in collaboration with the Nigerian Plant Quarantine Service, which receives all introduced germplasm upon entry into Nigeria. Since 1985, IITA has introduced almost 200 *Musa* accessions from all parts of the world following this procedure. More than 100 *Musa* cultivars have been distributed internationally.

The campaign against black Sigatoka consists of a short-term as well as a long-term strategy. First, black Sigatoka-resistant starchy alternatives are identified and rapidly multiplied *in vitro* for distribution to national programs. Currently, four ABB cooking bananas have been identified as resistant or tolerant to black Sigatoka; these are Fougamou 1, Foulah 4, Bom and Nzizi (*see* Table 1). One cultivar, Pelipita, was very susceptible to black Sigatoka during the first few months after planting; however, at the flowering stage it manifested some level of tolerance. As Pelipita was issued from *in vitro* plants, the *in vitro* passage might have caused a temporary leaf abnormality, resulting in initially susceptible plants.

IITA's long-term strategy focuses on the creation of black Sigatoka-resistant plantains through genetic improvement. To initiate the conventional breeding program, French plantain cultivars were screened for female fertility. Ten cultivars were found to be female fertile (Swennen and Vuylsteke, 1988). They were rapidly multiplied *in vitro* and pollinated with black Sigatoka-resistant male-fertile diploids. In some of these crosses, up to 145 seeds per bunch were retrieved. Seeds were viable, since hybrid plants were obtained with the aid of embryo rescue techniques. This technique of culturing zygotic embryos *in vitro* is currently being refined in order to increase the low germination rates. This will involve experiments with immature embryos.

Hybrid starchy bananas and plantains are under observation in the field. If found to be resistant and if the bunch size is acceptable, the selected hybrids will be rapidly multiplied *in vitro* for distribution and further use in breeding schemes.

One important aspect of *in vitro* propagation is the occurrence of somaclonal variation. At IITA, we have observed that variation frequencies in plantain range from 0.5 to 70% and are genotype-specific (Vuylsteke and Swennen, 1988b; Vuylsteke et al., 1988). This demonstrates that there are inherently stable and unstable cultivars. Somaclonal variation is a potential hindrance to the propagation and conservation of germplasm, but has also been highlighted as a potential benefit in terms of creating new variability for plant improvement. In plantains, the spectrum of variant phenotypes is not very diverse and most off-types are inferior to the parental clone. This limits the possibilities for introducing novel variability that may be used in breeding. However, some inflorescence type variants may prove to be extremely useful for the improvement of the preferred False Horn plantain. The switch from a False Horn to a French bunch type, and then possibly back to the original False Horn, could be of significant practical value in circumventing the extreme sterility of the False Horn plantains.

Other non-conventional techniques that are being researched to complement and support the conventional breeding activities are:

- the study of induced mutations; plants that were regenerated from I-irradiated shoot-tip cultures of plantains are under evaluation in the field to screen for useful mutations;
- somatic cell culture techniques, by which plants could be regenerated from single cells through somatic embryogenesis and which are a prerequisite to implementation of new biotechnologies (for example, recombinant DNA technology); so far, *Musa* species have responded poorly to such techniques;
- pollen viability tests; now under development, these tests are based on the culture of isolated, mature pollen on simple germination media.

In the later stages of the plantain/starchy banana breeding program, other characteristics such as nematode resistance, dwarfism and improved harvest index will be included.

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# 3.5

# *Cowpea Genepool Distribution and Crop Improvement*

N.Q. NG and S. PADULOSI

Cowpea, *Vigna unguiculata* (L.) Walp., is an important pulse in several African countries, particularly Nigeria, Niger, Burkina Faso, Ghana, Kenya, Uganda and Malawi; it is also important in Brazil and, to a lesser extent, in India (Rachie, 1985). The crop is an ancient African domesticate which is a nutritionally important, but minor, component of subsistence agriculture in the semi-arid and subhumid tropics of Africa. It is eaten in the form of dry seeds, green pods, green seeds and tender green leaves. It is also utilized for fodder and as a cover crop. In Asia, the long bean cultivar is more important than seed-type cultivar and its green pods are used as a vegetable. Recently, short season seed-type cultivars developed by the International Institute of Tropical Agriculture (IITA) have been adopted in South-East Asia as a fallow crop in rice paddy (IITA, 1983). Cowpea grain production may be increased in this region in the future.

IITA is part of the Consultative Group on International Agricultural Research (CGIAR) system and has the CGIAR global mandate for the improvement of cowpea and the collection, characterization, documentation and distribution of cowpea germplasm. A world collection of germplasm exceeding 15,100 accessions of cultivated cowpea drawn from over 100 countries and 560 accessions of wild *Vigna* and close relatives of cowpeas has now been assembled and evaluated, and it is being used extensively in IITA breeding programs (*see* Table 1 *overleaf*). This has been made possible by the generous support of numerous research organizations with which IITA has collaborated, particularly in Africa.

## ORIGIN AND TAXONOMY OF COWPEA AND ITS GENETIC RELATIONSHIPS WITH OTHER WILD SPECIES

The origin and taxonomy of cowpea and its closely related species has recently been reviewed (Ng and Maréchal, 1985). Cowpea belongs to the subgenus *Vigna*, genus *Vigna* (tribe

**TABLE 1** **Existing cowpea germplasm accessions at IITA and their geographical distribution (as of August 1987)**

| Countries or regions | No. accessions | Countries or regions | No. accessions |
|---|---|---|---|
| **Central and West Africa** | | **Asia** (contd) | |
| Burkina Faso | 222 | Israel | 8 |
| Cameroon | 600 | Iran | 29 |
| Central African Republic | 183 | Japan | 2 |
| Chad | 268 | Laos | 1 |
| Gambia | 4 | Nepal | 3 |
| Ghana | 282 | Pakistan | 7 |
| Guinea | 2 | Papua New Guinea | 12 |
| Côte d'Ivoire | 134 | Philippines | 114 |
| Liberia | 9 | Sri Lanka | 2 |
| Mali | 293 | Syria | 8 |
| Niger | 976 | Thailand | 1 |
| Nigeria (+ IITA) | 3,221 | Turkey | 47 |
| Republic of Benin | 331 | USSR | 42 |
| Senegal | 290 | **North and South America** | |
| Sierra Leone | 13 | Argentina | 1 |
| Togo | 103 | Brazil | 171 |
| Zaïre | 15 | Canada | 182 |
| **East and Southern Africa** | | Colombia | 2 |
| Angola | 1 | Cuba | 1 |
| Botswana | 457 | El Salvador | 1 |
| Congo | 47 | Guatemala | 11 |
| Ethiopia | 7 | Honduras | 1 |
| Madagascar | 34 | Jamaica | 1 |
| Malawi | 494 | Mexico | 23 |
| Lesotho | 42 | Nicaragua | 2 |
| Swaziland | 19 | Paraguay | 12 |
| Somalia | 3 | Peru | 3 |
| Tanzania | 495 | Suriname | 14 |
| Uganda | 71 | USA | 828 |
| Zambia | 587 | Venezuela | 3 |
| Zimbabwe | 158 | **Europe** | |
| **North Africa** | | UK | 282 |
| Algeria | 1 | Hungary | 36 |
| Egypt | 347 | Portugal | 5 |
| **Asia** | | Italy | 93 |
| Afghanistan | 65 | **Australia** | 23 |
| Bangladesh | 1 | **Unknown** | 610 |
| China | 35 | **Ex-mix** | 630 |
| India | 2,075 | | |
| Indonesia | 4 | TOTAL | 15,100 |

Phaseoleae, subfamily Papilionoideae). According to the revision of the tribe Phaseoleae by Maréchal and colleagues (Maréchal et al., 1981), the genus *Vigna* comprises 84 species, most of them from Africa, where 54 are endemic (*see* Table 2 *overleaf*). Cowpea is placed under section *Catiang* , which comprises only two distinct species, *V. unguiculata* and *V. nervosa*. According to Maréchal et al. (1981), a single cultivated subspecies and three wild subspecies are classified under *V. unguiculata* (*see* Table 3, *page 166*). All three cultivated subspecies recognized by Verdcourt (1970) are classified under the subspecies *unguiculata*, which comprises the cultivar groups Unguiculata (cowpea), Biflora (catjan bean), Sesquipedalis (yard-long bean) and Textilis. The three wild subspecies are: subspecies *dekindtiana* (Harms) Verdc. with four botanical varieties (*dekindtiana, mensensis, pubescens* and *protracta*); subspecies *tenuis* (E. Mey) M. M. and S; and subspecies *stenophylla* (Harvey) M. M. and S. The characteristics of the various subspecies of *V. unguiculata* are given in Table 4 (*see page 167*).

Although substantial new information regarding the distribution of wild relatives of cowpea has been accumulated recently, no concrete information exists to pinpoint a specific area as the cowpea's center of domestication. The botanical evidence indicates that the center of diversity of cowpeas lies in West Africa, in an area encompassing the savanna region of Nigeria, southern Niger, part of Burkina Faso, northern Benin and Togo. However, it is not certain whether this is the only region where domestication occurred or whether the process of domestication occurred concurrently in other regions, such as south-east Africa, the region in which lies the center of diversity of wild relatives of cowpea and a considerable diversity of cowpea. At any rate, it appears that cowpea had a diffuse center of diversity over a very wide area of Africa. Cowpea is the basis status among cultigroups of the subspecies *unguiculata*; three other cultigroups evolved or were selected from cowpeas under human selection pressure (Steele and Mehra, 1980; Ng and Maréchal, 1985).

After observing many samples of wild taxa within the wild subspecies complex of *unguiculata* which IITA scientists have recently explored and collected from many countries in Central and Southern Africa and other parts of Africa, and in light of new information emerging from studies on interspecific hybridization (Mithern, 1987; Ng, 1988; Ng, unpubl.), we believe that the scheme of classification proposed by Maréchal et al. (1978) or Verdcourt (1970) should be modified. At least, the variety *pubescens* should be classified as a separate subspecies to distinguish it from other varieties of the subspecies *dekindtiana*. There is a probable new taxon within section *Catiang* from Swaziland; its dense and long pubescence on the pods, leaves and stem make it very different from the other varieties or subspecies described by Verdcourt or Maréchal et al. We are preparing a description of this new taxon for publication.

For the first time, studies at IITA have shown that subspecies *stenophylla*, subspecies *tenuis* and variety *protracta* of the subspecies *dekindtiana* can be hybridized with cowpea (Ng, 1988; Ng, unpubl.). Thus these taxa must belong to the primary genepool of cowpea. The diversity of this genepool can be exploited by cowpea breeders for improving the quality of cowpea cultivars. On the other hand, preliminary results, both at IITA in Ibadan and at IBPGR in Zimbabwe have shown that *V. nervosa* cannot be hybridized with cowpea (Mithen, 1987; Ng, 1988). At present, *V. nervosa* cannot be assigned to the primary or secondary genepool of cowpea. Further studies, with a wider range of genotypes, should be conducted to verify whether the species is crossable with cowpea. No species outside section *Catiang* has ever been successfully crossed with cowpea; the species *V. unguiculata* is strongly isolated from other species.

**TABLE 2** **African indigenous wild *Vigna* species and the germplasm accessions available at IITA Genetic Resources Unit**

| Subgenus | Section | Species | No. accessions |
|---|---|---|---|
| *Ceratotropis* | - | *V. radiata* var. *sublobata* | 2 |
| *Haydonia* | *Glossostylus* | *V. nigritia* | 3 |
| | | *V. venulosa* | 4 |
| | *Haydonia* | *V. juncea* var. *juncea* | - |
| | | *V. juncea* var. *major* | - |
| | | *V. monophylla* | 1 |
| | | *V. triphylla* | 1 |
| | *Microspermae* | *V. microsperma* | - |
| | | *V. richardsiae* | - |
| | | *V. schimperi* | 1 |
| *Macrorhynchus* | *Macrorhyncha* | *V. macrorhyncha* | - |
| | | *V. praecox* | - |
| | *Plectotropis* | *V. davyi* | 1 |
| | | *V. hundtii* | - |
| | | *V. kirkii* | 5 |
| | | *V. vexillata* var. *angustifolia* | 5 |
| | | *V. vexillata* var. *dolichonema* | - |
| | | *V. vexillata* var. *macrosperma* | 3 |
| | | *V. vexillata vexillata* | 56 |
| | *Pseudoliebrechtsia* | *V. lobatifolia* | - |
| | | *V. longissima* | - |
| | | *V. nuda* | - |
| *Vigna* | *Catiang* | *V. unguiculata* subsp. *dekindtiana* var. *dekindtiana* | 18 |
| | | *V. unguiculata* subsp. *dekindtiana* var. *pubescens* | 11 |
| | | *V. unguiculata* subsp. *dekindtiana* var. *mensensis* | 6 |
| | | *V. unguiculata* subsp. *dekindtiana* var. *protracta* | 35 |
| | | *V. unguiculata* subsp. *stenophylla* | 2 |
| | | *V. unguiculata* subsp. *tenuis* | 3 |
| | | *V. nervosa* | 4 |
| | *Comosae* | *V. comosa* subsp. *comosa* var. *comosa* | 1 |
| | | *V. comosa* subsp. *comosa* var. *lebrunii* | - |
| | | *V. comosa* subsp. *abercornensis* | 1 |
| | | *V. haumaniana* var. *haumaniana* | - |
| | | *V. haumaniana* var. *pedunculata* | - |
| | *Liebrechtsia* | *V. antunesii* | - |
| | | *V. debanensis* | - |
| | | *V. decipiens* | 1 |
| | | *V. frutescens* subsp. *frutescens* var. *buchneri* | - |
| | | *V. frutescens* subsp. *frutescens* var. *frutescens* | 4 |
| | | *V. frutescens* subsp. *incana* | - |
| | | *V. frutescens* subsp. *kotschyi* | - |
| | | *V. longiloba* | 1 |

**TABLE 2 (contd)**

| Subgenus | Section | Species | No. accessions |
|---|---|---|---|
| *Vigna* | *Macrodontae* | *V. friesiorum* var. *angustifolia* | - |
| | | *V. friesiorom* var. *friesiorum* | - |
| | | *V. friesiorum* var. *ulugurensis* | - |
| | | *V. membranaces* subsp. *caesia* | 2 |
| | | *V. membranaces* subsp. *hapalantha* | 2 |
| | | *V. membranaces* subsp. *macrodon* | - |
| | | *V. membranaces* subsp. *membranacea* | - |
| | *Reticulatae* | *V. dolomitica* | - |
| | | *V. kassneri* | - |
| | | *V. phoenix* | - |
| | | *V. platyloba* | 2 |
| | | *V. pygmaea* | - |
| | | *V. radicans* | 11 |
| | | *V. reticulata* | 50 |
| | | *V. tisserantiana* | - |
| | | *V. wittei* | 4 |
| | *Vigna* | *V. ambacensis* var. *ambacensis* | 12 |
| | | *V. ambacensis* var. *pubigera* | 18 |
| | | *V. angivensis* | - |
| | | *V. bequaertii* | - |
| | | *V. desmodioides* | - |
| | | *V. filicaulis* var. *filicaulis* | 2 |
| | | *V. filicaulis* var. *pseudovenulosa* | 2 |
| | | *V. fischeri* | 1 |
| | | *V. gazensis* | - |
| | | *V. gracilis* var. *gracilis* | 1 |
| | | *V. gracilis* var. *multiflora* | - |
| | | *V. heterophylla* | 1 |
| | | *V. hosei* var. *hosei* | 2 |
| | | *V. hosei* var. *pubescens* | 1 |
| | | *V. laurentii* | 1 |
| | | *V. luteola* | 75 |
| | | *V. marina* | 1 |
| | | *V. multineris* | 3 |
| | | *V. oblongifolia* var. *oblongifolia* | 25 |
| | | *V. oblongifolia* var. *parviflora* | 5 |
| | | *V. parkeri* subsp. *parkeri* | - |
| | | *V. parkeri* subsp. *maraguensis* | 1 |
| | | *V. racemosa* | 50 |
| | | *V. subterranea* var. *spontanea* | 3 |
| Total | | | 560 |

**TABLE 3 Classification and nomenclature of the taxa within section *Catiang* of the subgenus *Vigna* (Savi) Verdc.**

| Maréchal et al. (1978a) | Verdcourt (1970) | Status |
|---|---|---|
| *V. unguiculata* | *V. unguiculata* | Cultivated |
| subsp. *unguiculata* | | |
| cv-gr. Unguiculata E. Westphal | subsp. *unguiculata* (L.) (Walp.) Verdc. | Cultivated |
| cv-gr. Biflora E. Westphal | subsp. *cylindrica* (L.) Van Eseltine | Cultivated |
| cv-gr. Sesquipedalis E. Westphal | subsp. *sesquipedalis* (L.) Verdc. | Cultivated |
| cv-gr. Textilis E. Westphal | - | |
| subsp. *dekindtiana* | | |
| var. *dekindtiana* | subsp. *dekindtiana* (Harms) Verdc. | Wild |
| var. *mensensis* (Schweinf.) M.M.&S. | subsp.*mensensis* (Schweinf.) Verdc. *V. unguiculata* (L.) Walp. | Wild |
| var. *protracta* (Wilczek) M.M.&S. | var. *protracta* (E. Mey.) Verdc. | Wild |
| var. *pubescens* (Wilczek) M.M.&S. | *V. pubescens* [1] Wilczek | Wild |
| subsp. *stenophylla* (Harv.) M.M.&S. | *V. angustifoliolata* Verdc. | Wild |
| subsp. *tenuis* (E. Mey.) M.M.&S. | *V. tenuis* [1] (E. Mey.) Dietr. | Wild |
| *V. nervosa* Markotter | *V. nervosa* Markotter | Wild |

1 Verdcourt noted that this species might be a variant or variety of *V. unguiculata*.

Source: Based on Ng and Maréchal, 1985.

## DISTRIBUTION OF WILD RELATIVES OF COWPEA

Subspecies *unguiculata* variety *dekindtiana*, found throughout sub-Saharan Africa, including Madagascar, has the widest distribution of any cowpea subspecies (Verdcourt, 1970; Maréchal et al., 1981; Ng and Maréchal, 1985; Padulosi et al., 1988) (*see* Figure 1 *overleaf*). The variety *mensensis* is found in Central and East Africa, particularly Zaïre, Uganda, Tanzania, north-eastern Zambia and Malawi. It is also found in north-eastern Ethiopia, Swaziland and a coastal region in Sierra Leone.

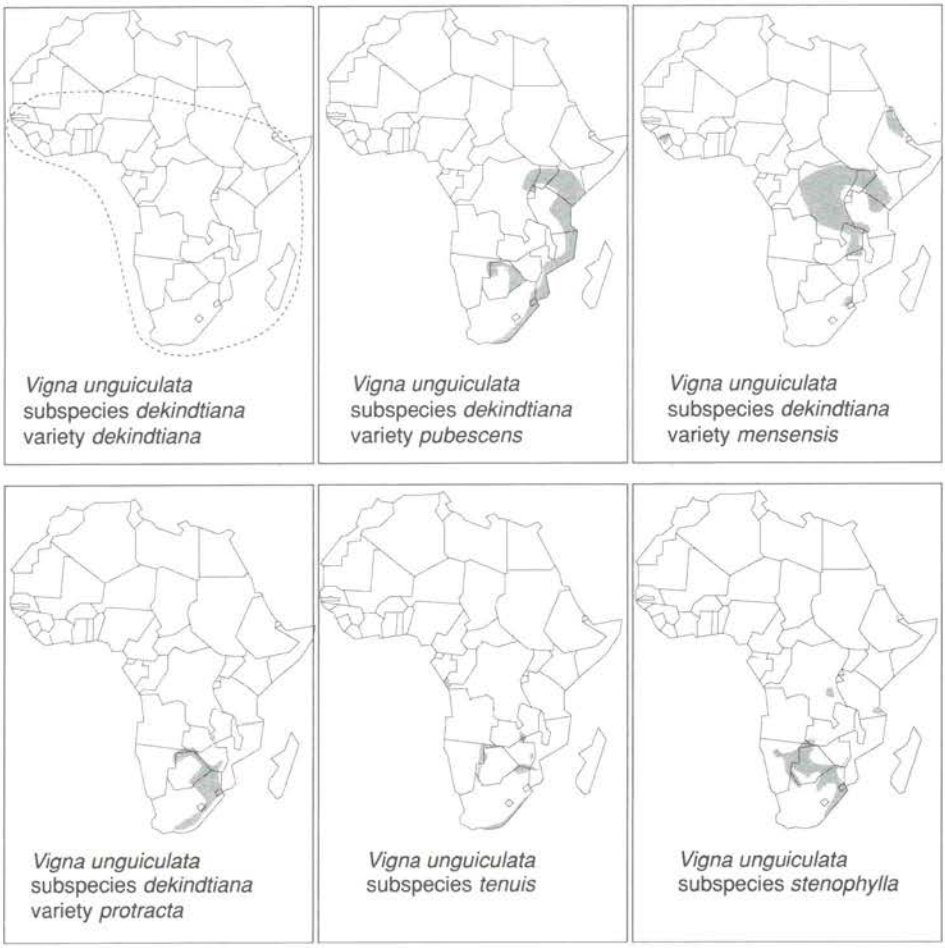
The variety *pubescens* is found in the coastal region of East Africa, stretching from as far north as Kenya to the southern tip of Africa (Verdcourt, 1970), whereas *protracta* is found mainly in northern and north-eastern South Africa, Swaziland, northern and eastern Botswana, part of Zimbabwe and Zambia. A sample collected from Niger by our plant explorer resembles *protracta*, and would represent the first record of its presence in West Africa.

After surveying the literature and herbaria and examining the records from extensive plant exploration conducted by IITA's Genetic Resources Unit (GRU) in many countries in Africa (IITA, 1988), we discovered that the area of distribution of subspecies *tenuis* and *stenophylla* was much wider than expected. New records of *tenuis* were found in Zimbabwe and Congo (Padulosi, 1987; Goli, 1988). It is more common in the northern and the north-eastern areas of South Africa and has also been recorded in the region where the borders of Botswana, Namibia and Zambia meet. The subspecies *stenophylla* is common in north-eastern South Africa, Botswana, Namibia and Zimbabwe (Maréchal et al., 1981). New records have been found in Tanzania and eastern Zaïre. *V. nervosa* is distributed from Zimbabwe to north-eastern South Africa (Maréchal et al., 1981; Crompton, 1976; Mithen, 1987; IITA, 1988).

**TABLE 4** **Characteristics of various cv-gr., botanical varieties and subspecies of *V. unguiculata***

| Character | cv-gr. Unguiculata | cv-gr. Biflora | cv-gr. Sesquipedalis | cv-gr. Textilis | var. *mensensis* | var. *dekindtiana* | var. *pubescens* | var. *protracta* | subsp. *stenophylla* | subsp. *tenuis* |
|---|---|---|---|---|---|---|---|---|---|---|
| Flower color | white, purple | white, purple | white, purple | white, purple | purple | purple | purple | purple | purple | purple |
| Standard petal | | | | | | | | | | |
| Width (mm) | 24-30 | 24-28 | 28-35 | 24-27 | 33-42 | 23-42 | 28-40 | 23-34 | 24-26 | 23-33 |
| Length (mm) | 18-23 | 18-24 | 21-24 | 19-21 | 26-28 | 17-34 | 19-28 | 23-28 | 18-20 | 18-23 |
| Pod | | | | | | | | | | |
| Length (cm) | 6.5-2.5 | 7-13 | 15-90 | 7-14 | 7.5 | 6.0-11.6 | 6-8 | 7.5-9 | 4.5-6.5 | 5-6 |
| Width (mm) | 3-12 | 4-6 | 5-11 | - | 3.6 | 2.8-7 | 3.2-3.5 | - | 3.3 | 3.2-3.3 |
| Orientation | mostly pendant, vertical | mostly vertical | all pendant | vertical | vertical | vertical | vertical | vertical | vertical | vertical |
| Texture | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated shrinking after maturity | succulent: inflated toward maturity | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated | fibrous, hard, firm, not inflated |
| Dehiscence | nil | nil to moderate | nil | nil to moderate | shatters | shatters | shatters | shatters | shatters | shatters |
| Locules/pod | 7-23 | 12-16 | 15.8-23 | -= | 16-19* | 14-17 | 10-16 | 15-17 | 8-13 | 8-15 |
| Calyx lobe (mm) | <5 | <5 | <5 | <5 | >5 | <5 | <5 | <5 (>5?) | <5 | <5 |
| Seed | | | | | | | | | | |
| Length (mm) | 6-11 | 5-7 | 7-11 | 5.1-7.6 | 2.5-4.2 | 3-6 | 2.7-4 | 3.1-4.9 | 3.2-3.4 | 3.1-3.5 |
| Width (mm) | 4-9 | 3-5 | 5-8 | 4-5.6 | 2.0-3 | 2-4 | 2.6-3 | 2.3-3.2 | 2.2-2.4 | 2.4-2.7 |
| Orientation | crowded | crowded | far apart | crowded | crowded | crowded | crowded | crowded | crowded | crowded |
| Breeding system | inbreeder | inbreeder | inbreeder | inbreeder | outbreeder | inbreeder | inbreeder | outbreeder | outbreeder | outbreeder |
| Growth habit | erect, prostrate | prostrate, climbing | sem-ierect, climbing | prostrate | prostrate, climbing | semi-erect, prostrate, climbing | semi-erect, prostrate, climbing | semi-erect, prostrate, climbing | prostrate, climbing | prostrate, climbing |
| Shoots | glabrous | glabrous | glabrous | glabrous | glabrous/ pubescence | glabrous | pubescence | pubescence | glabrous | glabrous |
| Inverted V-shaped pigmentation on leaves | nil | nil | nil | nil | some | some | all | all | some | some |
| Life span | annual | annual | annual | annual | annual | annual/ perennial | annual/ perennial | annual/ perennial | perennial | perennial |

**FIGURE 1 Distribution of wild species belonging to the primary genepool of cowpea**




## THE IMPORTANCE OF GERMPLASM FOR THE IMPROVEMENT OF COWPEA CULTIVARS

The significance of cowpea germplasm collection, evaluation, preservation and use for improving cowpea cultigens has recently been reviewed (Steele et al., 1985; Ng, 1987). Before the establishment of internationally oriented research programs, several national agricultural research programs in Africa, particularly Nigeria, Senegal, Tanzania and Uganda, devoted some of their effort to cowpea improvement, focusing on specific regions and objectives within national boundaries. National programs had limited access to the diverse germplasm resources scattered throughout Africa and other parts of the world. Through hybridization work involving locally adapted cultivars and carefully selected exotic germplasm, some progress in the improvement of cowpea was made in Nigeria and Senegal

(Sene and N'Diaye, 1971, 1973; Ojomo, 1973, Franckowiak et al., 1973). However, few national programs are strong enough to prosper alone, particularly in assembling and maintaining large collections of germplasm materials from around the world for plant breeding. After IITA's Grain Legume Improvement Program was established in 1970, a concerted effort was made to assemble, collect and evaluate cowpea germplasm for use in breeding programs and a systematic hybridization program for cowpea improvement was initiated in 1971. After the establishment of IITA's GRU in 1975, more systematic efforts were made to collect landraces of cowpeas throughout Africa to expand the diversity of cowpea and to further the work on characterization, evaluation, documentation and preservation of the valuable germplasm materials.

The large and diverse cowpea germplasm collection available at IITA and elsewhere provides the opportunity and challenge for scientists around the world, especially in Africa, to exploit naturally useful resources to improve cowpea cultivars. Through intensive screening of the existing collections at IITA, many sources of insect pest resistance to cowpea aphids, *Aphis craccivora*, leafhoppers, *Empoasca signata* and *E. dolichi*, legume bud thrips, *Megalurothrips sjostedti*, pod borers, *Maruca testulalis*, pod-sucking bugs, *Clavigralla shadabi*, and bruchids, *Callosobruchus maculatus*, have been identified (Singh, 1977, 1980; IITA, 1978, 1983; Singh and Allen, 1979; Singh et al., 1983). These sources are summarized in Table 5.

Although the success in identifying germplasm resistant to insect pests has been remarkable, the achievement in disease resistance screening has been even greater. Sources of resistance against many major diseases have been found, and some of the sources are resistant to multiple diseases (Williams, 1975, 1977a, 1977b; Ladipo and Allen, 1979; IITA, 1978;

**TABLE 5 Sources of cowpea germplasm resistance to various insect pests**

| Pest | Sources of resistance (IITA's TVu No.) |
|---|---|
| Aphids (*Aphis craccivora*) | 36, 57, 134, 157, 191, 200, 310, 308, 410, 801, 2000, 2657, 2755, 2845, 2896, 3273, 3346, 3433, 9836, 9914, 9929, 9930, 9944 |
| Pea aphid | 408, 410, 3629 |
| Leaf hoppers (*Empoasca* spp.) | 50, 59, 123, 305, 418, 662, 1037, 1045, 1190 |
| Legume bud thrips (*Megalurothrips sjostedti*) | 1509, 2870 (all moderate resistance) |
| Legume pod borers (*Maruca testulalis*) | 946, 13271, VITA-5 (all moderate resistance) |
| *Cydia ptychora* | 4579, 4328 (moderate resistance) |
| Pod-sucking bugs (*Clavigralla shadabi*) | 1977, 7274 |
| Bruchids (*Callasobruchus maculatus*) | 2027, 11952, 11953 |

Source: Based on Ng, 1987.

Singh and Allen, 1979; Singh et al., 1983). Sources resistant to viruses and to multiple diseases are listed in Tables 6 and 7, respectively.

Several elite germplasm lines were directly selected from the existing germplasm collections, and named as VITA lines (VITA 1-5). These were released and widely adopted in many countries (Singh and Ntare, 1985). Some lines, such as VITA 3, have a combined resistance to multiple diseases and insect pests (Allen, 1983).

These VITA lines and other selected materials resistant to insect pests and diseases, which are photo-insensitive and early-maturing and have erect and determinate plant growth, have been used at IITA as the basis for further improvements in cowpea cultivars. The aim was to develop cultivars with good yield potential, resistance to multiple diseases and pests, and good adaptability to various ecological regions in the dry Sahel and humid tropics, while maintaining a seed quality acceptable to consumers. Indeed, high-yielding cultivars with resistance to multiple diseases and to several insect pests (including leafhoppers, aphids, flower thrips and bruchids) have been developed and distributed to national programs by IITA (Singh et al., 1983; IITA, 1984; Singh, 1985). Cultivars that are suitable for extremely

**TABLE 6 Sources of cowpea germplasm resistance to selected viruses**

| Virus | Resistant germplasm accessions (IITA's TVu No.) |
|---|---|
| CYMV [1] (2 isolates from Nigeria, 1 from India) | 113, 274, 310, 410, 433, 470, 866, 746, 1190, 2769, 2563, 3650, 7941 |
| SBMv (3 isolates from Nigeria) | 79, 113, 238, 310, 347, 393, 445, 470, 486, 493, 697, 746, 1185, 1851, 1878, 1888, 1948, 1985, 1986, 2672, 2755, 2896, 6365 |
| CMeV (2 isolates from cowpea and Bambara groundnut in Nigeria) | 36, 42, 205, 471, 488, 566, 947, 1171, 1261, 1477, 2269, 2885, 2886, 2887, 2933, 2939, 2962, 2964, 2968, 2971, 3043, 6433 |
| CMeV + CYMV | 274, 346, 393, 493, 1888 |
| CMeV + CYMV + CAMV | 266-1, 433, 393, 493, 1888, 1947, 2755 |
| CMeV + CYMV + SBMV | 238, 393, 470, 2896, 493, 2755, 1185, 1888, 1849 |
| CMeV + CYMV + SBMV + CAMV | 493, 697, 746, 1185, 1888, 1948, 2755, 393, 1185, IT85F-867-5, IT85F-2687, IT84D-449, IT82D-442 |
| CMeV + CYMV + SBMV + CAbMV + GM | 393, 493, 2755, 1185 |

1 CYMV: cowpea yellow mosaic virus; SBMV: southern bean mosaic virus; CMeV: cowpea mottle virus; CAbMV: cowpea aphid-borne mosaic virus; GM: golden mosaic virus

Source: Based on Ng, 1987.

short (60-day) and intermediate (75- to 80-day) cropping seasons have also been developed. They are resistant to major diseases and to bruchids, aphids or flower thrips; they have an erect and determinate growth habit, mature uniformly, and have acceptable seed quality for various regions. These materials are also suitable for large-scale production, and the crops can be combine-harvested. Bush-type vegetable cowpeas that do not require staking have also been selected and developed at IITA. Some of IITA's advanced breeding lines that have resistance to multiple insect pests and diseases, combined with high yield potential, good plant type and good seed quality, are listed in Table 8 (*see overleaf*). These improved germplasm materials are freely available on request.

Many improved germplasm materials which have been developed at IITA have combined resistance to more than 10 major diseases and two insect pests, aphids and bruchids. They have been released and adopted by farmers. However, these materials are deficient in resistance to the three most important insect pests: pod borers, coreid bugs and legume bud thrips. So far, only low to moderate levels of resistance to these pests have been found from existing germplasm of cultivated cowpeas. High levels of resistance are likely to come from wild relatives of cowpea or species from other sections of the subgenus *Vigna* or other subgenera.

**TABLE 7 Sources of cowpea germplasm resistance to multiple diseases**

| Disease | Locality of screening | Resistant germplasm accessions (IITA's TVu No.) |
|---|---|---|
| Brown blotch | Nigeria | 201, 1977 |
| Scab | Nigeria | 843, 1404, 1433, 1977 |
| Bacterial blight | Nigeria | 347, 410, 456, 483-2, 726, 745, 1190, 1977 |
| Septoria | Nigeria | 456, 483, 486, 726, 853, 1433, 1583, 2455, 1977 |
| Fusarium wilt | Nigeria | 109, 347, 984, 1000, 1016 |
| Fusarium root rot | Puerto Rico | 202, 231, 243, 266, 274, 320, 316, 393, 408, 1563 |
| Phakopsora rust | Nigeria and Uganda | 612, 1258, 1962, 2455, 4540 |
| Web blight | Nigeria | 317, 2483, 4539 (all are moderately resistant) |
| Synchytrium false rust | Uganda | 43, 222, 612, 4535, 4537, 4569, 6666 |
| Target spot | Nigeria | 1190 |
| Root knot nematodes | Nigeria | 264, 401, 857, 1560 |
| Bacterial blight + scab + Septoria + brown blotch | Nigeria | 1977 |
| Scab + Septoria | Nigeria | 853, 1433 |
| Bacterial blight + Septoria | Nigeria | 456, 4832, 726 |
| Anthracnose + bacterial pustule + Cercospora leaf spot + rust + Cowpea yellow mosaic virus | Nigeria | 201, 347, 408, 410, 537, 697, 746, 1190, 1283, 2430, 3415, 310, 345, 393, 645, 990, 1452, 1980, 2755, 3565 |

Surce: Adapted from Ng (1987).

**TABLE 8** **Some of the selected high-yielding cowpea breeding lines developed at IITA that possess multiple disease resistance, as well as resistance to some insect pests**

| Disease/insect | TVx 3236 | IT81D-1137 | IT83S-742-11 | IT82D-716 | IT81D-1020 |
|---|---|---|---|---|---|
| **Disease** | | | | | |
| *Cercospora* spp. | R[1] | R | R | R | R |
| Bacterial blight | R | R | MR | R | MR |
| Bacterial pustule | S | R | R | MR | MR |
| Anthracnose | R | R | R | R | R |
| Web blight | R | R | R | R | R |
| Brown blotch | R | R | R | MR | R |
| Septoria | S | R | MR | S | S |
| Scab | R | R | R | R | R |
| Cowpea yellow mosaic virus | S | R | R | MR | MR |
| Cowpea aphid-borne mosaic virus | S | R | R | R | R |
| Cowpea golden mosaic virus | R | R | R | R | R |
| **Insect** | | | | | |
| Flower thrips | MR | S | - | MR | S |
| Aphids | S | S | R | S | R |
| Bruchid | S | R | S | R | R |

1 R: resistant; MR: moderately resistant; S: susceptible

Source: Based on Singh, 1985.

## PROSPECTS FOR FURTHER IMPROVEMENT OF COWPEA

There are ample opportunities for further improvement of cowpea through the exploitation of the currently available germplasm.

Potential seed yield of improved cowpea levels at approximately 2 metric tons per hectare. This level can be increased, but further research in plant physiology is needed to identify more efficient genotypes and plant architecture. The characteristic of many pods (more than 4) per peduncle found in wild species *V. unguiculata* subsp. *dekindtiana*, if transferred to cowpea cultivars, could perhaps increase the yield level.

The major limiting factor for cowpea production in Africa is the susceptibility to pod borers, pod-sucking bugs and cowpea coreid bugs, as noted above. We should continue to assemble new germplasm and identify new sources of resistance. The pod pubescence of the closely related wild taxa of cowpea, found in *V. unguiculata* subsp. *dekindtiana*, var. *pubescence*, var. *protracta* and a probable new taxon within section *Catiang* mentioned earlier could be a useful characteristic in conferring resistance to these pests. The role of this character in conferring resistance should be investigated and exploited. In addition, phytochemicals which may be present in some of the wild genotypes of the subsp. *dekindtiana*, subsp. *tenuis* or subsp. *stenophylla* that control insect pests should be investigated.

It has been shown that secondary plant metabolites may be playing an important role in conferring resistance to *M. testulalis* and *C. tomentosicollis* in the wild species *V. vexillata* (Jackai et al., 1988). However, this wild species cannot be successfully crossed with cowpea (Ng, 1988). Efforts are now being made to employ genetic engineering techniques to accomplish this transfer (IITA, 1988).

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# 3.6

# *A Comprehensive Breeding System for Maize Improvement in Africa*

S. A. EBERHART, S. K. KIM, J. MARECK, L. L. DARRAH, and M. M. GOODMAN

Because maize, *Zea mays* L., is not an indigenous crop in Africa, many introductions have been made. Recent acquisitions by maize breeders, including populations from the Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT), represent great genetic diversity. This germplasm has been evaluated in most countries and the best material has been selected for further improvement. Subsequent progress depends on breeding objectives and methodologies.

Large increases in maize production in Africa can be achieved with improved cultivars developed with a comprehensive breeding system (Eberhart et al., 1967) by: selecting the best germplasm available; using an effective breeding system to develop disease-resistant, pest-resistant, stress-tolerant and high-yielding source breeding populations that respond to improved cultural practices; and developing superior hybrids and/or open-pollinated varieties from the improved breeding populations for the commercial products. Kenya has been extremely successful with hybrids developed with this program, whereas Zambia, Zimbabwe, South Africa, Egypt and Nigeria have developed hybrids with traditional inbreeding and hybridization methodologies.

The comprehensive breeding system requires the development of two diverse breeding populations that maximize the population-cross performance. This provides greater flexibility because the commercial product can be either: the advanced generation of the population cross that can be used as an elite open-pollinated variety until hybrids can be developed and produced; or single cross (SC), three-way cross (TWC) or double cross (DC) hybrids involving inbred lines derived from successive cycles of reciprocal recurrent selection.

## DEVELOPING ELITE SOURCE BREEDING POPULATIONS

Environmental factors within the five major ecological zones in Africa require somewhat different source materials and breeding objectives. In West Africa, the major zones are

lowland rainforest, lowland moist savanna and dry savanna. Central, East and Southern Africa can be divided into the medium-altitude and high-altitude zones

Recommendations are sometimes made that each micro-environment within each ecological zone will require hybrid or open-pollinated varieties developed specifically for that micro-environment, but extensive trials in Africa (Eberhart et al., 1973; Darrah, 1976; Darrah and Mukuru, 1978) and experiences of maize seed companies fail to confirm these suppositions when appropriate germplasm is used with appropriate breeding objectives. Genotype x environment interactions can be dramatically reduced by developing breeding populations and hybrids with higher levels of disease and insect resistance and higher levels of tolerance to such stresses as low soil pH (aluminum toxicity), low soil moisture, low nutrient availability and good resistance to root and stalk lodging (Kim et al., 1985; Fajemisin et al., 1985). Such cultivars will have a very broad area of adaptation within their ecological zone.

The correct maturity for growing seasons that are limited by the rainfall distribution is very important. Eberhart et al. (1973), Darrah (1976), and Darrah and Mukuru (1978) have demonstrated a large differential response to varying altitudes (*see* Table 1). Presumably, the response to night temperatures is a major factor, although diseases may be involved unless the cultivars have a higher level of resistance. Northern corn leaf blight, *Helminthosporium turcicum*, and common rust, *Puccinia sorghi*, often limit yields at high elevations, whereas southern corn leaf blight, *H. maydis*, and southern rust, *P. polysora*, are serious diseases at lower elevations throughout Africa. Cultivars developed from medium-altitudes failed to

**TABLE 1** **Observed yields with altitude ($b_A$) and environmental ($b_I$) responses for selected entries in the 1976-77 East African Maize Variety Trials**

| Entry | Observed Yields | | | Environmental Responses | | |
|---|---|---|---|---|---|---|
| | Low (0-0.9 km) | Medium (0.9-1.6 km) | High (1.6-2.2 km) | Mean | $b_A$ | $b_I$ |
| | g/ha | | | g/ha | g/ha/km | g/ha/I |
| **High elevation cultuvars** | | | | | | |
| H611 (R) C5 | 43.7 | 51.5 | 90.8 | 68.1 | 22.6 | 1.07 |
| EAH6302 | 34.6 | 53.3 | 95.8 | 70.1 | 30.2 | 1.09 |
| H5020 | 52.3 | 61.5 | 97.4 | 76.4 | 20.9 | 0.92 |
| Alemaya | 32.5 | 40.2 | 62.4 | 49.2 | 7.0 | 0.96 |
| **Medium elevation cultivars** | | | | | | |
| H512 | 47.6 | 45.6 | 55.7 | 50.3 | -10.7 | 1.01 |
| H632 | 42.0 | 48.7 | 57.2 | 51.7 | -11.6 | 1.20 |
| SR52 | 50.3 | 50.0 | 56.2 | 52.7 | -20.8 | 1.35 |
| ZCA | 35.0 | 37.6 | 34.2 | 35.8 | -19.2 | 0.91 |
| UCA (F) CO | 40.3 | 42.5 | 55.9 | 48.2 | -5.3 | 0.99 |
| KwCB | 47.4 | 46.2 | 44.3 | 45.5 | -18.8 | 0.90 |
| Mean | 42.6 | 47.7 | 65.0 | 54.8 | -0.6 | 1.04 |

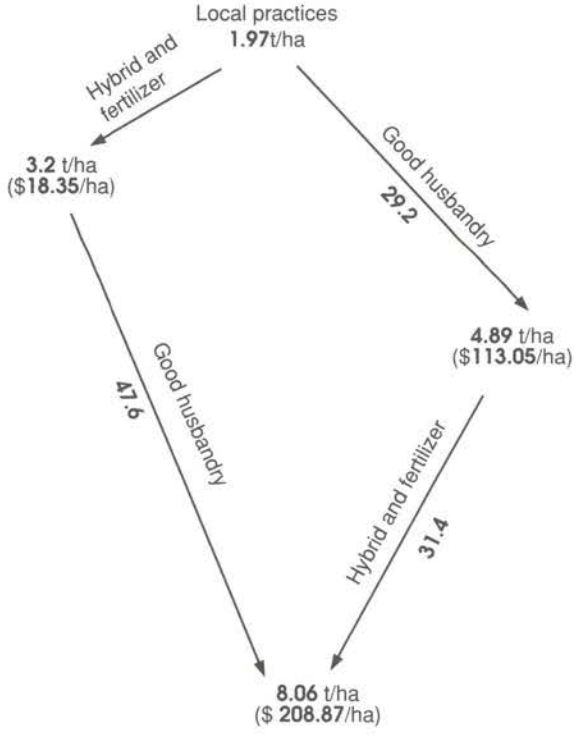
Source: Based on Darrah and Mukuru, 1978.

respond in higher-altitude environments, whereas the higher-altitude cultivars were extremely high-yielding at higher elevations and at least equal to the medium-altitude cultivars at the medium elevations. The hybrid H5050 gave outstanding yields at both medium and high elevations.

In addition to the leaf blights and rusts, Fajemisin et al. (1985) included maize streak virus, *Curvularia* leaf spot, maize mottle/chlorotic stunt and downy mildew in their list of significant maize diseases. They listed *Fusarium, Diplodia, Botryodiplodia, Rhizoctonia,* and *Macrophomina* as causal organisms for stalk and ear rots in Africa. The list of important insect pests of maize included: stem borers, *Sesamia calamistis, S. botanephaga, Busseola fusca, Chilo partellus, Eldana saccharina*; underground insects and nematodes, *Buphonella murina, Heteronychus licas,* and *Dereodus recticollis*; and army worms, *Spodoptera exempta.* The parasitic weed *Striga hermonthica* often causes serious losses. Kim et al. (1985) reported differential responses to *Striga* among maize hybrids.

Allan (1971) demonstrated that hybrid seed and fertilizer must be accompanied by improved husbandry, including timely planting and weed control (*see* Figure 1). With the total package, Kenya farmers had the potential to increase yields from 2 to 8 t/ha, and most

**FIGURE 1 Maize improvement in Kenya through improved husbandry and the use of hybrid seed and fertilizer (increased profit per hectare shown in parentheses)**




Source: Allan, 1971.

farmers succeeded in at least doubling their yields. The increased production resulted produced cash profits. Hybrids have been used on over 50% of the maize acreage in Kenya for the past decade.

The heterotic pattern is a key factor in selecting germplasm for the breeding populations in order to maximize the population-cross performance. If breeders in all countries would use a common superior heterosis pattern, the exchange of elite inbreds among breeding programs over time would result in improved commercial hybrids and would permit more effective population improvement, with minimum risk of the loss of genetic variability from genetic drift with small effective population sizes.

Wellhausen (1978) reported excellent population-cross performance from Tuxpeño and its related Caribbean and USA dents with Cuban Flint and Coastal Tropical Flint. Several tropical breeding programs (including those in Brazil, Colombia and Peru) have utilized the Tuxpeño (dent)/Caribbean Flint heterosis pattern (Paterniani 1985). The Brazilian dent population, ESALQ-VD-2, is mainly Tuxpeño germplasm, while the flint population, ESALQ-VF-1, is mostly Caribbean Flint. Recent experimental trials with improved CIMMYT populations continue to show high population-cross yields from this pattern (Vassal et al., in prep.). Evaluations of populations formed by mixing these germplasm types within each population demonstrate good population performance but almost no heterosis (Vassal et al., in prep.).

Wellhausen (1978) recommended that: 'Instead of the formation of 12 populations, in which racial complexes and hybrid patterns are disregarded, it seems to me that tropical maize breeders might better focus their main cooperative efforts on the development of two broad-based, high-yielding, widely adapted, fertilizer-responsive, more nutritive, biologically efficient populations, as follows: (a) a dent composite consisting of the combination of Tuxpeño and related dents, such as the Cuban, West Indies and US dents, and their precursors; and (b) a flint composite, consisting mainly of the Cuban, Coastal Tropical and Cateto flints and their precursors.' Goodman (1972, 1978) used morphological and geographic data to show a close relationship between the Tuxpeño, Vandeño and Celaya races.

In Kenya, a large amount of heterosis was obtained with two narrow base populations, a Tuxpeño-derived Kitale Station maize and a high-altitude flint collection (Ec573) from Ecuador (Darrah et al., 1978; Darrah, 1986). The population-cross yield of two elite broad base populations, KCB x KCE, was only 86% of the population-cross yield of the narrow base populations, Kitale II (R11) C1 x Ec573 (R12) C1 (Eberhart et al., 1973).

In the USA and central Europe, the Stiff Stalk Synthetic (dent)/Lancaster (semi-flint) heterotic pattern has been very successful. Kim et al. (in press) and others have demonstrated that introgression of Corn Belt inbreds into tropical germplasm can be useful for reducing plant height and increasing responses to fertilizer as long as the disease resistance of the tropical germplasm is retained.

Grain color and texture of the commercial product must be acceptable to the consumer. However, these traits are highly heritable and can and must be rapidly changed by selection once the breeding populations are formed. Experience has shown that selection from mixed yellow/white to white grain color requires intense selection because of minor genes and epistatic gene action in some germplasm.

Seed size and shape and the performance of seed parent SCs, and especially seed parent inbred lines, is critical to the reliability and cost of hybrid seed production. Hence, the designation of the Tuxpeño-Stiff Stalk breeding populations as seed parent populations and the Caribbean Flint-Lancaster breeding populations as pollen parent populations is war-

ranted. Selection for important seed parent traits will then be required in only the seed parent breeding populations. Many populations and some inbred lines which have been developed and released are adapted to environmental conditions in Africa (*see* Tables 2 *below*; Tables 3 and 4 *overleaf*). The best population-cross performance can be expected from populations improved by recurrent selection, especially reciprocal recurrent selection, and from the introgression of the best inbred lines back into these superior populations. Many breeders have already formed elite breeding populations with high population-cross performance and

**TABLE 2 Improved white maize breeding population for hybrid development**

| Semi-dent (Seed parent) | Semi-flint (Pollen parent) | Country |
|---|---|---|
| **1 Medium-high elevation (1600-2200 m)** | | |
| a Long season | | |
| KCB (F) C4 | KCE (F) C4 | Kenya |
| Kitale II (R11) C8 | Ec573 (R12) C8 | Kenya |
| **2 Medium elevation (900-1600 m)** | | |
| a Medium season | | |
| Mg A (M) | Mg B (M) | Kenya |
| Embu I | Embu II | Kenya |
| GS11 (R) C1 | GS12 (R) C1 | Kenya/USA |
| TZMSR-W | | Nigeria/IITA |
| **3 Low elevation (0-900 m)** | | |
| a Medium-long season | | |
| TZB-SR | Pop. 25-SR | Nigeria/IITA |
| TZSR-SR-W-1 | | Nigeria/IITA |
| DMR-LSRW | | Nigeria/IITA |
| 7921-SR | | Nigeria/IITA |
| 8443-DMRSR | | Nigeria/IITA |
| 8422-SR | | Nigeria/IITA |
| 8429-SR | | Nigeria/IITA |
| CMS 8507$C_1$ (TZB-SR) | Ekona White | Cameroon |
| ESALQ-VD-2 | ESALQ-VF-1 | Brazil |
| | Mayorbella (R) C3 | Puerto Rico |
| b Medium season | | |
| TZUTSR-W | 8730-SR | Nigeria/IITA |
| TZESR-W | | Nigeria/IITA |
| TZL Comp 1 | | Nigeria/IITA |
| DMR-ESRW | | Nigeria/IITA |
| 8149-SR | | Nigeria/IITA |
| 8440-SR | | Nigeria/IITA |
| 8444-SR | | Nigeria/IITA |
| Pool 16-SR | | Nigeria/IITA |
| Mex 17E | | Cameroon |
| CMS 8501$C_1$ (Pool 16-SR) | | Cameroon |
| CMS 8503$C_1$ (8249-SR) | | Cameroon |

**TABLE 3 Improved yellow maize breeding populations**

| Semi-dent (Seed parent) | Semi-flint (Pollen parent) | Country |
|---|---|---|
| **1 Low elevation (0-900 m)** | | |
| a Medium-long season | | |
| TZSR-Y-1 | Suwan 1-SR | Nigeria/IITA |
| Pool 16-SGY | | Nigeria/IITA |
| 8428-SR | | Nigeria/IITA |
| | Ekona Yellow | Cameroon |
| | CMS 8602 (30SR) | Cameroon |
| | Pop. 36 | Mexico/CIMMYT |
| b Medium season | | |
| TZUT-Y | 8431-SR | Nigeria/IITA |
| TZESR-Y | | Nigeria/IITA |
| DMR-ESRY | | Nigeria/IITA |
| 8435-SR | | Nigeria/IITA |

have improved them by recurrent selection. In these cases, further introgression should be extremely limited. For instance, introgression of a very small amount of GS11 (R) C1 into Kitale II (R11) Cn and KCB Cn might be of value in reducing plant height and increasing the frequencies of genes for high yield and fertilizer response from the improved Stiff Stalk Synthetics used to develop GS11 (R) C1. Similarly, a very small amount of GS12 (R) C1 might be introgressed into Ec573 (R12) Cn and KCE Cn. The elite Tuxpeño derived inbred lines developed in Nigeria should be introgressed back into TZPB-SR and the others into the Caribbean type populations. Such introgression should enhance the population-cross performance.

Eberhart et al. (1967) emphasized that the final population-cross mean can be predicted from data obtained in a partial diallel when epistasis is negligible. If varieties $M_1$ to $M_m$ are composited into the seed parent population (A) and varieties $N_1$ to $N_m$ are composited into the pollen population (B), the population-cross performance (A x B) can be predicted as follows:

$$(A \times B) = (1/mn)\,(M_1N_1 + M_1N_2 + \ldots + M_mN_n)$$

Kim et al. (1985) reported good population-cross yields in Africa from the Tuxpeño Pop. 21 with TZB. Vassal et al. (in press) reported that Pop. 24 (Tuxpeño-Antigua) gave the highest yields with both Suwan 1 (a Caribbean flint type improved breeding population from Thailand) and Pop. 36 (Caribbean composite) in a CIMMYT regional variety-cross diallel evaluation. Development of white versions of Suwan 1-SR and Pop. 36 may merit high priority to more fully utilize the Tuxpeño/Caribbean Flint heterosis pattern.

## IMPROVEMENT OF THE ELITE BREEDING POPULATIONS

Population improvement is the foundation of a maize breeding program seeking to maximize long-term genetic gain per year. Additionally, both short- and medium-term goals can be

**TABLE 4 Released maize inbred lines (with source)**

| Semi-dent (Seed parent) | Semi-flint (Pollen parent) | Country |
|---|---|---|
| **A White** | | |
| 1 Low elevation (0-900 m) | | |
| a Medium-long season | | |
| TZi3 (7721 x TZSR) | | Nigeria/IITA |
| TZi4 (7729 x TZSR) | | Nigeria/IITA |
| TZi5 (TZB x TZSR) | | Nigeria/IITA |
| | ICA L25 (Cuban flint) | Colombia |
| | ICA L27 (ETO) | Colombia |
| b Medium season | | |
| TZil2 (N28 x TZSR) | | Nigeria/IITA |
| TZil5 (N28 x TZSR) | | Nigeria/IITA |
| **B Yellow** | | |
| 1 Low elevation | | |
| a Medium-long season | | |
| TZi25 (B73 x RppSR) | | NIgeria/IITA |
| TZi30 (Hi29 x RppSR) | | Nigeria/IITA |
| | A-6 | Honduras |
| | A-21 | Honduras |
| | Hi27 | Hawaii |
| | Hi29 | Hawaii |
| | Hi34 | Hawaii |
| | KU1411 (Suwan 1) | Thailand |
| | KU 1413 (Suwan 1) | Thailand |
| b Medium season | | |
| TZi24 (H95 x RppSR) | TZill (Mol7 x RppSR) | Nigeria/IITA |
| TZi25 (B73 x RppSR) | | Nigeria/IITA |
| TZi28 (F44 x RppSR) | | Nigeria/IITA |
| | Hi28 | Hawaii |
| | ICA L29 | Colombia |
| | ICA L36 | Colombia |
| | ICA L210 (Cuban flint) | Colombia |

achieved. Much greater improvement of maize production in Africa can be achieved from hybrid and open-pollinated varieties developed with effective population improvement programs in fewer populations than from ineffective programs in many populations.

Maximizing the rate of improvement of the population cross is vital because this determines the rate of improvement of the derived hybrids and the advanced generation of the population cross when this is used as the commercial product (*see* Figure 2 *overleaf*). Choosing the appropriate recurrent selection method is critical. Reciprocal recurrent selection has been used successfully (*see* Table 5 *overleaf*) in several programs to improve the variety-cross performance (Eberhart et al., 1973; Darrah et al., 1978; Moll and Hansen, 1984; Darrah 1986; Helms et al., in press [a]).

**FIGURE 2** **Expected improvement of the breeding populations, the population cross, and the best single cross with reciprocal recurrent selection**

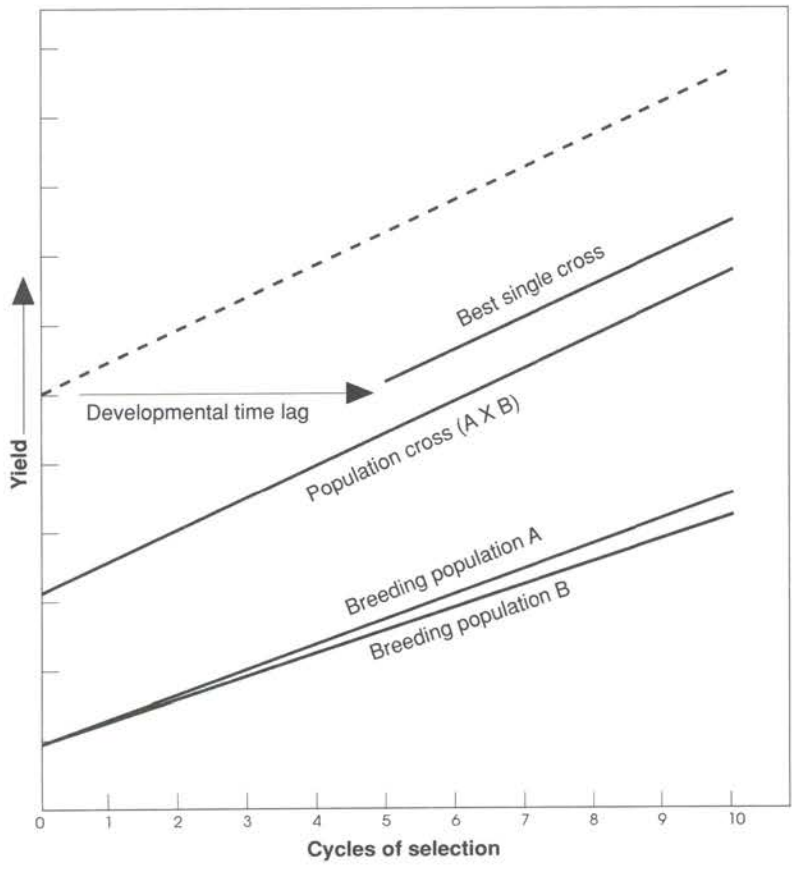



**TABLE 5** **Population improvement from reciprocal recurrent selection**

| Populations | Cycles | Yield | | | Moisture | | Lodging | |
|---|---|---|---|---|---|---|---|---|
| | | CO x CO | Cn x Cn | Gain (%) | CO x CO | Cn x Cn | CO x CO | Cn x Cn |
| BSSS (R) x BSCB1 (R) [1] | 10 | 5.13 | 7.30 | 42.3 | 23.7 | 24.5 | 33 | 18 |
| Jarvis (R) x I. Chief (R) [2] | 10 | 6.22 | 7.45 | 19.8 | | | | |
| Jarvis (R) x I. Chief (R) [2] | 10 | 8.93 | 10.84 | 21.4 | | | | |
| Kitale II (R11) x Ec573(R12) [3] | 3 | 5.91 | 7.16 | 21.2 | | | | |
| Kitale II (R11) x Ec573(R12) [4] | 5 | 6.36 | 8.11 | 27.5 | | | 63 | 48 |

1 From Helms et al. (in press); 2 Moll and Hansen (1984); 3 Darrah et al. (1978); Darrah (1986).

The modification of using inbred lines from the reciprocal population for the tester, as suggested by Eberhart et al. (1973) and Russell and Eberhart (1975), has real merit because selection for specific hybrid combinations is initiated in the population improvement cycle, and gain from selection may benefit from the increased variation among test crosses. Multiple testers will make this system somewhat more effective and should be used whenever feasible for two reasons: multiple testers provide a more representative sample of the reciprocal population; and the probability of quickly identifying useful commercial hybrids is increased. The experimental lines should be divided into two to four sets, with a different elite inbred line tester for each set (any given experimental line will be crossed to only one tester). Selection of lines for recombination will be made within each set, but selected lines from all sets will be recombined to form the next cycle of the population. The testers will usually be inbreds used in commercial hybrid production and will often be of different maturities. Testers will change with cycles of selection as improved inbreds are developed. SC testers can be used, but genetic variation among test crosses will be less than that for inbred line testers. The use of the reciprocal population may be necessary for the first two cycles in a new breeding program until inbred lines can be developed, but suitable testers are often available from other breeding programs (*see* Table 4).

The formula for gain per year for the population cross from reciprocal recurrent selection (Sprague and Eberhart,1977) can be used to identify key factors affecting the rate of improvement. This formula is as follows:

$$G_y = \left(\frac{1}{y}\right)\sqrt{\frac{k\frac{1}{4}(1+F)\ S^2_{A1}}{\frac{S^2_{e1}}{rm} + \frac{\frac{1}{4}S^2_{AE1}}{m} + \frac{1}{4}S^2_{A1}}} + \left(\frac{1}{y}\right)\sqrt{\frac{k\frac{1}{4}(1+F)\ S^2_{A2}}{\frac{S^2_{e2}}{rm} + \frac{\frac{1}{4}S^2_{AE2}}{m} + \frac{1}{4}S^2_{A2}}}$$

where:

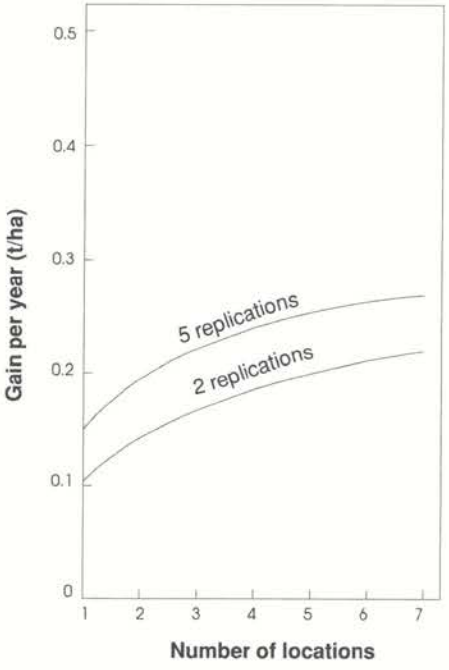
y = years per cycle; k = the standardized selection differential; F = the coefficient of inbreeding of the parental plants of the experimental lines used for the test crosses; $S^2_e$ = the pooled experimental error variance; $S^2_A$ = the additive genetic variance; $S^2_{AE}$ = the additive by environmental interaction; r = the number of replications in each of m locations (or environments)

Increasing $S^2_A$ should increase gain, but estimates of additive genetic variance from very diverse populations have not been shown to be larger than those from $F_2$ populations (Hallauer and Miranda, 1981), probably because of the finite number of experimental lines that can be evaluated. Very diverse breeding populations often have higher mean yields *per se*, but the population-cross mean will be lower (Darrah et al., 1972; Vassal et al., in prep.).

Increasing the level of parental inbreeding is strongly recommended. F increases from zero for $S_1$ lines (where the parental $S_0$ plants are non-inbred) to 0.5 for $S_2$ lines (where the $S_1$ parental plants are 50% inbred). Although the time for a cycle of selection is extended from y = 2 to y = 3, gain per year will be the same (assuming only one $S_2$ line is retained from each selected $S_1$ family) and test cross evaluations must be made every 3 years instead of every 2 years (Sprague and Eberhart, 1977). Furthermore, the $S_1$ lines can be screened for disease resistance and other agronomic traits so that only elite $S_2$ experimental lines are advanced to the test cross evaluations.

Decreasing the phenotypic variance (the denominator) will increase gain. Number of plants per plot has a minimal effect. The error variance $S^2_e$ can be expressed as $[(S^2_w/n) + S^2]$, where $S^2$ is the plot-to-plot error variance and $S^2_w$ is the within plot error variance. Between 20 and 25 plants per plot will reduce $S^2_w/n$ to a negligible factor in relation to $S^2$ (Eberhart, 1970). When test crosses vary greatly in plant height, use of two-row plots reduces the competition bias. Because $S^2_{AE}$ is usually a significant factor, only two replications per location (environment) and four or five locations are recommended (Sprague and Eberhart, 1977) (*see* Figure 3). Increasing the number of locations reduces the contribution of both $S^2_e$ and $S^2_{AE}$ to the phenotypic variance. Brewbaker (1985) recommends the use of varied planting dates at a location to provide different environments.

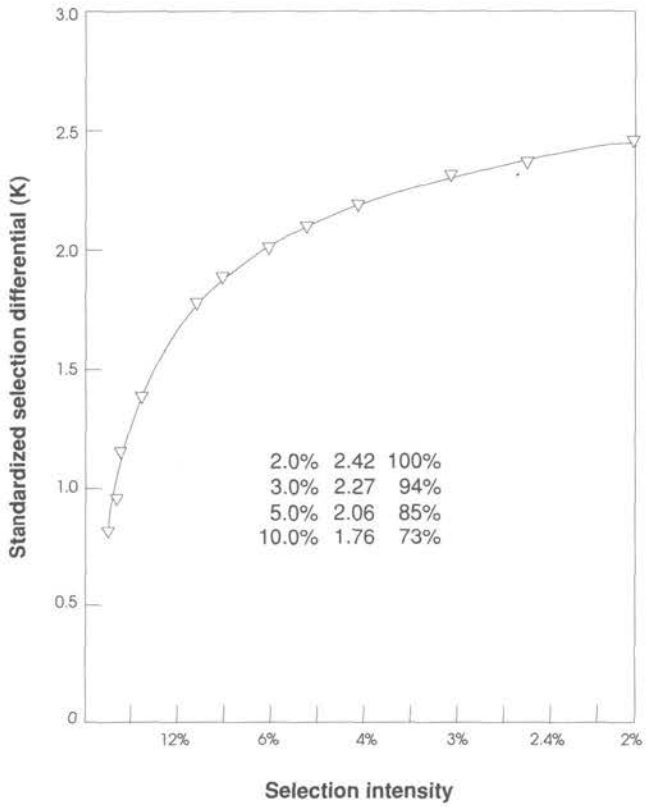
**FIGURE 3 Expected gain from reciprocal recurrent selection with Jarvis and Indian Chief in North Carolina with 2 and 5 replications per location**




Increasing the selection intensity is an important means of increasing gain (*see* Figure 4) because gain is proportional to k. Gain with a 5% selection intensity (k = 2.06) is 147% compared to a 20% selection intensity (k = 1.40), and 117% compared to 10% (k = 1.76). Selection intensity can be increased only by: increasing the number of experimental lines evaluated in test cross trials; or decreasing the number of lines selected for recombination to form the next cycle. The development of only two elite reciprocal populations per breeder (with test cross yield trials for the seed parent and pollen parent population phased in successive years) will permit a higher selection intensity for the same resources than will the use of larger numbers of populations. The number of selected lines that are recombined

affects the effective population size (Rawlings, 1970). Results from studies at USDA-ARS/ Iowa State University (Helms et al., in press [b]) show considerable genetic drift when only 10 lines are selected and recombined in closed populations. When several breeders work within a general heterotic pattern, exchange derived inbred lines, and then introgress these lines into their breeding populations, the effective population size of each population will be greatly increased. Lines for introgression must be carefully selected to maintain or enhance the population-cross performance. Experience and theoretical considerations suggest that test crosses from 400 $S_2$ lines should be evaluated in order to select 16 to 20 $S_2$ lines for recombination in each cycle in order to achieve a selection intensity of 4-5%.

**FIGURE 4 Relation of k and selection intensity in breeding populations**




## SIMULTANEOUS IMPROVEMENT OF TRAITS WITH MULTI-STAGE SELECTION

Farmers seek both risk avoidance and high yield in the maize hybrids and cultivars they select to grow. Hence, resistance to disease and insect pests, tolerance to moisture stresses throughout the growing season, resistance to root and stalk lodging and tolerance to low soil

pH (aluminum toxicity) are among the important agronomic traits for many African farmers. They also have strong preferences for grain texture and color, which must be included in the breeding objectives.

Multi-stage selection is the key to this multi-trait improvement because large numbers of $S_0$ plants and $S_1$ lines can be screened in these two generations. The rate of gain for any trait is reduced when additional traits are selected; hence, selection in early cycles of improvement should be limited to yield and only the most important other agronomic traits. Because correlations among agronomic traits are usually low (Suwantaradon et al., 1975), selection in the early stages usually has negligible effect on the variation among families in the last stage (test cross yield trials).

A recommended scheme with multi-stage selection is shown in Table 6. Selection among $S_0$ plants that are being selfed in season C is the first opportunity to eliminate undesirable material. Because the phenotype of the individual plant is the selection unit, gain will be expected only for traits with high heritabilities, including maturity, disease resistance (if a heavy uniform infection can be achieved), and ear height. In season D, $S_1$ families will be available as the unit of selection which will provide the opportunity to select for less heritable traits such as insect resistance, streak virus resistance, resistance to lodging, and tolerance to low soil pH. Adequate seed will be available for laboratory screening, multiple field plantings and even replication within screening tests if this is required to increase the heritability of the mean rating. $S_2$ lines can be planted in season E in isolation blocks and detasseled to obtain test cross seed. Selection in season F among test crosses should be based primarily on yield, but some selection pressure may be required for resistance to root and stalk lodging. As many as 30,000 $S_0$ and 3,000 $S_1$ lines may be required for reasonable gain in the agronomic traits.

Tolerance to moisture and heat stresses is one of the most difficult traits to improve. Use of higher plant densities in all field plantings appears to be extremely desirable as this intensifies moisture stress. Plant densities for the $S_1$ lines in season D should be especially high because of the reduced plant size due to inbreeding. Allan and Darrah (1978) reported that yields of the H611 (R) C3 variety cross — Kitale II (R11) C3 x Ec573 (R12) C3 — were increased at 33, 44 or 55 thousand plants per hectare when selection was done at 44 thousand plants per hectare. When this reciprocal recurrent selection program was initiated, this plant density in test cross trials was 33% higher than the rate of 33 thousand plants per hectare

**TABLE 6 Recommended schedule for reciprocal recurrent selection-inbred tester**

| | |
|---|---|
| **Year 1** | |
| Season A: | Recombine selected $S_2$ lines |
| Season B: | Recombine $F_1$'s |
| **Year 2** | |
| Season C: | Self and mass select among $S_0$ plants for highly heritable traits |
| Season D: | Plant $S_1$ progeny rows |
| | $S_1$ selection among rows |
| | Self and mass select within rows |
| **Year 3** | |
| Season E: | Produce test crosses of selected $S_2$ lines with inbred tester |
| Season F: | Conduct yield trials of test crosses |

normally used by farmers (Eberhart and Sprague, 1973). A planting date later than the optimum date often intensifies the moisture stresses, or at least varies the growth stages at which the moisture stresses occur in comparison with the optimum planting date.

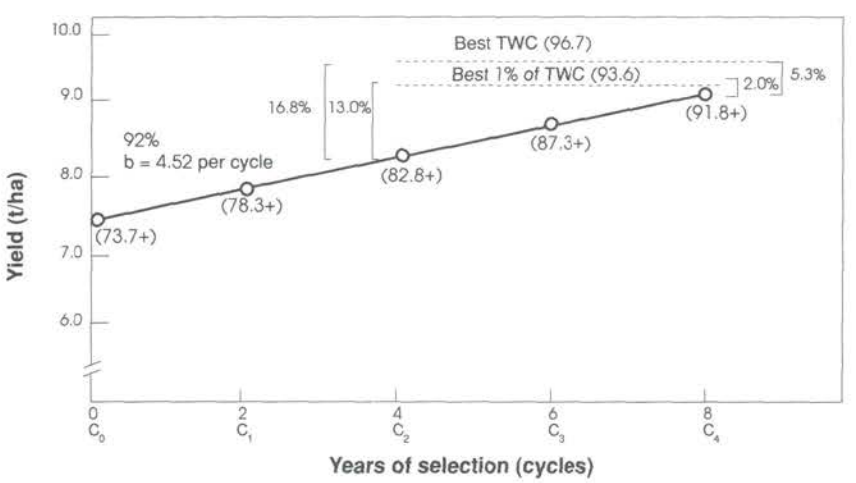
Selection for a higher level of prolificacy reduces the number of barren plants under stress. The Kenyan variety-cross hybrid KCB (F) C4 x KCE (F) C4, with 136 ears per 100 plants at 16 high-altitude sites, was developed by selecting the parental populations for increased prolificacy and yield for four cycles (Darrah, 1976).

## DEVELOPING COMMERCIAL HYBRIDS FROM IMPROVED BREEDING POPULATIONS

The rate of improvement of hybrids will be proportional to the improvement of the population cross between breeding populations (*see* Figure 5). Hence, the efficient development of new hybrids after each cycle of population improvement will be an important phase of the comprehensive breeding system. Procedures normally used in the traditional pedigree breeding system can be used to develop superior hybrids from the improved breeding populations, but greater efficiencies will be obtained by use of the yield evaluation trials from reciprocal recurrent selection as the early testing phase for inbred line development.

In the reciprocal recurrent selection-inbred tester phase, M test cross families will have been evaluated for general combining ability in population A and N test cross families in population B. When the elite m x n hybrids among inbred lines derived from the selected $S_2$ lines are evaluated in an AB factorial mating design, selection of the best hybrid will result in a selection intensity approaching 1/(M x N), rather than 1/(m x n).

**FIGURE 5** **Gain in the H611 (R) Cn variety cross versus extraction and evaluation of three-way crosses. Slope of the H611 (R) Cn line obtained from a separate evaluation of progress from selection and scaled through the common entry H611 (R) C3**



Hybrids involving inbred lines of Stiff Stalk Synthetic (BSSS) from the USDA-ARS/Iowa State University maize breeding program illustrate the potential value of commercial hybrids developed from improved breeding populations. Hallauer (1983) points out the importance of B14 and B37 developed from cycle 0, B73 from cycle 5, and B84 from cycle 7. Hybrids involving B73 have been used extensively not only in the USA, but also in Italy and Spain, and B73 has been used as a source material for developing inbreds in many countries. The best cross between selected $S_2$ lines from cycle 5 outyielded the population cross, BSSS (R) C5 x BSCB1 (R) C5 by 35% (Russell and Eberhart, 1975). Suwantaradon and Eberhart (1974) reported that the best derived SC ($S_2$ x $S_2$) outyielded the variety cross, BSK (S) C5 x BSSS (R) C5 by 18%. Recently, B90 and B91 have been developed from cycles 7 and 8 of the BSCB1, respectively.

Darrah and Penny (1975) extracted inbred lines from the second cycle of Kitale II (R11) and Ec573 (R12) and evaluated TWC hybrids. When the lines were advanced to $S_3$ lines, the best hybrid exceeded the cycle 2 variety cross by 17% (*see* Figure 5). The Kenya Seed Company has produced DC hybrids from later cycle lines of these breeding populations.

Kim et al. (in press) report the development of elite hybrids in Nigeria from inbreds derived from the TZB, TZSR and 7721 improved breeding populations, using traditional pedigree breeding methodologies for developing the inbreds and hybrids.

Use of multiple inbred-line testers with reciprocal recurrent selection increases the effectiveness of developing new inbred lines in comparison with the reciprocal population as tester. When the testers are commercial or pre-commercial inbreds (or SCs), superior hybrids can be identified in the reciprocal recurrent selection yield trials (early testing) and then retested at advanced levels of inbreeding with the same tester. TWC and DC hybrid performance can be predicted from SC data and DC performance from TWC results (Otsuka et al., 1972; Sprague and Eberhart, 1977) :

$$\text{TWC (AB x C)} = (1/2)\,[\text{SC (AC)} + \text{SC (BC)}]$$

$$\text{DC (AB x CD)} = (1/4)\,[\text{SC (AC)} + \text{SC (AD)} + \text{SC (BC)} + \text{SC (BD)}]$$

$$\text{DC (AB x CD)} = (1/2)\,[\text{TWC (AB x C)} + \text{TWC (AB x D)}]$$

Use of $S_3$ and $S_4$ inbred lines in commercial hybrids is strongly recommended over $S_6$ to $S_{10}$ lines to restrict the loss of vigor of the inbred lines. However, cold storage vaults and appropriate inbred line maintenance programs are needed to prevent genetic drift within each line when $S_3$ and $S_4$ levels of inbreeding are used. Once elite inbreds have been developed from the breeding populations, a limited amount of pedigree breeding with $F_2$, first backcross (BC1) and TWC source materials from these elite inbreds may be useful in achieving short-term breeding objectives, especially in seeking disease resistance and stress tolerance. However, the inbreds to be used as source materials must be carefully selected. For a cross (A x B), the mean of all derived $F_n$ lines crossed to a reciprocal tester 'G' can be predicted (assuming negligible epistasis) as follows:

$$(1/2)\,(A \times G) + (1/2)\,(B \times G)$$

Corresponding formulas can be used for BC1 (A x B) A and TWC (A x B) C projects with a tester G, respectively, as follows:

(3/4) (A x G) + (1/4) (B x G)

(1/4) (A x G) + (1/4) (B x G) + (1/2) (C x G)

When information on the relative performance of all (A x G), (B x G) and (C x G) type SC hybrids is available for the traits of interest, the choice of source materials can be made objectively to select a limited number of projects with an improved probability of success.

Multi-stage selection for the key agronomic traits is essential for pedigree breeding projects, as well as for the population improvement program. Because larger numbers of derived $F_n$ lines are required for multi-stage selection, only a limited number of different $F_2$, BC1 and TWC projects will be feasible.

Significant increases in maize production require improved agronomic practices in addition to the improved hybrids. Timely planting dates, optimum planting rate, good weed control and rotation with a legume crop are important factors in maize yields that can be obtained with no cash expenditure. The application of modest levels of fertilizers is needed for further yield increases, which will require a cash input. Although hybrid seed requires a cash purchase each year, it can be used as a lever to get the farmer to adopt the total package.

The performance advantage of hybrids will be realized by farmers only when high-quality hybrid seed is available to them. Seed companies in Zimbabwe, Zambia, Kenya and Nigeria have provided this high-quality seed to their customers at very modest prices.

## DESCRIPTION OF BREEDING POPULATIONS

DMR-ESRY. IITA developed this population by crossing TZSR to downy mildew resistant germplasm from Thailand and the Philippines, with subsequent selection for downy mildew and streak resistance. A yellow version was selected.

Ec573 (R12) C8. The Kenya Kitale Station introduced the Ecuador 573 race collection from CIMMYT. Eight cycles of reciprocal recurrent selection (Kitale II (R11) Cn tester) for increased yield and disease resistance (*H. turcicum*) were completed. This high-altitude population also is highly resistant to *P. sorghi*.

ESALQ-VD-2. This population was developed mainly from Tuxpeño germplasm by the Escola Superior de Agricultura Luiz de Queiroz, Universidade de Saõ Paulo, Piracicaba, Brazil.

ESALQ-VF-1. This population was developed mainly from Caribbean Flint germplasm by the Escola Superior de Agricultura Luiz de Queiroz, Universidade de Saõ Paulo, Piracicaba, Brazil.

GS11 (R) C1. This population was formed by Funk Semillas at Guadalajara, Mexico from Kitale II (R11) C6 by introgressing 12.5% Stiff Stalk Synthetic (RSSC) and 12.5% Tuxpeño planta baja C7. One cycle of reciprocal recurrent selection (ETO tester) was completed. Available from the USA National Plant Germplasm System (PI 520764).

GS12 (R) C1. This population was formed by Funk Semillas at Guadalajara, Mexico from Ec573 (R12) C6 by introgressing 25% CIMMYT ETO Leaf and Tassel population. One

cycle of reciprocal recurrent selection (GS11 tester) was completed. Available from the USA National Plant Germplasm System (PI 520765).

KCB (F) C4. The Kenya Kitale Station developed this population from a wide range of Kenya Flat White maize strains and elite inbred lines. This was followed by an introgression of 25% Ecuador 573. Four cycles of full-sib selection for prolificacy and increased yield have been completed.

KCE (F) C4. The Kenya Kitale Station developed this broad base population from Kitale II and other improved Kenya Flat White maize varieties, elite inbred lines from Kitale II, Ec573, Cometico, Chalqueño, Colombian inbred lines, Costa Rica 77, Costa Rica Comp., and collections from the Oloton, Jala, Montaña, Amagaceño, Tuxpeño, Celaya and Cuzco races.

Kitale II (R11) C8. The Kenya Kitale Station developed this population from the Kitale Station strain of Kenya Flat White maize by one cycle of half-sib selection and eight cycles of reciprocal recurrent selection, Ec573 (R12) Cn tester, for increased yield and disease resistance (*H. turcicum* and *P. sorghi*). Kenya Flat White maize was derived from Kickory King, White Horsetooth, Ladysmith White, Salisbury White, Champion White Pearl and Iowa Silver Mine, which seem to have been derived from Tuxpeño source material.

Mayorbella (R) C3. This Puerto Rico variety was improved with three cycles of reciprocal recurrent selection (Diente de Caballa tester) for increased yield and disease resistance at the USDA-ARS Tropical Agricultural Research Station, Mayaguez, Puerto Rico.

Pop. 21-SR. IITA/CIMMYT developed this maize streak-resistant population from CIMMYT Pop. 21 in a BC project. Pop. 21 included the Tuxpeño race collections Veracruz 48, Veracruz 143, Veracruz 174, Michoacan 137, Michoacan 166, V-520C, Colima group 1-Mix. 1 and Pool 24 (Tuxpeño germplasm).

Pop. 22-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 22 in a BC project. The components of Pop. 22 were Tuxpeño, ETO, Antigua group 2, USA hybrids, Compuesto Centro-Americano, lines from El Salvador, V-520C, Nicarillo, and Pool 24 (Tuxpeño germplasm).

Pop. 28-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 28 in a BC project. Components of Pop. 28 include Tuxpeño, Caribbean, Brazilian germplasm, ETO and Pool 26.

Pop. 29-SR. IITA/CIMMYT developed this streak resistant population from CIMMYT Pop. 29 in a BC project. The components of Pop. 29 were Tuxpeño, Cuban Flints and ETO.

Pop. 31-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 31 in a BC project. Components of Pop. 31 include 96 families from Compuesto Selection Precoz C8, crosses of tropical and temperate materials, and four families from Pool 17 (Tropical Early Yellow Flint).

Pop. 35-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 35 in a BC project. Components of Pop. 35 include Republica Dominicana groups 2, 3, 7, 8, 9 and 15 crossed with Antigua Group 2.

Pop. 36. CIMMYT developed this population from 165 accessions from all the Caribbean Islands. Families from IDRN, Pool 22 (Tropical Intermediate Yellow Dent) and Pool 26 (Tropical Late Yellow Dent) were subsequently introgressed to form Pop. 36.

Pop. 43-DMRSR. IITA/CIMMYT developed this maize streak virus resistant, downy mildew resistant population from CIMMYT Pop. 43 (La Posta) in a BC project. Pop. 43 was formed from 16 elite inbred lines from the Tuxpeño race.

Pop. 44-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 44. Pop. 44 is the advanced generation of a cross of American Early (from Egypt) and Tuxpeño planta baja.

Pop. 49-SR. IITA/CIMMYT developed this maize streak resistant population from CIMMYT Pop. 49 in a BC project. Pop. 49 originated from initial selection of 240 fill-sib families from Tuxpeño Crema -1 planta baja cycle 17 (derived from Pop. 21).

Suwan 1-SR. IITA developed this maize streak resistant population from Suwan 1 in a BC project. Suwan is a broad base population developed by Kasetsart University, Bangkok, Thailand. Components are mainly elite Carribean varieties but Tuxpeño, Salvadoreño and USA germplasm were also included in the original population (Thai Comp. #1). After three cycles of $S_1$ selection, a BC project ($BC_3$) was completed to obtain downy mildew resistance with Philippine DMR1 and DMR5 as sources of resistance to develop Suwan 1. Subsequent cycles of $S_1$ selection were completed with emphasis on grain yield and other desirable characters.

TZB-SR. This broad base population was formed by IITA from Nigerian Comp. B (Tuxpeño germplasm) and Nigerian Comp. A (Caribbean Flint germplasm). Several cycles of $S_1$ selection for increased yield were completed. Streak resistance was obtained in a backcross project.

TZESR-Y. IITA developed this population by crossing some local varieties with introduced early maturing varieties with TZSR-Y. A yellow version was selected.

TZMSR-W. This maize streak virus resistant population was formed by IITA from the cross of TZSR with the best available varieties and hybrids from East, Southern and Central Africa.

TZSR-W-1. This streak resistant population was developed by IITA. Tuxpeño planta baja was the base population, with subsequent introgression of TZB, Pop. 21 and Pop. 22 x TZSR, and streak resistant plants from TZ-yellow.

TZSR-Y-1. This maize streak resistant population was developed by IITA. Tuxpeño planta baja was the base population with subsequent introgression of TZB, Pop. 21, 28, and 32, 096EP6, N28, Pop. 22 x TZSR, and streak resistant plants from TZ-yellow and IB32 x La Revolution. A yellow version was developed by selection.

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# 3.7

# *Germplasm Diversity in Bambara Groundnut and Prospects for Crop Improvement*

A.E. GOLI, F. BEGEMANN and N.Q. NG

Bambara groundnut is an old crop in tropical Africa with a high nutritional value. The dried seed contains 54.5-69.3% carbohydrates, 17-24.6% protein and 5.3-7.8% fat (Oliveira, 1976; Platt, 1965) and provides 367 to 414 Kcal per 100 g. The protein is of a high quality and is especially rich in lysine. Cultivation of bambara groundnut, which is undoubtedly of African origin (Doku and Karikari, 1971), was flourishing before the introduction of the now common groundnut of American origin (Rassel, 1960), but very little research has been devoted to it and, at present, it has no real commercial value. Until recently, there was no substantial reservoir of genetic materials to serve as a starting point for improvement of the crop. The International Institute of Tropical Agriculture (IITA), which has nearly 2,000 accessions in its genebank, is the major center for bambara groundnut germplasm. This paper reviews the taxonomy, origin and geographical distribution of bambara groundnut and discusses the scope of variability and yield potential of the germplasm maintained at IITA.

## TAXONOMY

Bambara groundnut belongs to the family Leguminosae, subfamily Papilionoideae, but further refinement of its taxonomic position has been the object of much controversy. According to Begemann (1988), the crop was first mentioned in the literature by Marcgrav de Liebstad in 1648, who referred to it as 'mandubi d'Angola'. In 1763, Linnaeus described the plant and gave it the botanical name *Glycine subterranea*, in accordance with his system of nomenclature. In 1806, Du Petit-Thouars found the crop growing in Madagascar under the vernacular name 'voanjo', subsequently pronounced 'voandzou' by the French. He adapted this to make the generic name *Voandzeia* to which he added Linnaeus' epithet. The scientific name then became *Voandzeia subterranea*.. This nomenclature was widely accepted by later workers, such as Harms (1912), Dalziel (1937), Jacques-Felix (1946, 1950), Hepper (1963)

and Doku (1968). As recently as the 1970s, Maréchal et al. (1978) undertook detailed botanical studies and found great similarities between bambara groundnut and plant species of the genus *Vigna*. Verdcourt (1978, 1980) found that their results corroborated those of his own study and seized the opportunity to propose the generic name *Vigna*. Henceforth, the accepted name of bambara groundnut was *Vigna subterranea* (L.) Verdc. Comb. Nov.

Although the exact place of origin of bambara groundnut is debatable, all investigators interested in the subject (Harms, 1912; Dalziel, 1937; Jacques-Felix, 1946; Rassel, 1960; Hepper, 1963) agree that the crop originated on the African continent. The name itself suggests a West African origin, since Bambara is the name of a district near Tombouctou in Central Mali. Key evidence to support the assignment of the origin of a plant species to a given area is the occurrence of its spontaneous or wild forms in that area, but such evidence has not yet been found in Mali, although Guillemin et al. (1832) reported the plant as probably growing wild in nearby Senegal. Rassel (1960) believed that the plant originated from Madagascar because it was seen growing there in the early 19th century (Du Petit-Touars, 1806). De Candolle's statement (1886) that some travelers found the plant growing wild on the banks of the Nile was later proved false (Hepper, 1970). Bambara groundnut in the true wild state was found in 1909 in North Yola province of Nigeria by Dalziel, who reported that Ledermann also found the wild plant the same year near Garoua in northern Cameroon. Their findings were confirmed when, in 1957, Hepper collected a wild strain in Nigeria, from the same area where Dalziel had found the plant.

The distribution of wild bambara groundnut is now known to extend from the Jos Plateau and Yola, in north-east Nigeria, to Garoua, in north Cameroon. As confirmation, Howell (pers. comm.) collected the wild strain in 1987 near Yola in Nigeria. Pasquet (pers. comm.) has also collected wild strains in northern Cameroon. The occurrence of all these wild forms supports Hepper's belief that the site of origin of bambara groundnut lies between north-eastern Nigeria and northern Cameroon.

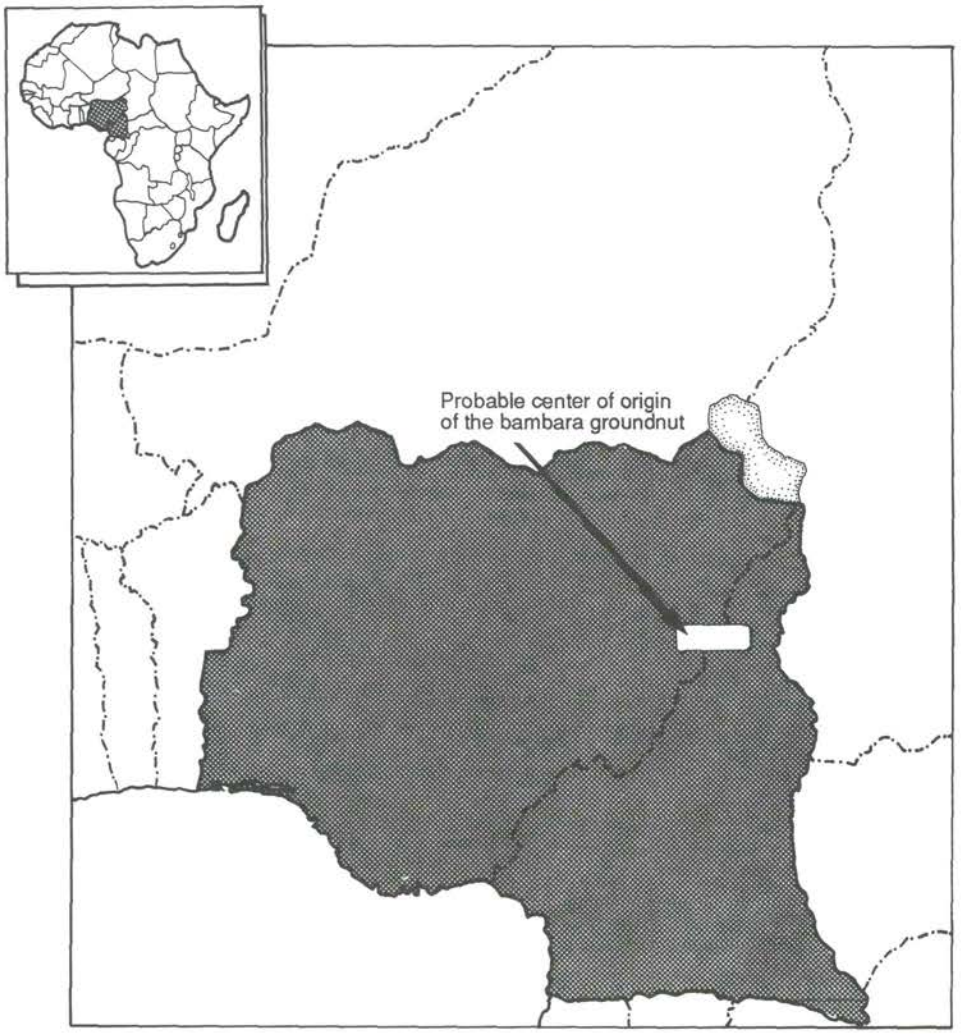
As further confirmation, Begemann (1988) carried out detailed analyses of the seed-pattern diversity and the variations of some qualitative characters in the collection maintained at IITA. Accessions from West Africa in general and Nigeria/Cameroon in particular were compared with those from East Africa in general and Zambia/Zimbabwe in particular. The seed-pattern diversity indices were determined by the Shannon-Weaver formula (Shannon and Weaver, 1949). The greatest seed-pattern diversity was concentrated in West Africa. All seed patterns exist in West Africa, although dotted seeds are less frequent. Apart from light and dark plain and dotted seeds, all other patterns are rarely found in East Africa. Samples collected less than 200 km from the putative center of origin between Yola and Garoua consistently show a great seed-pattern diversity. All major seed patterns occur frequently within the area.

Analysis of plant and seed quality characters also reveals more diversity among accessions from West Africa and particularly from Nigeria/Cameroon. Diversity indices for the number of days to maturity, pod length, pod width, number of stems per plant and internode length were comparatively higher for accessions from Nigeria and Cameroon.

In conclusion, the seed-pattern diversity, as well as the diversities of quantitative characters, confirm Hepper's hypothesis that the center of origin of bambara groundnut is in the region of north-eastern Nigeria and northern Cameroon (*see* Figure 1).

From the center of origin, bambara groundnut has been dispersed throughout tropical Africa, from Senegal to Kenya and from the Sahara to South Africa. The crop is usually grown for home consumption but in some countries, such as Nigeria and Cameroon, large quantities

**FIGURE 1 Probable center of origin of bambara groundnut**




Source: Begemann, 1988b.

of the fresh pods are often sold on local markets. Attempts at canning in Ghana and Zimbabwe did not significantly boost production of the crop. Outside the African continent, bambara groundnut has been reportedly carried as far as India, Sri Lanka, Indonesia, Malaysia, New Caledonia and South America, especially Brazil (Rassel, 1960), but overall it seems that the degree of cultivation outside Africa is insignificant.

## VARIABILITY WITHIN THE IITA COLLECTION

The accessions held at the IITA genebank exhibit an impressive variability at the plant level as well as at the seed level. In 1986, a total of 1,379 accessions were planted in Ikenne and

Ibadan in Nigeria for characterization and evaluation. There are still some 600 accessions, mainly from later collecting missions, to be characterized. The extent of variability is evident from the following outlines of some selected characters.

### Days to maturity

The number of days from planting to maturity varies from 90 to 165, but most of the accessions mature between 115 and 135 days after planting. The merit of early-maturing varieties is that they escape late-season fungal diseases, especially in humid ecologies.

### Growth habit

Bambara groundnut plants are classified as having a bunch, semi-bunch or spreading growth habit. In the first group, short stems and internodes produce plants with tightly clustered leaves. Such a configuration is advantageous during harvesting because most pods remain attached to the stem crown after the plant is pulled up. Detached pods lie in a cluster underneath. With spreading growth habit, stretching stems with usually long internodes give a much larger diameter to the plant foliar crown. Canopy width can reach 100 cm, as against the average of 30 cm in the bunch growth habit group. Harvesting becomes more difficult, and yield loss increases because most of the mature pods remain spread in the ground. Plants of semi-bunch growth habit lie between the two extreme cases.

### Leaflet shape and size

Narrow and lanceolate as well as broad and roundish leaflets exist in the bambara groundnut collection: about 8% of the accessions have narrow leaflets and 14% broad. The bulk of the accessions have intermediate oval leaflets. Cultivars with broad leaflets may be preferred when the crop is grown for foliage. No comparative study has been conducted to determine whether the leaf area index (LAI) associated with the broad-leaflet genotype is of any significant advantage in terms of yield or dry-matter production.

### Shell thickness

The average shell thickness in the collection is about 0.35 mm, but some accessions have shells as thick as 2 mm. Unlike cowpea, bambara groundnut is not seriously attacked by pod borers, but the thick shell may provide protection against attack by insects in the field and during post-harvest storage. Interestingly, a positive correlation has been found between shell thickness and seed yield (Goli et al., unpubl.).

### Seed coat patterns

Patterns and color of seed testa and eye are extremely variable in bambara groundnut. Plain, mottled, dotted or striped seed coats are found. Eye pattern ranges from eyeless seeds to very

prominent, butterfly-like eye. The color for both testa and eye ranges from milky to black through all the shades of brown and red. Whether consumers have a preference for a particular seed coat has not been established. Farmers tend to grow varieties with a plain whitish seed coat.

### Number of seeds per pod

Most pods contain only one seed but a few genotypes have two-, three-, or even four-seeded pods. It was observed during plant explorations that cultivars grown in Cameroon and, in particular, in the Congo, tend to have more seeds per pod (Goli, 1987, 1988). The length of the pod is generally proportional to the number of seeds it contains, and as a result, there is no negative correlation between seed size and number of seeds per pod. However, the number of pods per plant tends to decrease as the number of seeds per pod increases.

### Seed size

Seed size, measured by the weight of 100 seeds, averages 42 g for the accessions evaluated. Values as high as 98 g were obtained for some genotypes. Larger seeds contribute to higher seed yield, as shown by a significantly positive correlation between the two traits (Goli et al., unpubl.). No studies have yet been carried out on consumer preferences regarding seed size or the relationship between seed size and cooking time or chemical composition.

### Viral and fungal diseases

Attacks by viral diseases are widespread in most environments, especially in areas where other grain legumes such as cowpea are grown. Common diseases are cowpea mottle virus (CMeV) and cowpea aphid-borne mosaic virus (AbMV). A combination of unusually heavy virus attacks and Cercospora leaf spot on one particular accession (TVSu 218) resulted in zero yield during a trial at Kaboinse, Burkina Faso (IITA, 1988). However, not all the accessions are highly susceptible; data from a field evaluation in Ikenne indicated that there was little or not effect on up to 11% of the accessions.

Fungal diseases can cause significant damage in wet environments. The most fungal diseases are Cercospora leaf spot, Fusarium wilt, Sclerotium rot and Rhizoctonia blight. In a field experiment at IITA, up to 40% of the pods were found to be rotten as a result of fungal attack when the crop was harvested late, at a time when humid conditions prevailed (IITA, 1988).

### Yield potential

Because of its relative resistance to diseases and insect pests, bambara groundnut could be a stable, low-cost and profitable food crop. Unfortunately, its cultivation remains marginal in most countries lying in its zone of adaptation and thus no substantial effort has been made to improve crop productivity. With no inputs, average yield at the peasant level ranges from

300 to 800 kg/ha, but during trials conducted at IITA and at Mokwa, Nigeria, yields from the best accessions were as high as 3,500 kg/ha (Goli and Ng, unpubl.). With sound cultural practices, such as optimum plant density and soil fertilization, yields could probably be higher in accessions which have been carefully selected for specific environments.

## PROSPECTS FOR IMPROVEMENT

There is ample room for the improvement of bambara groundnut, in terms of agronomy as well as breeding.

It is clear that accessions in the collection do not have the same adaptability in different environments — an accession that performs well in the forest zone may give poor results in dry savanna areas, for example — and thus preliminary efforts should be made to regroup ecotypes of comparable adaptability. As growth habit is such an important trait in bambara groundnut, a more objective, unequivocal system should be developed to classify the various morphotypes; recommendations concerning plant density, for example, should be based on a cultivar's growth habit. Following the ecological and morphological classification, optimal cultural practices should be laid down for maximum productivity. Adequate plant density, level of soil fertilization, planting date and level of plant protection should be established for some basic environments.

Breeding is obviously the ultimate tool for crop production improvement. It should be emphasized that genetic improvement of a crop is possible only when the genetic base is sufficiently broad, as seems to be the case with bambara groundnut. Furthermore, single plants from some accessions should be selected in order to utilize the potential still hidden in the heterogeneity of these samples. Breeding strategies involving crosses between accessions could be used to increase the yield potential or to incorporate valuable genes into desirable cultivars. Unfortunately, no reports on such experiments are available. The underground nature of the pods makes artificial hybridization difficult. An attempt was made at IITA to intercross bambara groundnut genotypes differing in seed coat and petiole color; hand pollination was carried out at different times of the day. However, these efforts were unsuccessful and more elaborate techniques may be needed to obtain positive results.

## CONCLUSION

Bambara groundnut needs more publicity, both as a crop and as a food. Even in tropical Africa, few people in the forest zone are aware of its existence. Peasants should know that it is a low-cost, dependable crop that grows in harsh environments where many other crops fail, and to this end production figures need to be collected from all producing countries to give an idea of the crop's present importance. The fact that it is a highly nutritious food should be made known to the general public and, in particular, to people with limited resources in rural areas. However, the general mode of consumption of bambara groundnut is not appetizing; in many countries, the whole fresh pods are boiled until completely cooked and the beans are eaten plain, often as a snack. Food technologists and experts in the culinary arts should develop more diverse and appetizing ways of consuming the beans.

Problems associated with grain conservation could arise should the crop become popular and production increase. One way of overcoming these problems would be to initiate mass

processing (such as canning, which is being undertaken in Ghana). Other forms of processing should be encouraged in order to distribute the product to non-producing areas.

A commodity without a market is not viable, and that is precisely why bambara groundnut is still a subsistence crop. Prospective large-scale growers must be able to sell their product in order to survive. Government agencies or private processing companies which buy the beans at a fair price should be established. A growth in the number of consumers would make production more sustainable.

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# 3.8

# *Soybean Germplasm Diversity, Uses and Prospects for Crop Improvement in Africa*

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The total annual production of soybean, *Glycine max.* (L.) Merr., in Africa has increased gradually over the past 10 years. Breeding programs in Zimbabwe, Zambia, Zaïre, Cameroon, Ghana, Côte d'Ivoire and Nigeria have had extensive variety testing programs and have released a number of improved varieties. Although these varieties are good, significant improvements are needed and can be made.

The main objective of this paper is to describe useful traits that could be incorporated into improved varieties and identify germplasm sources that have the desired traits. All the germplasm described here is available from IITA and will be supplied to scientists upon request. For elaboration on the breeding methodologies mentioned, the reader should consult the list of references.

## SEED VIABILITY, NODULATION AND SHATTERING

In the more humid regions of tropical Africa, farmers often find that germination of their soybean seed is very poor. One of the main reasons for this is that the seed has lost its ability to germinate during storage. A number of varieties have been developed that have improved seed storability, including TGx 342-345D, TGx 536-01D, TGx 709-01E and TGx 923-2E (IITA,1981, 1984, 1986). Breeding methodologies for improving seed storability have been well described (Kueneman and Wien, 1981).

Most soybean varieties have poor or no nodulation when grown in Africa without being inoculated with *Rhizobium japonicum.* However, in 1977 and 1978, 400 soybean lines were tested and 10 were found to have good nodulation at all five test locations in Nigeria (Pulver et al., 1985). Many improved varieties have been developed that are able to nodulate well without inoculum (TGx 814-49E, TGx 539-5E, M-351, Samsoy 1 and Samsoy 2) (Dashiell et al., unpubl.).

If the weather is hot and dry at the stage when the soybean crop is maturing, most varieties will quickly shatter. Lines which have good resistance to shattering include TGx 995-22E, TGx 923-2E and TGx 984-2E (Dashiell et al., unpubl.) A reliable screening technique for evaluation of shattering resistance has been used to develop and evaluate these varieties (IITA, 1985).

## SOYBEAN DISEASES

Bacterial pustule, caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, occurs in most soybean-growing areas in Africa. Many varieties show good resistance to this disease. The variety TGx 1025-8E had the best resistance to bacterial pustule in trials conducted in Nigeria in 1987 (Dashiell et al., unpubl.).

Frog eye leaf spot, caused by *C. sojina* Haro, is becoming a major problem in some areas of Africa, but some good breeding lines with good resistance are available (TGx 996-28E, TGx 1019-2E and TGx 1083-24E) (Dashiell et al., unpubl.).

Red (or *Pyrenochaeta*) leaf blotch is caused by the fungus *Dactuliochaeta glycines* (Stewart) Hartman and Sinclair (synonym *Pyrenochaeta glycines* Stewart) (Hartman and Sinclair, 1988). Severe leaf blotching and up to 75% defoliation were reported on soybean grown at the Jimma Agriculture Research Station in Ethiopia (Stewart, 1957). The disease has since been reported from Central, East and Southern Africa, including Cameroon, Malawi, Rwanda, Uganda, Zaïre, Zambia and Zimbabwe (Hartman et al., 1987); in Southern Africa, particularly in Zambia and Zimbabwe, the incidence of the disease has increased concomitantly with production of soybean. The disease and its causal fungus have been reported on only one other host, a perennial relative of soybean, *Neonotonia wightii* (Arnott) Lackey, in Ethiopia, Zambia and Zimbabwe.

In the mid-1970s, red leaf blotch was reported as a potentially serious disease of soybean in Zambia when severe defoliation was recorded in field plots (Javaid and Ashraf, 1978). In 1977, a 50% reduction in yield as a result of the disease was reported from Zambia and in 1984 estimated yield losses there ranged from 7 to 37% in the medium- to late-maturing cultivars. In 1985, on the basis of fungicide trials, it was estimated that a 34% yield reduction occurred over approximately 25% of the growing area of Zambia (Hartman et al., 1987). In Zimbabwe in 1982 red leaf blotch reportedly occurred throughout the soybean-growing area, with yield losses ranging from 10% to 50% in Mashonaland Province (Levy, 1987). Most recent reports from researchers working in these two countries has continued to confirm the devastating effect of red leaf blotch on soybean yields.

The fungus causes lesions on foliage, petioles, pods and stems throughout the growing season (November to April in Zambia and Zimbabwe). Lesions are often associated with the primary leaf veins and are visible as dark red to brown, circular to angular spots, 1-3 mm in diameter, initially on unifoliolate leaves. The lesions enlarge and may coalesce to form irregular blotches, 3-10 mm in diameter, with buff-colored centers and dark margins. The lesions merge to form large necrotic blotches up to 2 cm in diameter. Necrotic tissue frequently drops out, giving a shot-hole appearance to severely infected leaves. Diseased plants may defoliate prematurely and senesce 5-10 days before normal maturation. As lesions enlarge, sclerotia develop primarily on the lower leaf surface, but occasionally on the upper leaf surface. Pycnidia are formed primarily within the blotches on the upper leaf surface, although some form on the lower surface.

Stewart described the fungus by morphological characteristics, including the dimension and shape of conidia, pycnidia and pycnidial setae. Leadey (1964) described the sclerotia as dark brown with external bristles and internally colorless and undifferentiated. Sclerotia collected from lesions on soybean leaves or screened from soil of a soybean field will germinate in 1-3 days on culture media, in water or on moistened filter paper. Sclerotia germinate directly by producing hyphae, which grow and give rise to pycnidia; sclerotia may produce additional pycnidia on their outer surfaces. The fungus can be cultured *in vitro* and will produce pycnidia on common nutrient media.

The disease frequently develops on soybean planted in newly cleared land (Hartman et al., 1987). The source of primary inoculum for such epidemics has not been shown. Primary inocula may be conidia or sclerotia from infected plants of *N. wightii* or from other unknown alternative hosts previously established on virgin land or growing adjacent to new soybean fields. A host range study has not been reported. Inoculum may be introduced into new fields either by cultivation, seed transmission, wind storms moving soil peds, conidia, or other means. Dispersal from field to field probably is probably effected viasclerotia, as they are moved easily in soil during routine farm operations. The microflora from seeds of diseased soybean plants in Zambia were assayed and *Dactuliochaeta glycines* was not recovered from them (Hartman et al., 1987). However, incidental transmission of the pathogen may be through seed lots contaminated with infected plant debris or soil peds carrying sclerotia of the fungus.

Chemical methods for the control of red leaf blotch have been studied in Zambia and Zimbabwe (Hartman et al., 1987; Levy, 1987). In addition, a concerted effort has been made to evaluate germplasm and breeding lines for resistance to the pathogen. In field trials in Zambia and Zimbabwe, conducted between 1982 and 1984, more than 2,500 lines, cultivars, accessions and breeding lines were evaluated for resistance. Most commercial cultivars now grown in the USA and included in these trials proved to be susceptible. When symptoms on late- and early-maturing cultivars were compared at equal growth stage, both types were susceptible and had similar disease severity ratings. More research on cultural practices to control the disease, such as date of planting, long rotations, plant spacing and varying tillages, must be done before a recommendation can be made. Some farmers use longer maize rotations when red leaf blotch is a serious problem, but this practice does not always alleviate the disease.

In Zambia, fentin acetate effectively controlled disease, which resulted in higher yields than in non-sprayed plots from 1982 and 1985. During the 1983-84 and 1984-85 growing seasons, soybean in plots sprayed with one or more applications of fentin acetate at 0.9 or 0.6 kg a.i./ha had significantly ($P<0.5$) higher total grain yields and seed weights than plants in non-sprayed plots.

Based on observations in Zambia and Zimbabwe, red leaf blotch can limit soybean production. The disease has been known in the region since 1956, but did not become economically significant until the early 1970s when soybean production intensified. Red leaf blotch is the most important disease of soybean in that region. The disease is probably also economically important in Ethiopia and Zaïre, but there are no published reports on yield losses from these countries. Most germplasm lines tested are susceptible, although some have been reported resistant in Zimbabwe. In general, all lines tested at Mpongwe, Zambia, where the disease pressure is great, are susceptible. Finding resistance in other accessions that have not been tested and in exotic lines would provide soybean breeders with germplasm to develop resistant cultivars.

## SOYBEAN INSECT PESTS

Until recently, most records showed that insect pest depredation on soybean was not a serious problem in most parts of Africa. However, increased soybean hectarage coupled with recent climatic aberrations has resulted in increased pest incidence at economically damaging levels. For example, during the past three years there has been unprecedented infestation of soybean by grasshoppers (IITA, 1986) and more recently by lepidopterous defoliators, mainly loopers and armyworms.

The most important pests of soybean in Africa are: stink bugs, particularly *Nezara viridula* in Nigeria (Ezueh and Dina, 1979; Jackai et al., 1985); semi-loopers, *Trichoplusia orichalcea*, *Chrysodexis chalcites* and *C. acuta* (Taylor, 1980); and *Heliothis armigera* (Khamala, 1978). Leafhoppers and foliage beetles attack soybean in the Plateau and Benue States of Nigeria, respectively, but the associated yield losses appear to be insignificant. In storage, soybean seeds are not easily attacked by pests, but there are clear indications that once the coat is broken (for example, during the making of flour), soybean becomes predisposed to damage by various insect pests (Williams, 1986). Overall yield losses on soybean are still lower in Africa than in Asia (Talekar, 1987), although at one location in Nigeria stink bugs alone have caused up to 60% yield reduction at one location and, at another, a 20% reduction (Jackai and Singh, 1987).

Research efforts are directed mainly at the development of varieties with sufficient resistance to the existing pests, particularly stink bugs and defoliators, so as to maintain the status quo where many growers do not use insecticides. A few lines have already been identified with field resistance to *Nezara viridula* (Jackai et al., 1988). The variety with multiple insect resistance, PI 171444 (Talekar, 1987), has also been shown to be resistant to *N. viridula* and the leafworm, *Spodoptera littoralis* (Jackai et al., unpubl.; Ojo, 1988). A systematic breeding effort is underway to incorporate this resistance into elite genotypes. Using recently developed assays for both pests, the entire soybean germplasm available at IITA will be screened for other possible sources of resistance. It is hoped that pest-resistant varieties will ensure that farmers can continue to grow soybean without recourse to insecticides.

## SOYBEAN AS A FOOD

Africa's serious malnutrition problem is especially acute in terms of protein deficiency. Livestock constitute the major source of protein for human consumption, but a combination of factors — including the recent persistent drought, the fall in foreign exchange earnings and the poor performance of indigenous animals — have led to situation where the prices of such conventional livestock products as meat, eggs and milk have risen beyond the reach of the ordinary man. There is, therefore, an urgent need to seek alternative sources of high-quality, cheap protein. Soybean has the potential to meet part of this need.

In many Far Eastern countries, soybean has been used as food for centuries and its exceptionally good nutritional value is well known. Elsewhere, since the early 1950s special formulae utilizing isolated soy proteins have been developed for use in hospital diets, particularly for post-operative diets, and soyflour, protein concentrate and isolates have been incorporated into infant foods, such as rice- and wheat-based foods, in order to increase the protein content.

### Food analogs

Several meat-like products (or analogs) are being produced using soy flour, soy concentrates and isolated protein. These usually contain other sources of protein such as wheat gluten, egg albumin and yeast.

### Dairy-type foods

In addition to the use of soy protein products as replacements of non-fat dry milk in baked goods and the use of isolated soy protein infant formulas, soy proteins are also used in such products as non-dairy coffee whiteners. They are also being used in evaporated milk products.

### Use in meat products

One of the major uses of isolated soy proteins is as complementary protein to the meat proteins in emulsified meat products, such as frankfuters, not only because of their moisture-binding, fat-emulsifying and emulsion-stabilizing properties but also for their flavor and nutritional characteristics. The use of soy protein products allow meat emulsions to be prepared in a wider range of emulsion temperatures than is possible with meat proteins only.

### Use in baked products

For many years, soy proteins have found favor in the baking industry. Low levels of full-fat soy flour added to wheat flour at levels of 0.5%-1% of the wheat flour allows the production of bread which has increased crumb softness and improved keeping quality. Soy flour is also used at varying levels in doughnut and cake mixes. The soy flour helps to regulate the amount of oil absorbed during the frying stage in doughnut production. Soy flour is now commonly used in some varieties of crackers to the level of 2-5% of the total ingredient weight.

### Oil

Soybean oil has a composition similar to other vegetable oils, such as groundnut and sunflower. It is highly digestible and has a high degree of unsaturation, containing about 85% unsaturated and 15% saturated fatty acid, making it especially suitable for people who have a high level of blood cholesterol and are therefore highly susceptibile to cardiovascular disease.

It is believed that the use of polyunsaturated fats in the diet reduces the level of cholesterol in the blood. Cholesterol, a fat-like substance, is found in all animal tissue. It is also believed that when too much cholesterol has accumulated in the blood, it is deposited along the walls of the arteries, making the arteries narrower. This may increase the chances of a blood clot forming and the possible occurrence of a heart attack. Consumption of soybean oil is one way of preventing cholesterol accumulation in the blood.

Soybean oil also has a high percentage (60%) of the essential fatty acids (EFA) but the presence of large amounts of linoleic acid in soybean has been implicated in the development of the undesirable 'beany' flavors in the stored oil and in food products containing the oil. However, hydrogenation lowers the linoleic acid content.

## Processing problems

Soybean, like most legumes, contains some biologically active substances in their raw state; these substances are also referred to as antinutritional factors. Examples of such substances are lipoxygenase, trypsin inhibitors, phytic acids, tannins and haemaglutinin. Lipoxygenase and trypsin inhibitors pose particular problems.

### *Lipoxygenase*

One of the most important factors limiting the acceptance of soybean products is the 'beany' flavor problem (Wolf, 1975). This flavor is hardly evident in the raw whole beans but develops after the breakdown of the cell structure and is still evident after cooking (Nandaine et al., 1987). After the rupture of the soybean cell, the inactive lipoxygenase is activated by its contact with oxygen, and the enzyme lipoxygenase then catalyses the oxidation by molecular oxygen of polyunsaturated lipids containing a cis-cis 1:4 pentadiene moiety, resulting in rancid off-flavors and poor storage stability (Arai et al., 1970; Hinchcliffe, 1975). The oxidation products further decompose to middle chain aldehydes and alcohols, and these are major contributors to the undesirable beany flavor and sometimes bitter taste in soybean products (Fujimaki et al., 1965; Wolf, 1975).

It is now known that normal soybean seeds contain at least three lipoxygenase isozymes, called L-1, L-2 and L-3 (Yamamoto et al. (1970), and these isozymes are responsible for the beany flavor. In order to deactivate lipoxygenase to improve the flavor of soybean products and enhance their acceptability, several methods have been investigated. Eldridge et al. (1977) reported the possibility of deactivating lipoxygenase in soybeans by soaking them in ethanoic solutions at 25°C for 24 hours. Borhan and Snyder (1979) used a combination of heat and ethanol to destroy lipoxygenase. Other methods which have been used include pH adjustment and the addition of anti-oxidants (Baker and Mustakas, 1973; Nelson et al., 1976) and decomposition of beany flavor by aldehydedehydrogenase (Sasaki et al., 1982).

However, these treatments are expensive to implement on a commercial scale and, in any case, are not entirely satisfactory because the heat treatment used lowers the protein solubility. If the lipoxygenase can be reduced genetically in the seeds, then the deactivation of the enzymes will be easier to accomplish commercially. Fortunately, efforts in this area have met with some success. Several workers, including Hildebrand and Hymowitz (1981), Kitamura et al. (1983) and Kitamura (1984), have found three types of mutants lacking L-1, L-2 and L-3 and have shown that the absence of these isozymes from the seeds is under the control of single recessive alleles.

### *Trypsin inhibitors*

Another area where germplasm diversity is being exploited to improve soybean processing and enhance its nutritional value and acceptability is in finding lines that are free of Kunitz

trypsin inhibitor, which interferes with protein utilization. Efforts to reduce or eliminate this inhibitor have included autoclaving, fermentation, sprouting and direct heating (Oke, 1987; Ologhobo, 1980; Liener, 1962; Aykroyd and Doughty, 1982). As with lipoxygenase, these efforts have not been particularly successful, largely for the same reasons.

However, Hymowitz and Bernard, collaborating scientists in the International Soybean Program (INTSOY), have recently announced the development of three soybean lines that are free of the Kunitz trypsin inhibitor. These lines may reduce the cost of processing needed to make soybean suitable for human food and animal feed.

## EXOTIC GERMPLASM AND SOYBEAN IMPROVEMENT IN AFRICA

One of the most important issues that must be addressed in a breeding program is whether there is sufficient genetic diversity within the germplasm pool to allow improvement of traits that will ultimately lead to the development of superior cultivars (Dudley and Moll, 1969). The use of exotic germplasm in soybean breeding programs would increase genetic variability in the breeding populations, and Vello et al. (1984) observed that it may enhance genetic improvement for yield beyond levels possible with local germplasm.

Genetic variability studies in soybean involving crosses between domestic and exotic germplasm have been made by Thorne and Fehr (1970), Shoener and Fehr (1979), Khalaf et al. (1984) and Vello et al. (1984). All these studies showed increased genetic variability in crosses of exotic and domestic germplasm. The studies by Khalaf et al. (1984) also showed there was greater variability for yield in populations where the exotic cultivar contributed 25% to the cross (that is, a three-way cross of [domestic x exotic] x domestic). Most of the studies showed that a greater number of superior lines were identified from the more variable populations than from the less variable populations.

The use of exotic soybean germplasm in most breeding programs has depended primarily on their being sources of resistance genes to diseases and insect pests or of certain nutritional quality traits. Such traits are usually controlled by one or a few genes and can easily be transferred by backcrossing into the existing adapted varieties. Beside qualitative traits, soybean breeding programs in Africa can improve quantitative traits such as grain yield and protein/oil content by infusing exotic germplasm into their breeding populations.

Dudley (1984a, 1984b) proposed a method for identifying exotic lines with favorable alleles not present in local adapted cultivars or lines. The method allows a relatively quick screening of exotic germplasm that may be available to the breeder. It is currently being used at IITA to determine which of 10 high-yielding varieties from the northern USA have favorable alleles for grain yield which are not present in four IITA elite varieties. Since some of the best soybean yields are recorded from varieties developed and grown in the northern USA, we expect that they may carry some alleles for yield and yield-related traits absent in the varieties currently being grown in Africa. These American varieties will be used in crosses with the local elite varieties to develop breeding populations for the extraction of superior lines.

Preliminary observations from our current study show that there is substantial variability for plant height and maturity within the $F_2$ populations derived from the exotic x domestic crosses. While we cannot predict the yield potential and the chances of extracting high-yielding lines from these crosses until yield data are analyzed, there are strong indications (based on field observations) that early-maturing lines may be identified from some of the

crosses. The average number of days from planting to flowering for the IITA parents, the USA parent and their crosses were 43, 25 and 29, respectively. We hope to identify high-yielding lines among these early-maturing progeny which will be suitable for the second growing season in southern parts of West Africa, an area which has a bimodal rainfall pattern.

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# 3.9

# *African Rice Diversity: Conservation and Prospects for Crop Improvement*

N. Q. Ng, T. T. Chang, D. A. Vaughan and C. Zuno-Alto Veros

Rice, the principal staple food for millions of people in most Asian countries and parts of Africa and Latin America, feeds more people in the world than any other crop. Feeding the world's growing population will depend heavily on increased rice production. Although Africa produces less than 3% of the world total, the potential for the expansion of rice cultivation is great, particularly in the inland valleys and basins (Andriesse, 1986), and the area of cultivation on the continent is growing (Kaung Zan et al., 1985).

Successful expansion of rice cultivation depends not only on cultural practices and management but also on the suitability of rice varieties, which must be drawn from existing germplasm or developed by combining desired characters from various germplasm sources. Genetic resources centers play a vital role in collecting and preserving these important resources and making them available for use by researchers.

## RICE RELATIVES IN THE AFRO-TROPICAL REGIONS

Africa contains representatives of four of the six known genomes in the genus *Oryza*; these are AA, BB, CC and FF (*see* Table 1 *overleaf*). In addition to the indigenous domesticated species *O. glaberrima*, grown in West Africa, the Asian domesticated species, *O. sativa*, is widely grown in Africa. The wild species of *Oryza* indigenous to Africa are *O. barthii* (annual) and *O. longistaminata* (perennial), which both have the same genome (AA) as those of the two cultivated species. Their distribution is shown in Figure 1 (*overleaf*). *O. punctata* and *O. eichingeri* have the genomes BB and CC. *O. brachyantha* is the only *Oryza* species known with the genome FF. All these species are diploids except *O. punctata*, which is both diploid and tetraploid (*see* Figure 2, *page 216*) (Tateoka, 1965; Chang, 1985; Ng et al., 1983).

In addition to *Oryza*, three related genera occur on the African continent: *Leersia*, *Maltebrunia* and *Prosphytochloa* (*see* Table 2 *overleaf*). The 10 African species of the genus *Leersia* have been studied in detail by Launert (1965). In the past, some *Leersia* species were

**TABLE 1 The species of *Oryza* in Africa**

| Species | Genome group | Remarks |
|---|---|---|
| *O. sativa* L. | AA | Introduced cultigen from Asia |
| *O. glaberrima* Steud. | $A^gA^g$ | Indigenous West African cultigen; also occasionally found in East Africa |
| *O. barthii* A. Chev. | $A^gA^g$ | Annual relative of *O. glaberrima* |
| *O. longistaminata* A. Chev. et. Roehr | $A^lA^l$ | Rhizomatous, partially self-incompatible, perennial relative of *O. glaberrima* |
| *O. eichingeri* A. Peter | CC<br>BBCC | Found in forests in East Africa; also occurs in Sri Lanka |
| *O. punctata* J.S. Presl.ex C.B. Presl. | BB<br>BBCC | Diploid form with annual characteristics; tetraploid with perennial characteristics[1] |
| *O. brachyantha* A. Chev. et Roehr. | FF | Commonly found in temporary pools in laterite soils |

1 From Sano, 1980.

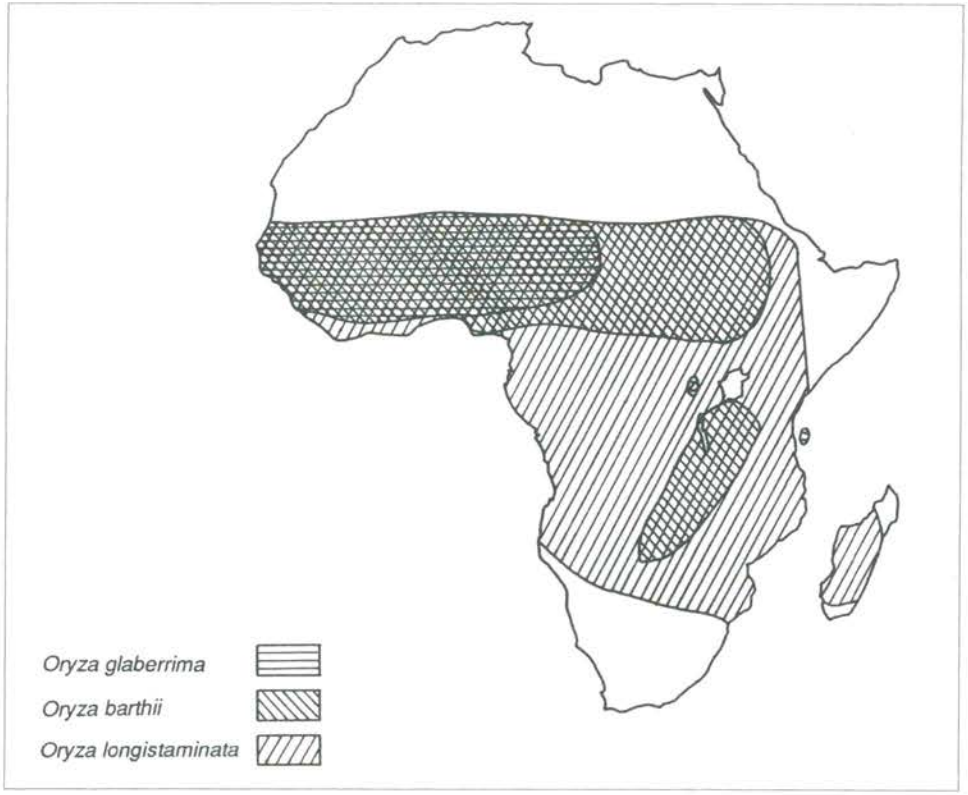
**TABLE 2 African genera and species related to *Oryza* in the tribe *Oryzaea* and their distribution**

| Genus | Species | Distribution |
|---|---|---|
| *Leersia* | *L. angustifolia* Munro ex. Prod | Central African Republic, S. Sudan, N. Zaïre |
| | *L. denudata* Launert | Kenya to South Africa |
| | *L. drepanothrix* Stapf. | West Africa, Sudan, Uganda |
| | *L. friesii* Melderis | Angola, Tanzania, Zaïre, Zambia |
| | *L. hexandra* Swartz | Throughout the continent |
| | *L. nematostachya* Launert | Angola, Zambia |
| | *L. oncothrix* Hubb. | Zambia |
| | *L. perrieri* (Camus) Launert | Madagascar |
| | *L. tisseranti* (A.Chev.) Launert | Central, East and West Africa |
| | *L. triandra* Hubb. | Liberia, Sierra Leone |
| *Maltebrunia*[1] | *M. gabonensis* Hubb. | Gabon |
| | *M. leersioides* Kunth. | Madagascar |
| | *M. maroana* A.DC. | Madagascar |
| | *M. petiolata* A.DC. | Madagascar |
| | *M. schliebenii* (Pilger) Hubb. | Tanzania |
| *Prosphytochloa*[1] | *P. prehensilis* (Nees) Schweichkerdt | South Africa |

1 Species in these two genera have been considered within the generic limits of *Potamophila*, a genus with one Australian species. *Prosphytochloa prehensilis* was formerly in the genus *Maltebrunia*. Species of these genera are forest grasses (Duistermaat, 1987).

Source: Based on Chase and Niles, 1962; Jackson, 1970; Launert, 1965.

**FIGURE 1** **Distribution of *Oryza glaberrima* and its relatives *Oryza barthii* (annual) and *Oryza longistaminata* (perennial)**

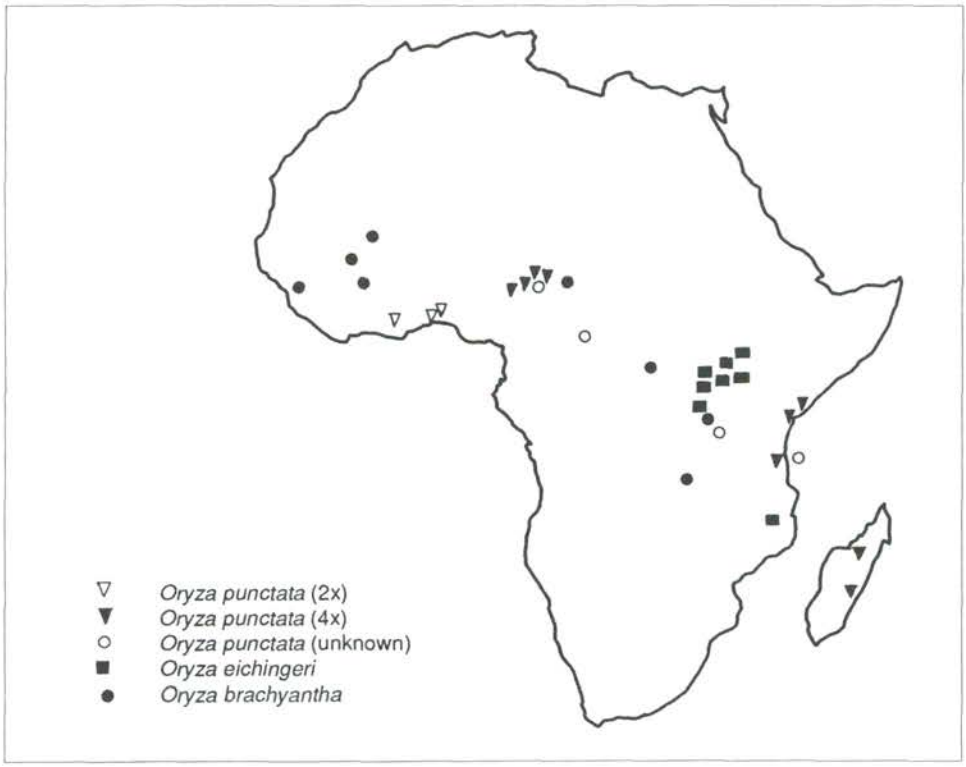



placed in the genus *Oryza* and frequently grow sympatrically. At present, three of the 10 African *Leersia* species are in the world's germplasm collection: *Leersia perrieri*, *L. tisseranti* and *L. hexandra*. The genera *Maltebrunia* and *Prosphytochloa* have been little studied, and representatives of these two genera are not available in germplasm collections. Efforts to collect representatives of all the species in these genera should be made, although they are only distantly related to *Oryza*. Techniques for studying evolutionary relationships at the molecular level are now available, and techniques for the transfer of chromosome segments from distantly related species will soon be available for rice (Murty and Cocking, 1988). With the innovations of biotechnology, superior genes from wild species can be isolated and incorporated into cultivars.

## ORIGIN OF AFRICAN RICE AND INTRODUCTION OF ASIAN RICE INTO AFRICA

Little is known with certainty about the origins of rice domestication in West Africa, and archaeological evidence is lacking. As a result, the subject remains at the hypothetical-

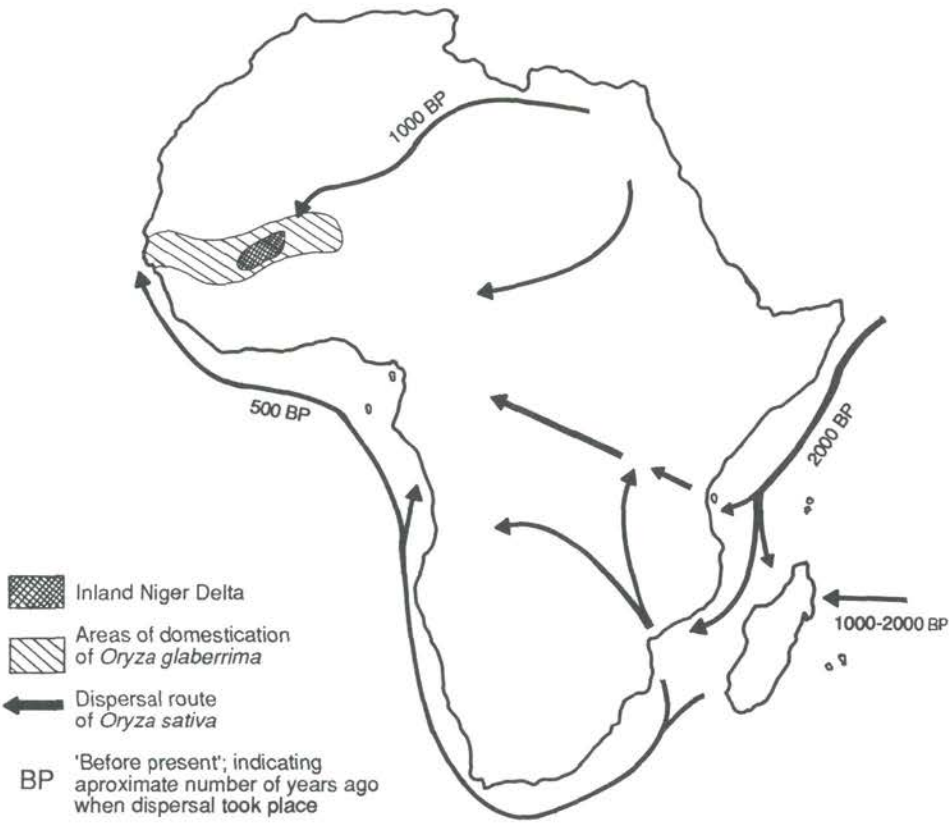
**FIGURE 2** **Distribution of *Oryza brachyantha*, *O. eichingeri* and chromosome races of *O. punctata***




deductive stage. According to Porteres (1950, 1976), African rice originated or was first domesticated in the inland delta area of the Niger river in about 1500 BC by the indigenous inhabitants of the area. From there it would have spread through the upper Niger and Senegal valleys. The areas of domestication could have occurred in areas stretching from Niger Bend along the Niger valley and delta, then westward to the Senegal valley and Casamance (*see* Figure 3).

Asian cultivated rice, *O. sativa*, was probably introduced into East Africa some 2,000 years ago, when the sea trade between East African ports and India was flourishing (Harlan and Stemler, 1976). This assumption is based on the fact that indigenous African crops such as cowpea, *Vigna unguiculata*, had already been introduced to India and has a Sanskrit name (Ng and Maréchal, 1985) and that pearl and finger millet had been found on the Indian subcontinent at Rangpur and Hallur, respectively, datable at about 1000 BC (Harlan and Stemler, 1976). It is likely that so important and ancient a crop as rice would have been imported into East Africa from Asia during the same period. It is also probable that Asian rice was carried to East Africa at a later date (by the 10th century AD) by Polynesians using the southern Indian Ocean sea route from Indonesia to Madagascar and the east coast of Africa (Carpenter, 1978; Chang, 1976). This Asian cultigen was subsequently introduced to West Africa, either by the Portuguese in about 1500 AD via the sea coast (Porteres, 1950) or by

**FIGURE 3** **Area of domestication of *Oryza glaberrima* and dispersal route of *Oryza sativa***




traders or Muslim missionaries traveling across the Sahara from North Africa or across Central Africa in the 9th to 10th centuries AD (Nayar, 1973; Carpenter, 1978).

Asian rice is a good 'colonizer'. It established well in many African ecologies soon after it was introduced into West Africa and, particularly after 1500, spread quickly to where African rice, *O. glaberrima*, was being cultivated. Today it is an important staple food in several African countries, including Egypt, Guinea, Liberia, Madagascar, Mozambique, Nigeria, Sierra Leone and Tanzania.

It is believed that *O. glaberrima* had replaced other cereal crops (Guinea corn, sorghum and millets) in parts of West Africa in precolonial times, before the introduction of Asian rice (Harris, 1976). Since its introduction to West Africa, Asian rice has been replacing *O. glaberrima* in many parts of the region (Porteres, 1950), so much so that the genetic resources of *O. glaberrima* may have been eroded (Ng, 1979). On the other hand, introgression between Asian rice and the African indigenous species may have created new arrays of genetic materials, which would have enriched the genetic diversity of rice (Ng et al., 1983). In recent years, however, the diversity of rice has gradually been eroded by the introduction of selected

and improved Asian rice cultivars to African farmers who may abandon the old traditional landraces in favor of the improved germplasm, and by clearance of new land for agriculture, which destroys the habitats of wild relatives of rice. Fortunately, progress has been made by many organizations in collecting and preserving these valuable resources for preservation and use.

## COLLECTION OF AFRICAN RICE GERMPLASM

The earliest known *Oryza* germplasm collecting missions in Africa were led by Japanese scientists of the National Institute of Genetics, Mishima. In 1959, one of their scientists, Furusato, explored West Africa, but details of this collection are not available (Sharma and Steele, 1978). Oka and Chang collected many wild and cultivated species in West Africa for the study of the evolution of rice and related subjects (Oka and Chang, 1964). Tateoka explored in East Africa in 1964 for taxonomic studies (Tateoka, 1964). Harlan collected *Oryza* species on his trips to Africa in the 1960s for the collection and study of African cereals (Harlan, 1973). Oka and his colleagues returned to West Africa in 1977 to collect and study African *Oryza* (Oka et al., 1977). In 1984 and 1985 another Japanese team, led by Katayama of Kagoshima University, collected some wild and cultivated *Oryza* in East and West Africa for a study of the distribution and ecotypic differentiation of *Oryza* in Africa (Katayama, 1987). All these collections, undertaken mainly for academic interest, contributed to our knowledge of rice in Africa but, unfortunately, many of them no longer exist or are unavailable to the public.

With a modest financial contribution from the International Board for Plant Genetic Resources (IBPGR), systematic exploration and collection missions for *Oryza* germplasm were initiated by IRAT and the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) in 1974 and by the International Institue of Tropical Agriculture (IITA) in 1976 (Ng et al., 1983). IITA holds the Consultative Group on International Agricultural Research (CGIAR) mandate for the collection, conservation, characterization, documentation and distribution of rice germplasm from Africa and since 1972 it has been assembling *O. sativa* germplasm from around the world for immediate use in its Rice Improvement Program. Between 1976 and 1988, IITA, in collaboration with African national institutes and scientists, organized 54 missions to 28 countries in which the collection of *Oryza* species was a main objective (*see* Chapter 1.2). The current composition of the rice germplasm collection at IITA consists of 9,451 *O. sativa*, 2,489 *O. glaberrima* and 270 wild rice strains (*see* Table 3). IITA germplasm collection activities continue.

At the 1983 International Rice Germplasm Workshop held at the International Rice Research Institute (IRRI), participants recognized the role of IITA as a regional center for the long-term conservation and the distribution of African forms of *O. sativa* and other African indigenous rice species. They also recommended that samples of all collected materials from Africa should be sent to IITA for preservation. IITA has, in its recent strategic planning, reaffirmed its commitment to continue as a regional center for these activities.

Analysis of the material collected by the 15 IRAT and ORSTOM missions undertaken between 1974 and 1986 (Bezancon and Second, 1984) reveals that 83% of the *Oryza* species collected were of the two rice cultigens and 17% were wild. Although these collection efforts placed considerable emphasis on wild species, only 2% of the samples collected were of species with the BB, CC or FF genomes. Many of the collections of the two rice cultigens have

**TABLE 3 Existing rice germplasm collections in the Genetic Resources Unit of IITA and their geographical distribution (as of mid-1988)**

| Countries/regions | *O. sativa* | *O. glaberrima* | Wild rice | Subtotal |
|---|---|---|---|---|
| **Central and West Africa** | | | | |
| Burkina Faso | 710 | 155 | - | 865 |
| Cameroon | 110 | 42 | 1 | 153 |
| Central African Republic | 54 | 1 | 14 | 69 |
| Chad | 45 | 17 | 27 | 89 |
| Congo | 9 | - | - | 9 |
| Gambia | 334 | 49 | 1 | 384 |
| Ghana | 173 | 41 | - | 214 |
| Guinea | 224 | 61 | 14 | 299 |
| Guinea Bissau | 70 | 15 | - | 85 |
| Côte d'Ivoire | 778 | 85 | 1 | 864 |
| Liberia | 1,024 | 624 | - | 1,648 |
| Mali | 44 | 142 | 36 | 222 |
| Mauritania | 8 | - | - | 8 |
| Niger | 77 | 41 | 45 | 163 |
| Nigeria (+ IITA) | 1,159 | 1,071 | 60 | 2,290 |
| Republic of Benin | 28 | - | - | 28 |
| Senegal | 541 | 96 | 20 | 657 |
| Sierra Leone | 360 | 26 | 1 | 387 |
| Togo | 5 | 15 | - | 20 |
| Zaire | 23 | - | - | 23 |
| **Southern and East Africa** | | | | |
| Botswana | 8 | - | 11 | 19 |
| Egypt | 126 | 2 | - | 128 |
| Madagascar | 511 | - | - | 511 |
| Malawi | 310 | - | 15 | 325 |
| Seychelles | 11 | - | - | 11 |
| Tanzania | 458 | 3 | 20 | 481 |
| Zambia | 502 | - | 2 | 504 |
| Zimbabwe | 57 | 2 | 2 | 61 |
| **Asia** | | | | |
| Afghanistan | 2 | - | - | 2 |
| Bangladesh | 26 | - | - | 26 |
| Burma | 9 | - | - | 9 |
| India | 153 | - | - | 153 |
| Indonesia | 34 | - | - | 34 |
| Japan | 70 | - | - | 70 |
| Korea | 104 | - | - | 104 |
| Laos | 10 | - | - | 10 |
| China | 5 | - | - | 5 |
| Malaysia | 15 | - | - | 15 |
| Papua New Guinea | 2 | - | - | 2 |
| Philippines | 160 | - | - | 160 |
| Sri Lanka | 11 | - | - | 11 |
| Taiwan | 12 | - | - | 12 |
| Thailand | 21 | - | - | 21 |
| USSR | 72 | - | - | 72 |

**TABLE 3 (contd)**

| Countries/regions | *O. sativa* | *O. glaberrima* | Wild rice | Subtotal |
|---|---|---|---|---|
| **North and South America** | | | | |
| Argentina | 1 | - | - | 1 |
| Bolivia | 1 | - | - | 1 |
| Brazil | 143 | - | - | 143 |
| Canada | 57 | - | - | 57 |
| Colombia | 10 | - | - | 10 |
| Costa Rica | 1 | - | - | 1 |
| Cuba | 1 | - | - | 1 |
| Ecuador | 2 | - | - | 2 |
| El Salvador | 24 | - | - | 24 |
| Hispaniola | 9 | - | - | 9 |
| Jamaica | 4 | - | - | 4 |
| Mexico | 4 | - | - | 4 |
| Panama | 3 | - | - | 3 |
| Peru | 16 | - | - | 16 |
| Puerto Rico | 14 | - | - | 14 |
| Suriname | 17 | - | - | 17 |
| USA | 40 | - | - | 40 |
| Venezuela | 2 | - | - | 2 |
| **Europe** | | | | |
| UK | 118 | - | - | 118 |
| **Pacific** | | | | |
| Australia | 16 | - | - | 16 |
| **Unknown** | 503 | 1 | - | 484 |
| **Total** | 9,451 | 2489 | 270 | 12,210 |

been duplicated at IITA, and IITA has begun making arrangements to acquire ORSTOM's collection of about 400 accessions of the wild and weedy species of *Oryza*. A major international conference on African rice species was sponsored by IRAT and ORSTOM in 1976 (IRAT/ORSTOM, 1977).

IBPGR has recently provided funds to some African national programs, including those in Burkina Faso, Kenya and Madagascar, for the collection of cultivated rice germplasm.

IRRI has participated directly in collection of rice germplasm with national scientists in Madagascar. In 1984 and 1985, in collaboration with the Département de Recherches Rizicole Nationale, 221 samples of *O. sativa* were collected. The International Rice Germplasm Center (IRGC) at IRRI acts as a back-up site for a duplicate of the base collection of African cultivated rice germplasm held at IITA (IRRI/IBPGR, 1978) and currently holds 6,528 *O. sativa* and 2,278 *O. glaberrima* accessions (*see* Table 4). IRGC has global responsibility for the wild species of *Oryza* (IRRI, 1978) and 457 accessions of wild taxa, including natural hybrids from Africa, are currently in its collection.

**TABLE 4** *Oryza sativa*, *O. glaberrima* and wild species germplasm from Africa conserved at the IRGC (as of July 1988)

| Countries | *O. sativa* | *O. glaberrima* | *O. barthii* | *O. longistaminata* | *O. brachyantha* | *O. eichingerii* | *O. punctata* | Natural hybrids | *Leersia perrieri* | Unspecified | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Central/West Africa** | | | | | | | | | | | |
| Burundi | 36 | | | | | | | | | | 36 |
| Burkina Faso | 35 | 68 | 7(1) | 3 | | | | 1 | | | 114 |
| Cameroon | 59 | 50 | 35(6) | 10 | 5 | | 5(1) | | | | 164 |
| Chad | 33 | 101 | 31(10) | 7 | 1 | | 1(1) | | | | 174 |
| Congo | | | 1 | | 1 | | | | | | 2 |
| Gabon | 1 | | | | | | | | | | 1 |
| Gambia | 140 | 3 | 5(4) | | | | | | | | 148 |
| Ghana | 139 | 10 | 1 | | | | 3(3) | | | | 153 |
| Guinea Bissau | 53 | 31 | 1(1) | 1(1) | | | | | | | 86 |
| Guinea (Conakry) | 615 | 213 | 4(1) | 1 | | | | | | | 833 |
| Côte d'Ivoire | 823 | 236 | 1 | 10 | | | | | | | 1070 |
| Liberia | 1317 | 480 | | | | | | | | | 1797 |
| Mali | 39 | 764 | 48(10) | 23(2) | 3(1) | | | 2(1) | | | 879 |
| Niger | 2 | 2 | 4(2) | 3 | | | 2 | 1(1) | | | 14 |
| Nigeria | 150 | 175 | 21(3) | 13(1) | | | 9(2) | 3 | | | 371 |
| Senegal | 752 | 114 | 21(4) | 19 | | | | 3 | | 8(1) | 917 |
| Sierra Leone | 787 | 14 | 7 | 3 | 10(5) | | | 1 | | | 822 |
| Togo | 6 | | | | | | | | | | 6 |
| Zaïre | 43 | | | | | | | | | | 43 |
| **East/Southern Africa** | | | | | | | | | | | |
| Ethiopia | 6 | | | 12 | | | | | | | 18 |
| Kenya | 225 | | | | | | 2(1) | | | | 227 |
| Madagascar | 889 | | | 3 | | | | | 1 | 2 | 895 |
| Malawi | 11 | | | 2 | | | | | | | 13 |
| Mozambique | 8 | | | | | | | | | | 8 |
| Tanzania | 123 | 3 | 7 | 15(1) | 2 | | 10(10) | | | 4(3) | 164 |
| Uganda | | | | | | 20(8) | 1 | | | | 21 |
| Zambia | 29 | | 7 | | | | | | | 2 | 38 |
| Zimbabwe | 97 | | | | | | | | | | 97 |
| **North Africa** | | | | | | | | | | | |
| Sudan | 17 | 8 | 8(3) | 3(1) | | | | | | | 36 |
| **Africa general** | 103 | 6 | 10 | 9 | 2 | 1 | 5 | 1 | | 1 | 138 |
| **TOTAL** | **6538** | **2278** | **219** | **137** | **24** | **21** | **38** | **12** | **1** | **17** | **9285** |

1 Numbers in parentheses indicate the number of accessions screened against various insect pests.

The West Africa Rice Development Association (WARDA) has participated in three explorations in Mali, Nigeria and Senegal (Ng et al., 1983). Most of WARDA's existing germplasm consists of breeding stocks and varieties from other continents (*see* Chapter 1.3).

The collection efforts to date have enjoyed a high degree of international cooperation, with the result that an impressive array of *Oryza* germplasm from Africa has been built up. However, major gaps in the germplasm collection for *Oryza* remain and there is an urgent need to fill these gaps. Priorities include:

- collection of cultivated rices and their wild relatives from Mozambique;
- collection of traditional varieties from isolated pockets in Southern, Central and East Africa;
- collection of wild *Oryza* taxa from Uganda, southern Sudan, southern Chad and elsewhere in East and Central Africa;
- collection of samples of related genera from East and Southern Africa.

## PRESERVATION, CHARACTERIZATION AND EVALUATION OF AFRICAN RICE SPECIES

The conditions under which rice germplasm is preserved at IITA and IRRI are shown in Table 5. Long-term studies on the viability of Asian rice varieties have shown that tropical varieties have good storage ability — greater than 90% viability after 23 years when stored at 2°C and 40% relative humidity (Chang, 1988). However, rice varieties from temperate regions stored

**TABLE 5 Storage conditions of the International Rice Germplasm Center (IRGC) at IRRI and the IITA Genetic Resources Unit and projected life span**

| Room designation | Temp. (°C) | RH (%) | Seed M.C. | Seed container | Expected longevity (years) |
|---|---|---|---|---|---|
| IRGC | | | | | |
| Short-term | 19±1 | 50 | 10 | Paper bags | 5-7 |
| Medium-term (Active collection) | 2±1 | 40 | 6 | Aluminum cans | 20-40 |
| Long-term (Base collection) | -10±1 | 37 | 6 | Aluminum cans | > 50 |
| IITA | | | | | |
| Temporary store | 16±1 | 20 | 7 | Paper and cloth bags | 20-40 |
| Active collection | 5±1 | 30-35 | 9 | Plastic jars, metal foil envelopes | 20-40 |
| Base collection | -20±1 | < 30 | 5-6 | Aluminum cans | > 100 |

in the same conditions begin losing viability after only about 10 years. No comparable studies have been made for African germplasm, and this area needs investigation.

Both IITA and IRRI systematically characterize the *O. glaberrima* and *O. sativa* landraces from Africa. Passport and characterization information are part of the computer database for African rice germplasm. An earlier account of characterization and evaluation of African germplasm can be found in Chang et al. (1977) and Ng (1988). The principal morphological characteristics of *O. glaberrima* that distinguish it from *O. sativa* are the reduced secondary branching (169 out of 999 accessions at IRRI show no secondary branching), truncated or round-shaped ligules and reduced or lack of pubescence on the leaf blade surface (13 out of 1,110 accessions at IRRI are glabrous and 557 accessions have intermediate leaf pubescence). The glabrous leaves of some varieties of *O. glaberrima* is a trait which breeders of deepwater rice are interested in because it may reduce silt build-up on the leaves of submerged rice.

Morphological and other characteristics of *O. glaberrima* show less diversity than is the case with *O. sativa* , as would be expected from its fairly limited distribution (Chang et al., 1977). For instance, almost all *O. glaberrima* varieties have red pericarp. Only four accessions at IITA have white pericarp.

However, studies conducted at IITA on the reaction of hundreds of accessions of *O. glaberrima* and *O. sativa* to the indigenous Africa insect pests *Diopsis longicornis* and *Malaiarpha sparatella* and to rice yellow mottle virus (RYMV) have shown that *O. glaberrima* has a higher level of resistance than *O. sativa* (Alam, 1988; Ng, 1988; Alam, unpubl.). *O. glaberrima* is also well adapted to the diverse, and often adverse, environments of West Africa. This would indicate that this species offers potentially useful genes for scientists breeding rice for such environments. In addition, attributes of grain quality, which make this species a preferred rice for many people in the region (Bangura, pers. comm.), contribute to its value, which, because of its limited yield potential, continues to decline in the area in which it is planted.

Field screening of *O. glaberrima* has shown this species to be a good source of resistance to drought at both the vegetative and reproductive stages. Of the 800 accessions screened in recent years, most flowered early in the dry season at IRRI's sites in the Philippines, at about 50-60 days after seeding. Many accessions of *O. glaberrima* mature in less than 90 days after seeding during the main growing season (June to October) at IITA's sites in Nigeria. Thus, drought escape through early maturity is an important contributory factor to drought resistance in this species. The African rices also have strong vegetative vigor and they recovered well after a long stress period (Chang et al., 1977).

*O. glaberrima* has also been shown to be a possible source of tolerance to saline conditions. Of nine different *Oryza* species screened in salt water, the greatest seedling survival was found in *O. glaberrima* (16%), in comparison with 8% for *O. punctata* and none for *O. brachyantha* (Akbar et al., 1987).

Of 230 strains of *O. glaberrima* tested for resistance to rice tungro virus in the Philippines, 44 were resistant. Of the 75 strains of *O. glaberrima* inoculated with the African indigenous RYMV at IITA, 45 were resistant, and some even immune, to the virus. However, the majority of the more than 1,500 accessions of *O. sativa* tested against this virus were highly susceptible, while only a very few showed a high level of resistance (Ng et al., 1980; Ng, 1988). At IRRI, 681 accessions of *O. glaberrima* have been screened for resistance to the white-backed planthopper, *Sogatella furcifera* (Horvath), and 45.4% were found to be resistant.

Wild rices from Africa have been screened at IRRI for their tolerance or resistance to: brown planthopper biotypes 1, 2 and 3; green leafhopper; white-backed planthopper; zigzag leafhopper; yellow stem borer; rice whorl maggot; leaf folder; and case worm. High levels of resistance and tolerance have been found, with 72 accessions showing tolerance and/or resistance to all these pests. These accessions include *O. barthii* from Chad, the Gambia, Mali, Nigeria, Senegal and Sudan; collections of *O. punctata* from Ghana and Uganda; and collections of *O. brachyantha* from Sierra Leone.

A number of wild rices from Africa have also been screened at IRRI for resistance to rice tungro, grassy stunt and ragged stunt viruses. Only a few *O. punctata* and *O. eichingeri* accessions showed resistance to one virus, the rice ragged stunt virus. At IITA, two accessions of *O. barthii* and one of *O. longistaminata* were found to be immune to RYMV (IITA 1984; IITA 1985).

Evaluation of African rice germplasm is far from complete. There is a need to continue to find and make use of new and diverse sources of germplasm to improve the rice plant's ability to withstand attacks by pests of perennial importance as well as pests that emerge as new problems. With the limited information available so far, it appears that the African indigenous rice species are better equipped to combat some of the indigenous pests and diseases in Africa. Their role in rice improvement, especially in Africa, will increase as more research is conducted on African rice species and their potential is understood, and as the area of rice cultivation expands.

## POTENTIAL OF AFRICAN RICE

Thus far we have emphasized the need to evaluate and make use of African rice germplasm for the improvement of Asian rice cultigens. Perhaps, however, we should ask whether it would not be better to select superior cultivars of *O. glaberrima* from existing collections and then to improve them by combining better characteristics from *O. sativa* to suit African conditions. Yield potential of some *O. glaberrima* cultivars has been shown to be better than some *O. sativa* cultivars under poor management conditions (Morshima et al., 1962; Ng, 1988). In some areas, African farmers still grow a greater proportion of *O. glaberrima* in a mixed-rice cultivation of *O. glaberrima* and *O. sativa*, and in others *O. glaberrima* is alone, particularly in deepwater areas as a floating rice.

Through its Rice Research Program, IITA had already initiated interspecific hybridization between *O. sativa* and *O. glaberrima* with the aim of transferring the RYMV-resistant gene(s) from *O. glaberrima* to improve Asian rice cultigens (IITA, 1987).

## DEMAND FOR GERMPLASM

Germplasm provides the fuel for research as well as for crop improvement. Nineteen landraces and one wild *Oryza* population were involved in the development of IR64 at IRRI. The IRGC distributes between 40,000 and 50,000 samples of rice a year to rice scientists around the world. However, the distribution of germplasm to African countries has not been as great as might be expected (*see* Table 6). During the past two decades in Africa, much effort was made to test improved or selected rice varieties from around the world, but only few of those varieties performed well and were accepted by farmers. Several of the IITA and IRRI

**TABLE 6 Germplasm accessions in the International Rice Germplasm Center (IRGC) distributed to Africa, 1983-87**

| Country | 1983 | 1984 | 1985 | 1986 | 1987 | Total |
|---|---|---|---|---|---|---|
| Burkina Faso | 22 | | | | 8 | 30 |
| Cameroon | 8 | 6 | | | | 14 |
| Chad | | | 14 | | | 14 |
| Ethiopia | | 3 | 9 | 23 | | 35 |
| Gambia | | 2 | | | | 2 |
| Ghana | | | | 31 | | 31 |
| Kenya | 16 | 40 | | | 16 | 72 |
| Liberia | | | | 1 | | 1 |
| Madagascar | | 75 | 2 | 77 | 29 | 183 |
| Mali | | 18 | | | | 18 |
| Nigeria | 18 | 19 | 30 | 28 | 144 | 239 |
| Senegal | 78 | 986 | 68 | 42 | | 1,174 |
| Sierra Leone | | | | 18 | | 18 |
| Tanzania | 4 | 14 | 5 | | 35 | 58 |
| Uganda | | 5 | 8 | | 5 | 18 |
| Zaïre | | | 2 | 1 | | 3 |
| Zambia | | | 2 | | | 2 |
| Subtotal | 146 | 1,168 | 140 | 221 | 237 | 1,912 |
| Total | | | | | | 1,912 |

breeding lines have been released to and adopted by African farmers. Increased use of the germplasm collections could make a marked contribution to increasing rice production in Africa.

At the IRGC, the demand for exotic germplasm, which includes wild *Oryza* species, *O. glaberrima* and the *sinica* (*japonica*) race of *O. sativa*, has increased fourfold in recent years. Interest in the exotic germplasm of wild relatives has grown as more progress has been made with hybridization. For biotechnologists, the extremes of diversity available in rice germplasm can provide appropriate material for research. Germplasm curators have a continuing responsibility to describe the diversity to their clientele.

Another role that is increasingly being played by the genetic resources programs of the international agricultural research centers is the return of entire national collections to countries where they are no longer available. Two examples of this are the recent return to Senegal of 517 Senegalese accessions, received by the IRGC in 1962, and to Tanzania of 79 accessions, collected by IITA in Tanzania in 1977-78.

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# PART 4

# Plant Quarantine Issues in the Movement of Germplasm

# 4.1

# *Plant Quarantine and the Global Transfer of Plant Genetic Resources*

R. P. KAHN

This paper aims to provide some background information on the interaction of plant quarantine with the transfer of plant genetic resources. The author hopes that a brief discussion of principles and concepts involved will set the stage for the following papers (Chapters 4.2 to 4.5). Since this does not represent a review of the literature *per se*, but rather a distillation of some of the author's publications, literature citations are not presented in the text. Instead, the reader is referred to a short bibliography at the end of the paper listing some papers where the relevant literature citations may be found.

## THE HISTORICAL BASIS OF PLANT QUARANTINE

The early history of plant quarantine is deeply rooted in the early history of plant pathology and entomology. The early control of plant diseases was effected by farm practices based on farmers' observations over time, and was attempted through religious appeals to various gods to control weather conditions that were associated with disorders, centuries before the causal agents were known. Plant quarantine practices in the 19th and 20th centuries stem from much earlier human medical practices for contagious diseases, and from the development of the biological sciences, including the discovery of causal agents.

The word 'quarantine' is derived from the Latin *quadraginta* and the Italian *quaranta*, meaning 'forty', and *quarantina*, meaning 'quarantine'. *Quarantina* was the word used for the 40-day period of isolation (to allow time for symptoms to appear) required for a ship, with its passengers and cargo, to remain anchored in the port of arrival if the ship arrived from a country where certain epidemic diseases (such as bubonic plague and cholera) were known to occur. The practice began in the 14th century in Venice and some other European ports.

In modern agricultural usage, the numerical connotation has been dropped, but the procedural meaning has been retained and broadened. 'Quarantine' is now an umbrella term

that covers all regulatory actions taken to exclude animal or plant pests or pathogens from a site, area, country or groups of countries. It can now be defined as the use of exclusion as a control strategy.

Although the actual detention of plants in quarantine or isolation, in the sense of the 14th-century practice, is still a component of modern quarantine, accounting for only a relatively small percentage of all plant quarantine regulatory actions, it still has a disproportionately large impact on the international transfer of plant genetic resources for some important crop species.

## THE LEGAL BASIS OF QUARANTINE

The legal basis of plant quarantine activities are the laws enacted by a national government and the regulations promulgated under these laws. Governments establish policies, guidelines, safeguards or procedures, or take regulatory actions, to reduce the chances of hazardous organisms being inadvertently introduced via a wide spectrum of imported articles, including plants and plant parts.

The mechanisms for enacting laws or promulgating regulations are as follows: legislation enacted by national, and sometimes state or provincial, governments as acts under which rules or regulations are promulgated; or enabling legislation that authorizes a governmental agency, usually the Ministry or Department of Agriculture, to issue decrees, orders or directives which are in themselves rules or regulations or under which rules and regulations are promulgated; and, for some countries, quarantine acts or decrees passed by parliaments of intergovernmental organizations that are binding on member countries. Regional plant protection organizations, such as the European and Mediterranean Plant Protection Organization (EPPO), set standards for member countries, and seek to harmonize regulations within the region, but such actions are not binding on member countries.

In addition, the International Plant Protection Convention (IPPC) of 1951, as amended (the so-called Rome Convention), is a treaty that provides additional legal obligations according to international law. The depository for this treaty is the Food and Agriculture Organization of the United Nations (FAO), based in Rome. The phytosanitary certificate (PC) issued by the exporting country, in accordance with the requirements of the IPPC, is an instrument of that treaty. Controversies between nations over phytosanitary certification are usually settled by joint discussion but can be judged in an international court of law — although nations have not found it necessary in the past to do so. By 1987, 85 countries had signed the IPPC. Several other nations issue phytosanitary certificates in accordance with the model phytosanitary certificate of the IPPC.

The quarantine services of most importing countries require that plant genetic resources be accompanied by a PC issued according to the FAO model. The PC must be addressed to the quarantine service of the importing country and signed by an authorized officer of the quarantine service of the exporting country. The upper part of the certificate must furnish information about the consignment, including the place of origin and the botanical name of the plant. This information is a prerequisite for a pest risk assessment by the importing country.

The PC contains a certifying statement to the effect that: 'This is to certify that the plants or plant products described above have been inspected and found free from quarantine pests and substantially free from other injurious pests, and that they are considered to conform with the phytosanitary regulations of the importing country.' The quarantine service of the

exporting country must be familiar with the regulations of the importing country in order to issue a meaningful PC. The PC may also contain added declarations (for example, that a growing-season inspection or specified treatment has been conducted), as required by the permit issued by the importing country. In essence, the PC is a certificate of inspection at place of origin.

However, just because a consignment of plants or seeds is accompanied by a PC does not necessarily mean that the plant material will be admissible at a port of entry. Countries with well-developed quarantine services usually do not rely on the PC as the sole safeguard, although they may require the document as a condition of entry. The consignment, or a sample, is inspected upon entry and, depending on pest finds, is admitted, sometimes with treatment, or denied entry. The PC becomes more acceptable to a given importing country if the exporting country has a past history of successfully meeting the certifying statement. Other countries with less than adequate facilities and technical support may give more weight to the PC and the added declarations required by permits.

## PLANT QUARANTINE CONCEPTS

### Geographic basis

The known distribution of crop pests and pathogens, as well as several biological factors, form the geographic basis upon which regulations are promulgated and regulatory actions taken. Many organisms are ubiquitous, occurring in most of the area of their hosts. Many others have narrower ecological ranges than those of their hosts. Quarantine officers are concerned with the entry and establishment of certain exotic organisms into their countries.

Plant disease agents and pests can move or be moved between countries along natural and man-made pathways (*see* Table *1 overleaf*), depending on at least four factors: life cycle; passive or active movement or dispersal; the environment; and man's exporting and importing activities. The life cycle includes the sequence of events that takes place from the appearance of a given stage of an organism to the reappearance of the same stage in the next generation. During the development of the life cycle, one or more active or dormant stages of the organism may appear. Some disease agents or pests have stages or life forms that enable the organism to survive the biological, chemical or physical stresses which occur along the various pathways. Some organisms have stages or spore forms with physiological, morphological or anatomical characteristics that facilitate not only survival on natural or man-made pathways but also active or passive movement or dispersal. Nevertheless, there are many pest organisms of quarantine significance that have very inefficient means of natural movement or spread and/or life cycles that are not conducive to spread in a plant quarantine sense. However, such organisms could be moved passively along man-made pathways when articles, particularly plants or plant parts, are exported or imported.

Quarantine actions are taken in an attempt to block the movement of certain exotic pests and pathogens, or to reduce the changes that they will make along man-made pathways. Quarantine is not designed to be applied to natural pathways.

### Biological basis

Interwoven in the fabric of plant quarantine is a mixture of administrative, economic, social, political and biological threads. The strength of fabric lies in the content of its biological

**TABLE 1 Natural and man-made pathways for the movement of plant pests and pathogens**

| |
|---|
| **Natural pathways** |
| 1 Winds, storms, jet streams |
| 2 Air currents, convection currents |
| 3 Ocean currents |
| 4 Surface drainage |
| 5 Natural seed dispersal |
| 6 Fliers (insects and mites) |
| 7 Migratory species (locusts) |
| 8 Self-locomotion (zoospores) materials |
| 9 Vectors (insects, nematodes, etc.) |
| 10 Other carriers (birds and other higher animals) |
| **Man-made pathways** |
| 1 Cargo (agricultural and nonagricultural) |
| 2 Mail |
| 3 Baggage |
| 4 Common carriers (ships, vehicles, airplanes) |
| 5 Dunnage, crates, packing |
| 6 Smuggling |
| 7 Farm practices (irrigation, movement of used farm equipment) |

threads. The concept is illustrated in Figure 1, which shows the interaction of pest risk, safeguards and entry status, defined as follows:

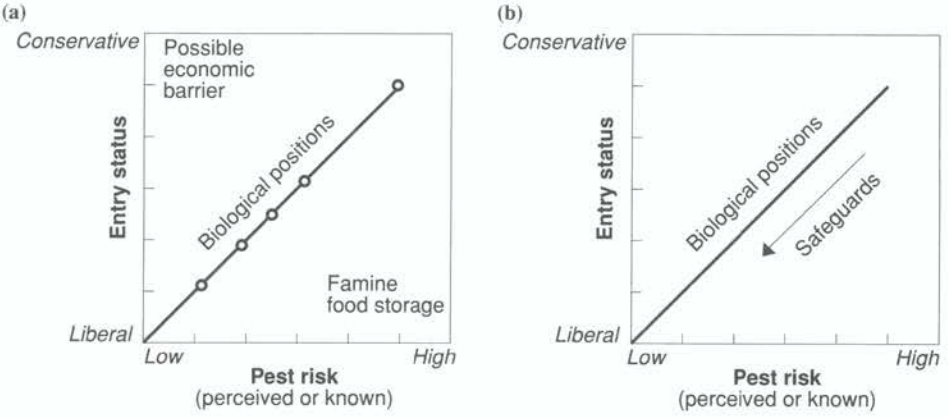
*Pest risk* The known or perceived chance that a hazardous organism will enter on an imported article moved along a man-made pathway; since quarantine risks have not often been quantified, the level of risk is generally referred to as low, medium or high.

*Safeguards* Actions taken to lower the risk of introducing hazardous agricultural organisms along man-made pathways; such actions include issung permits, certification and treatment at origin, inspection and treatment upon arrival, isolation or post-entry quarantine after arrival, and other specified procedures.

*Entry status* Whether or not an article may enter, determined on the basis of rules, regulations, guidelines, procedures, policy statements and decisions of quarantine officers at ports of entry, any or all of which might govern whether such imported articles as plant genetic resources are enterable, and if so, under what conditions; the entry status is ranges from liberal to conservative.

The high biological content of the regulatory fabric is based on a matching of known or perceived pest risk with entry status. When the known or perceived pest risk is high, the entry

**FIGURE 1** **Graphic representation of the relationship between pest risk, entry status of articles offered for importation, and safeguards. The line, representing biologically sound positions, is developed from points where entry status is matched against known or perceived pest risk. If the pest risk is low, the entry status should be liberal; if the pest risk is high, the entry status should be conservative**




status should be conservative and the protective action taken is likely to be drastic. When such risk is low, the entry status should be liberal, and the regulatory actions taken are mild.

Often, quarantine officers do not have sufficient data to make a biological decision — that is, the pest risk is unknown or is difficult to estimate. Under such circumstances, quarantine officers tend to be conservative and err on the side of protecting agriculture, hence the policy of 'when in doubt, keep it out'. Quarantine officers are less likely to be conservative if they can obtain data relating to risk determinations for exotic pests or pathogens. Usually, such information is not readily available in the importing country where the pest or pathogen does not occur.

When the risk is considered to be high, the entry of the host is often prohibited to the general or commercial public. This type of action is usually taken when the exotic pest or, more often, the pathogen cannot be inspected at an inspection station by an officer using conventional equipment and procedures. Most of the organisms that fall into this category are viruses, viroids, most bacteria and some fungi. Such pests may be latent or the host may be symptomless.

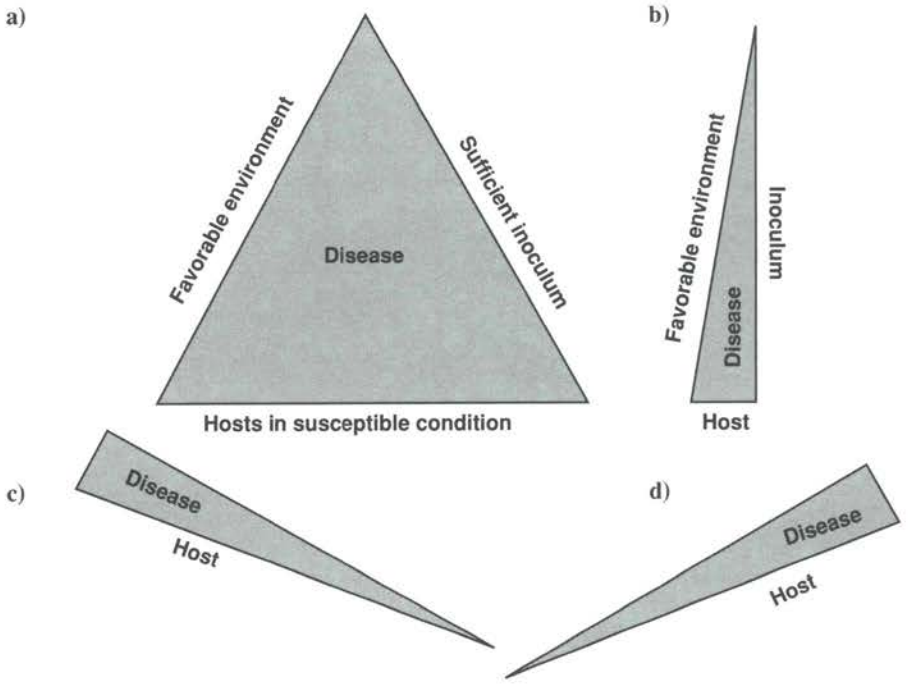
However, complete prohibition of high-risk crop species denies to the country the benefits from importing germplasm or new varieties to improve or diversify agriculture. Therefore, countries may allow prohibited plant materials to enter in small quantities for scientific purposes provided adequate safeguards can be established. Usually, a special permit is issued based on favorable benefit/risk considerations. Among the safeguards are passage of the imported plants through post-entry quarantine where laboratory and greenhouse pathogen detection tests are conducted, certain types of isolation, passage through third-country or intermediate-quarantine, entry of tissue cultures from approved sources, and special certification from approved sources abroad. Decisions to issue a special permit for scientific materials are generally made on a case-by-case basis.

## THE DISEASE TRIANGLE AND PATHOGEN ESTABLISHMENT

Quarantine officers are concerned not only with the entry of organisms but also with their colonization and establishment. An organism does not become established every time it enters a country. Certainly, a country is peppered with spores and other propagules of organisms entering along natural and man-made pathways, but usually there is no establishment.

In order for an establishment to take place after an organism reaches the end of either a natural or man-made pathway, three factors must be operating: a host in a susceptible condition; sufficient inoculum (pathogen propagules) to initiate an infection or a large enough population to develop a pest infestation; and favorable environmental conditions. The three factors must not only be synchronized, but no factor must be limiting. The concept may be depicted as the disease triangle (*see* Figure 2).

**FIGURE 2** **Graphic representation of a disease in which the area of the triangle represents the extent and development of a disease. The sides represent the three factors that must be operative and synchronized if a disease is to develop:**
**a) = none of the factors is limiting; b), c) and d) = the factor that is limiting is shown by the shortened side, and the failure of the disease to develop is shown by the smaller area of the triangle**
**The diagram also applies to pests if 'inoculum' is changed to 'pest population' and 'disease' to 'infestation'.**



Exclusion, or plant quarantine, as a pest and pathogen control strategy is aimed at breaking up the disease (or infestation) triangle so that the three factors are not operational and synchronized, and an exotic pest is not established. The most widely employed regulatory action is to eliminate or reduce the inoculum or population by, for example, not issuing permits for high-risk plant material that might be carrying pests and pathogens, requiring treatment as a condition of entry, denying entry of a consignment if an exotic pest is found at a port of entry, and requiring certain post-entry safeguards.

## IMPACT OF PLANT QUARANTINE ON INTERNATIONAL TRANSFER OF PLANT GERMPLASM

Although most countries require permits for the entry of plants, seeds and vegetative propagations, most crop species are not prohibited by regulations. Thus, germplasm enters with little or no restriction unless a pest or pathogen of quarantine importance to the importing country is detected at a port of entry or inspection station. If there is an effective eradicant treatment, the material is treated and sent on to the importer. If there is no known treatment, on a case-by-case basis the consignment is destroyed or enters under specified safeguards.

However, some important plant species are prohibited by regulation (but often a country reserves the right to allow entry for scientific purposes under specified safeguards on a case-by-case basis). In 1983, the author reviewed the quarantine regulations of 124 countries to determine the extent to which each country prohibited plant genera from one or more countries (*see* Table 2). The range of plant genera prohibited was from 0 to 132 but the average was 15. The range of pests and pathogens named in regulations was between 0 and 275, with an average of 35.

The 38 most frequently prohibited genera in the quarantine regulations of the 124 countries are listed in Table 3 (*overleaf*). Of these, the following are mandated crops of the international

**TABLE 2** **Range and average number of plant pests and pathogens, and plant genera prohibited by the plant quarantine regulations of 124 countries**

| Geographic region | Number of countries whose regulations were reviewed | Number of pests and pathogens named by species or common name [1] | | Number of plant genera prohibited [2] | |
|---|---|---|---|---|---|
| | | *Range* | *Average* | *Range* | *Average* |
| North America | 22 | 0 - 111 | 12 | 0 - 51 | 7 |
| Europe | 29 | 0 - 232 | 54 | 0 - 30 | 14 |
| South-west Pacific | 10 | 0 - 103 | 25 | 0 - 40 | 22 |
| South America | 12 | 0 - 71 | 21 | 0 - 27 | 7 |
| Africa | 30 | 0 - 275 | 46 | 0 - 132 | 26 |
| Asia | 21 | 0 - 129 | 28 | 0 - 24 | 9 |
| All regions | 124 | 0 - 275 | 35 | 0 - 132 | 15 |

1 Figures do not include all the species in a genus when the genus is named; in such cases, the number 1 is used in calculations.

2 Figures do not include all the genera in a family when an entire family is prohibited, or all the genera when a group of plants (e.g., 'forest trees') is prohibited; in both cases, the number 1 is used in calculations.

**TABLE 3** **The 38 genera or crops most frequently prohibited (as plants, seeds or both) in the quarantine regulations of 124 countries**

| Most frequently prohibited genera | No. countries prohibiting | % of countries prohibiting | | |
|---|---|---|---|---|
| | | Plants only[1] | Plants[1] and seeds | Seeds only |
| **Fruit crops** | | | | |
| Citrus | 62 | 55 | 45 | 0 |
| *Cocos* | 28 | 29 | 64 | 7 |
| *Fragaria* | 20 | 65 | 35 | 0 |
| *Musa* | 39 | 54 | 46 | 0 |
| Pome fruits[2] | 37 | 85 | 15 | 0 |
| *Prunus* | 37 | 85 | 15 | 0 |
| *Ribes*[3] | 16 | 69 | 31 | 0 |
| *Vitis* | 41 | 90 | 10 | 0 |
| **Vegetable crops** | | | | |
| *Ipomoea* | 23 | 61 | 39 | 0 |
| *Solanum* | 48 | 77 | 23 | 0 |
| **Forest crops** | | | | |
| *Acer* | 14 | 100 | 0 | 0 |
| *Castanea* | 34 | 76 | 24 | 0 |
| Conifers[4] | 27 | 100 | 0 | 0 |
| *Crategus* | 14 | 100 | 0 | 0 |
| *Juglans* | 21 | 100 | 0 | 0 |
| *Populus* | 27 | 93 | 7 | 0 |
| *Quercus* | 25 | 92 | 8 | 0 |
| *Salix* | 22 | 100 | 0 | 0 |
| *Sorbus* | 24 | 96 | 4 | 0 |
| *Ulmus* | 32 | 84 | 16 | 0 |
| **Other crops** | | | | |
| *Camellia sinensis* | 20 | 45 | 55 | 0 |
| *Coffea* | 49 | 24 | 57 | 18 |
| *Elaeis* | 16 | 56 | 44 | 0 |
| *Gossypium* | 52 | 25 | 61 | 14 |
| *Helianthus* | 15 | 20 | 80 | 0 |
| *Hevea* | 28 | 29 | 71 | 0 |
| *Nicotiana* | 26 | 35 | 58 | 7 |
| *Oryzae* | 33 | 21 | 67 | 18 |
| *Rosa* | 22 | 100 | 0 | 0 |
| *Theobroma* | 43 | 19 | 79 | 2 |
| *Saccharum* | 40 | 63 | 37 | 0 |

1 Includes plant parts capable of vegetative propagation.
2 *Chaenomeles, Cydonia, Malus, Pyrus.*
3 Currants, gooseberries *Abies, Larix, Picea, Pinus.*
4 *Abies, Larix, Picea, Pinus.*

agricultural research centers (IARCs) that enter under special permits of their host country and according to their own health standards: *Musa* species (banana); *Ipomoea* species (sweet potato); *Solanum* species (Irish potato); and *Oryza* species (rice). Nevertheless, although not among the top 38 prohibited crops, many IARC crops (such as cassava and many important legumes) are detained for seed health testing or other pest and pathogen testing.

Quarantine officers sometimes take into consideration the site at which germplasm is collected in determining the case-by-case entry status of certain imported crop species. Table 4 shows some collection sites for germplasm; the sites are listed in order, from those which, in the author's opinion, have the highest pest risk to those with the lowest risk.

**TABLE 4 Some collection sites for plant germplasm, listed in order from the highest to lowest perceived pest risk**

| | Source of germplasm | Risk- or hazard-related factors |
|---|---|---|
| 1 | Collected in the wild | Pest and pathogen occurrence and incidence is often unknown, no survey or control |
| 2 | Tubers, roots, seeds, collected in market places | Health of mother plant is unknown, etc., Without foliage, material is difficult to inspect; even so, pathogens may be latent; country of origin is uncertain |
| 3 | Farms | On the average, pest control activities are relatively low or absent |
| 4 | Orchards, plantations | Often a high level of inspection, pest survey and management, or control |
| 5 | Experimental fields | May have a high level of pest survey and control; personnel are often aware of pests, pathogens, symptoms and signs |
| 6 | Experimental plots isolated from commercial plantings | Risk is lowered by absence of specified pests or lower inoculum levels, survey or located where pests are not known to occur and control practiced; personnel are aware of pests, pathogens, symptoms |
| 7 | Greenhouse with floor beds | Risk is lowered by isolation, improved phytosanitation, but soilborne pests could create sanitary and management problems |
| 8 | Commercial greenhouses | Risk is lowered by plant isolation, with raised benches; high levels of phytosanitation, survey and control |
| 9 | Research greenhouses with raised benches | Similar to 8, but at a much higher level; highly trained personnel |
| 10 | Plant tissue cultures, aseptic plantlet culture, etc. | Propagations usually have the same health status as their mother plant in so far as obligate or fastidious pathogens are concerned, but other pests and pathogens are usually eliminated during processing; isolated from contamination |
| 11 | Approved certification | Pathogen-tested plants, approved procedures, survey, phytosanitation, precautions against recontamination |
| 12 | From certain quarantine stations, third-country | Plants grown under the highest levels of pest detection, eradication, phyto-quarantine, other high sanitation, treatment, isolation, containment locations; no recontamination |

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# 4.2

# *The Role of Plant Quarantine in Nigerian Agricultural Development*

M. O. ALUKO

Nigeria is naturally endowed with tremendous but untapped potential for agricultural production. One of the main tasks of the various agricultural research institutes, government ministries and the private sector is to tap this potential by plant breeding and research to suit the requirements of the country's various ecological zones that can support agricultural production. The research and production programs depend heavily on the availability of a large pool of improved plant germplasm that must be acquired through the introduction and exchange of plant materials from all over the world, but this carries high risks of introducing foreign dangerous pests, diseases and noxious weeds. The Nigeria Plant Quarantine Service was tasked with the responsibility to protect the nation against such risks.

In addition to the efforts of the Nigerian Government to develop a Post-Entry Plant Quarantine Station at Ibadan, between 1970 and 1978 the United Nations Development Program (UNDP), in cooperation with the Food and Agriculture Organization of the United Nations (FAO), funded a Quarantine Project to modernize the Plant Quarantine Service. As a result of this project, 68 glasshouse compartments, four laboratories and two phytotrons have been constructed and equipped at the Plant Quarantine Station, Ibadan, for use in processing imported plant materials. Thus the necessary infrastructure is in place to ensure that there is only minimal delay, if any at all, in the processing of plant materials passing through the Post-Entry Quarantine Station before use in Nigeria for agricultural development.

The aim of this paper is to review the risks posed to Nigeria by the uncontrolled introduction of improved plant germplasm from other parts of the world and to elaborate on Nigeria's quarantine system and the role it plays in eliminating risks at the plant introduction stage, in eradicating or confining any pests or diseases that may slip through, and in preventing the possibility of the spread of damaging pests and diseases that would seriously threaten the country's attempts to achieve, within the shortest possible time, self-sufficiency in the production of food, fiber and industrial raw materials.

## THE RISK POTENTIAL

The risk potential of any exotic pest or disease can best be assessed in relation to the damage being caused by similar pathogens that have been introduced into the country or by the losses it has caused in other parts of the world. In Nigeria's case, the quarantine system has been so effective that only a neglible number of foreign diesease and pests have found their way into the country, and thus it is difficult to assess the specific risk potential as it relates to Nigeria. However, a brief review of some destructive plant enemies that have been introduced and have become established in countries formerly free from them may serve to emphasize the risk potential inherent in the introduction and spread of foreign plant pests and diseases from which Nigeria is still free.

Among plant diseases, the spectacular occurrence and damage caused by maize rust, *Puccinia polysora*, in Africa and Asia illustrates what a relatively unimportant pest can do in new environments. This rust apparently never caused much loss in its native habitat in tropical America, but when it was found in Sierra Leone in 1949, the losses it caused were such as to necessitate the initiation of an expensive and largely unsuccessful research and breeding program to which millions of dollars were committed through the establishment of the West African Maize Research Unit (WAMRU). From Sierra Leone, the disease spread rapidly across Central Africa; after several years it reached Kenya and Uganda and finally crossed the Indian Ocean and appeared in South-East Asia.

Coffee leaf rust is another classic example of the danger of introducing a disease into countries where it was previously unknown. The fungus appeared originally to have been confined to Ethiopia and Uganda. In 1869, it appeared in Ceylon, which at that time was the world's main coffee producer. Within 10 years, coffee production was cut to half by the disease and after a further 10 years the coffee growers were ruined and many had to sell their estates and leave; this caused a major financial crisis on the island, to the extent that one of the principal banks had to close down. Eventually, nearly all the coffee was replaced by tea. The center of coffee production moved from the Old World to the New World, in which coffee rust has not yet occurred. Nigeria, too, is still free of coffee rust.

Inadvertent introduction of virus-infected material can have catastrophic consequences. Prior to the 1920s, the citrus industries of Argentina and Brazil flourished, despite the use of tristeza-susceptible rootstocks. Then tristeza-infected nursery stock was imported from South Africa and Australia, and within 20 years some 20 million trees perished. Serious, although lesser, catastrophes befell these and other countries from the importation of budwood and nursery stock containing viruses of psorosis, exocortis and xyloporosis. Today, there is considerbale danger of the spread of two highly destructive diseases: greening, caused by a mycoplasma-like organism, and stubborn, caused by *Spiroplasma citri*.

North America was originally free of citrus canker, caused by *Xanthomonas citri* (Hasse) Dowson. In 1910, the disease was introduced into the Gulf coast area by a shipment of nursery stock from Japan. Eradication was eventually achieved, but at a cost of US$ 6 million. In the process, 257,745 orchard trees and 3,093,110 nursery plants were put to the torch (Knorr, 1965).

The most recent examples of the risk component can be found in Nigeria itself, with the appearance of cassava bacterial blight, *Xanthomonas manihotis*, and the noxious weed, *Eupatorium odoratum*. In the Eastern Central States of Nigeria, cassava bacterial blight in 1972 was reported to have caused a loss of US$ 3.75 million, and a nationwide famine looked imminent. The problem of the *Eupatorium* weed necessitated the setting up of a National

Eupatorium Eradication Program, to which thousands of dollars were committed without satisfactory results. For the past 5 years, research on cassava by the International Institute of Tropical Agriculture (IITA) and the Nigerian National Root Crops Research Institute has concentrated on finding a solution to the problem of cassava bacterial blight which has, by its introduction, severely hampered the progress which was being made in cassava research and development for the tropics. This is in addition to the millions of dollars being spent annually by international organizations and the Nigerian Government in combatting this disease which, until very recently, was not present in the country.

Great concern has also been expressed about the new outbreak of the destructive Sigatoka disease in some plantain plantations in the Rivers State of Nigeria. This disease, caused by *Mycosphaerella fijiensis*, was first identified in 1963 in Sigatoka on the Pacific Island of Fiji, from where it later spread to Colombia and Honduras and, more recently, to Côte d'Ivoire, Ghana, Gabon and Cameroon. It is a devastating disease, as illustrated by the experience in Colombia. In 1974 Colombia exported 16.33 million kg of plantains, but when the country was struck by this disease, this figure fell to 3.29 million kg within 5 years, representing a reduction of 80% in the country's foreign exchange earning capacity. By 1981 Colombia could no longer export any plantains. Honduras has suffered a similar fate.

In Nigeria, plantain is a major staple food and a potential foreign exchange earner. Recently, there have been tremendous efforts to grow the crop in large plantations in the southern parts of the country. Unless adequate steps are taken now by the Nigerian Plant Quarantine Service to stop the disease from spreading, Nigeria's banana/plantain industry may be heading for disaster.

## FORMULATION OF PLANT QUARANTINE REGULATIONS

To formulate the necessary plant quarantine regulations and processing procedures, the most dangerous foreign pests and diseases not yet known to occur in Nigeria and/or the West African region are first determined. There are over 250 of such pests and diseases for various crops. Table 1 (*overleaf*) illustrates the postion with regard to rice, as an example. The exclusion of these important foreign pests and diseases in order to safeguard the agricultural economy of the nation, and a recognition of their potential damage, must always remain a central concern. A comprehensive list and descriptions of these pests and diseases and the damages they cause is provided by Aluko (1976).

The most serious threats are undoubtedly the virus and virus-like diseases of unknown etiology. Most of these diseases are not known to be seedborne, and thus the importation of all vegetative materials of most plant materials other than seeds are prohibited. The majority of the insect pests and nematodes can be eliminated by various plant treatments; seeds can be inspected for fungal and bacterial diseases in the laboratories and glasshouses; and inspection at the source of origin can be done to ascertain the health status of mother plants in the field.

The schedule of regulations set up to prevent the introduction of diseases range from complete prohibition (as for most vegetative materials) to conditional entry, as usually specified on the import permits. Of course, the conditions for entry for each consignment vary according to the plant pests and diseases occurring in the country of origin, the virulence and mode of transmission of the pests and diseases under consideration, and the plant part that is required (*see* Table 2 *overleaf*).

**TABLE 1 World distribution of important rice diseases not yet recorded in Nigeria**

| Disease organism | World distribution |
|---|---|
| *Xanthomonas oryzae* (bacterial blight) | Asia: India, China, Indonesia, Japan, Malaysia, Korea, Taiwan, Philippines, Thailand |
| Rice dwarf virus | Asia: India, Japan, Philippines |
| Hoja blanca virus | Asia: Japan<br>North America: Mexico, USA<br>Central America: British Honduras, Costa Rica, Cuba, Dominican Republic, Guatemala, Panama, Salvador<br>South America: Colombia, Surinam, Venezuela |
| *Neovossia horrida* (bunt disease) | Asia: Burma, China, India, Indonesia, Japan, Philippines, Vietnam |
| *Tilletia barclayana* (rice kernel smut) | Africa: Sierra Leone<br>North America: Mexico, USA<br>Asia: Burma, Cambodia, China, India, Japan, Indonesia, Korea, Malaysia, Pakistan, Philippines, Taiwan, Thailand, Vietnam<br>Australia and Europe: Australia, Fiji, Greece<br>South America: Brazil, Guyana, Suriname, Venezuela<br>North and Central America: Mexico, USA, Cuba, Nicaragua, Panama, Trinidad |
| *Sclerospora oryzae* | Africa: Eritrea, Ethiopia, South Africa<br>Europe: Bulgaria, Italy, Austria, Poland, Yugoslavia<br>Asia: Japan, Manchuria, India, Pakistan<br>Australasia and Oceania: Australia, New Zealand<br>North America: USA, Canada, Mexico |
| *Xanthomas translucens* | Asia: Cambodia, China, India, Indonesia, Malaysia, Thailand, Philippines |
| *Ditylenchus angustus* (UFRA) | Asia: Burma, India, Malaya, Pakistan, Philippines |
| Virulent biotypes of *Pyricularia oryzae* (rice blast) | Europe: Italy<br>Asia: India, Japan, Pakistan<br>North America: USA |

Source: CMI World Distribution of Pests and Diseases

**TABLE 2 Nigerian regulations schedule for the importation of rice**

| Parts of plants | Countries to which restriction applies | Entry conditions | Reasons and/or requirements |
|---|---|---|---|
| All parts except seed for propagation and milled or polished rice | All countries | Prohibited | Exclusion of dangerous pests and virus pathogens of rice |
| Seeds for propagation (rough or paddy rice) | All countries except Chad, Benin, Ghana, Guinea, Liberia, Mali, | Post-entry quarantine | Exclusion of black ring (*Ephelis oryzae*), smut (*Tilletia barclayana*), downy mildew (*Sclerospora oryzae*), bacterial leaf blight (*Xanthomonas oryzae*), and Ufra disease (*Ditylenchus angustus*) |
| Seed or propagation | Chad, Benin, Ghana, Guinea, Liberia, Mali, Niger, Burkina Faso | Permit | Phytosanitary certificate, plant treatment |
| Milled, polished or parboiled for consumption | All countries | No restrictions | None |

## PLANT QUARANTINE PROCESSING OF IMPORTED PLANT MATERIALS

Many expensive and sophisticated procedures are used to detect and intercept foreign pests, diseases and noxious weeds in imported plant materials. These range from laboratory seed health testing (Neergaard and Adib, 1962; Neergaard, 1969, 1973, 1977; Phetak, 1974; Kado and Hesket, 1970; Kulshrestha et al., 1976; Aluko, 1983) to grown-on tests within closed quarantine glasshouses.

Methods used for getting rid of detected infection and infestation range from various methods of plant treatments (such as vacuum fumigation and thermopeutic and chemopeutic treatments) to complete destruction of any given consignment. Where necessary, field inspections are carried out at the source of origin to ensure that only healthy materials are introduced.

For the purpose of setting up quarantine methods for processing plant material in Nigeria, the important plant diseases are classified into three categories. The classification is based on: the local pest/disease situation in Nigeria and/or West Africa in terms of the presence or absence of the pathogens; and the local epidemiological conditions pertaining to those pathogens. Furthermore, consideration is given to local and international distribution of the pathogenic races (pathotypes). The pathogenic races of any one disease organism are such that they cannot be distinguished in appearance but they vary greatly in their ability to attack

different varieties of the same crop. From a plant quarantine point of view, therefore, each race must be considered separately. The three categories are:

*Category A* This category contains plants pathogens which justify strict quarantine measures. Such pathogens are not present in Nigeria and/or in any member state of the West African subregion and would be a threat to the crop because of their harmful effects and/or dynamic potential to spread.

*Category B* This category contains quarantine objects which are of restricted local distribution in Nigeria and/or in some West African countries and against which quarantine can be adequately provided by field inspection and/or standard laboratory methods of seed health inspection and treatments. A representative sample of the seed consignment can be health tested to determine the need for an effective seed treatment.

*Category C* This category contains internationally widespread seedborne plant disease organisms which are of importance to the planting value of the seed. In such cases, seed lots can still be health tested and tolerance levels above zero may be accepted, depending on the certification standards required.

In general, the procedures in use depend on the types of crop and the pests or diseases suspected as being most likely to be introduced. These range from normal processes, as illustrated here for cassava, to highly complicated, brain-storming cases.

### Quarantine processing of cassava

Cassava is affected by more than 25 pathogens, including fungi, bacteria, viruses, virus-like diseases and mycoplasma (Lozano and Sequeira, 1974; Lozano and Wholey, 1974). Over 90 species of insects and six species of mites have also been recorded as pests of cassava (Montaldo, 1967). These organisms can cause considerable losses. The potential danger of introducing some of them into uninfested areas can be very serious. Efforts to increase yield and production are threatened by an under-estimation of the importance of diseases and insects in cassava.

Apart from *Cercospora henningsii* and *C. vicosae*, which have been observed in all warm cassava-growing areas of the world, cassava pathogens appear to be confined to specific zones, either continents or ecological regions within continents, as follows:

- *Cercospora caribaea* (tropical America) and some American strains of *Phoma* species (Colombia, Venezuela and Panama);
- Super-elongation disease caused by *Sphaceloma* species (South America), *Colletotrichum gloesporoides* fs. *manihotis* (Brazil, Venezuela and Mexico) and *Xanthomonas cassava* (Malawi).

As for insects, the cassava hornworm, *Erinnyis ello*, shoot flies, *Silba pendula*, fruit flies, *Anasterpha pickeli* and *A. Manihoti*, and gall midges, *Cecidomyidae* species, attack cassava only in the Americas. Besides, all cassava viruses and mycoplasma in the Americas invade the vascular system and are disseminated mainly by propagation of vegetative materials.

Propagating materials in the Euphorbiaceae (forest and ornamental crops) represent a serious risk of disseminating cassava diseases. This risk is emphasized by the recent discovery that the *Sphaceloma* species found on *Poinsettia* are also pathogenic to cassava. The Plant Quarantine Service, therefore, strictly controls the importation of such alternate hosts.

The contribution of the Nigerian Plant Quarantine Service towards safeguarding the cassava industry in Nigeria against devastation has been enormous. It uses a number of measures in efforts to stop the spread of vascular pathogens such as the causal agents of cassava bacterial blight (Lozano and Sequeira, 1974; Lozano and Wholey, 1974), the American viruses (Costa, 1972; Lozano, 1972) and the super-elongation disease. These measures include obtaining materials from disease-free sources only, chemicals treatments (combination of a fungicide such as thiram or chloronab, and an insecticide such as methamidophos or carbofuran), heat treatments (such as hot water dip at 50°C for 30 minutes against super-elongation disease), tissue culture and shoot-tip indexing within 20 days of germination for cassava bacterial blight (Lozano and Sequeira, 1974; Lozano and Wholey, 1974; Takatsu and Lozano, 1975). As all viruses and mycosplasma of cassava in the Americas invade the vascular system and are disseminated mainly by propagation of vegetative materials, the Nigerian Plant Quarantine Service adopted the policy of receiving mainly true seeds and not vegetative propagating materials from the Americas. In this way, such serious mycoplasma diseases as witches-broom of cassava has been kept out of Nigeria's cassava industry, although it has recently been reported in some less strict countries, including Côte d'Ivoire.

The implementation of measures aimed at minimizing the risk of introduction of pests and diseases is seen by the Plant Quarantine Service as the responsibility both of the donor country and the recipient country. The donor country is required to:

- select materials from disease-free sources only;
- treat the materials with a fungicide/insectide combination;
- disinfect and sterilize all tools and packing materials used for handling the materials.

When receiving materials, the Nigerian Plant Quarantine Service:

- burns all materials which, on arrival, show pest infestation and/or disease symptoms;
- re-treats healthy-looking materials with a fungicide/insecticide combination; materials from countries where super-elongation occurs are given the hot water dip treatment;
- establishes the materials in isolation within closed environment quarantine glasshouses and makes routine inspections and disease diagnostic tests over a period of one year;
- destroys any established plants with pest infestation and/or disease symptoms.

## INTERNAL QUARANTINE MEASURES

When a dangerous foreign disease suddenly appears in a country, as was recently the case in Nigeria with black Sigatoka disease of plantain, quarantine intervention must be instituted

without delay, as such a disease may quickly spread throughout the length and breadth of the country. To contain the situation, the Plant Quarantine Service may institute internal quarantine measures, culminating in an eradication program.

Internal quarantine is imposed in the state affected. An area of about 100 miles radius surrounding the site of infection is cordoned off and declared by law a 'quarantine area'. All roads and other avenues of transmission between the area and the other parts of the country are placed under 'internal quarantine' to ensure that no host plant material or any other plants known to be alternative hosts to the disease can be taken to other parts of the country. This measure is intended to confine the disease to the area concerned, prior to eradication.

An eradication program carried out in to eradicate the disease from the area to which it is being confined. Such a program will, of necessity, incorporate a combination of measures: sanitation measures (for example, cutting and burning infected plants), prophylactic measures (for example, preventive sprays) and therapeutic measures. When the eradication is complete, the quarantine authorities will undertake surveys for any reappearance of the disease for three consecutive years before lifting the legal restriction imposed on the affected part of the country.

## CONCLUSION

Over a 12-year period, 70,445 varieties/selections of 76 different crops and plant materials were processed. These included 51,107 varieties/selections of cereals, 11,364 of legumes, 1,242 of root crops, 576 of tree crops, 169 of forest trees, 5,151 of vegetables, 127 of industrial crops, 644 of ornamentals and 65 of miscellaneous crops such as mushrooms. The Plant Quarantine Service records show that 82 different dangerous pests and diseases were intercepted, many of them many times over. On rice alone, notable among the pests and diseases of quarantine importance that were constantly being intercepted was the notorious bacterial blight bacterium, *Xanthomonas oryzae*, the nematodes, *Aphelenchoides bessayi* and *Ditylenchus angustus*, and other diseases of economic importance such as *Phoma glumarum*, *Colletotrichum dermatium*, *Drechslera oryzae* and *Pyricularia oryzae*. But for the interceptions and treatments and/or destruction of the infected materials, most of these dangerous pests and diseases would by now have become established in Nigeria and would be diverting the attention of the research institutes from agricultural development programs to finding ways of eradicating the pests and diseases.

Plant Quarantine Service records in Nigeria show a total of 452 foreign pests, diseases and nematodes of quarantine importance on various crops. Taking into account that some of them appear on more than one crop, there remain over 250 different dangerous foreign pests and pathogens that have been successfully kept out of Nigeria by the Plant Quarantine Service. In the case of export and industrial crops, more than 120 dangerous diseases and pests still exist in other continents but are not present in Nigeria. For instance, the witches-broom disease of cocoa which is present in Central and South America could cause up to 20% loss in the yield of cocoa within a year. The South American leaf blight disease could cause the complete destruction of Nigeria's rubber industry in a very short time. Virulent strains of the fungi causing the bud rot and wilt in oil palms exist in South America and if introduced could decimate Nigeria's oil palm plantations. The groundnut 'rust' could cause crop losses of up to 50%. The boll weevil of cotton and the 'Fiji' virus diseases of sugarcane are other serious diseases that could seriously reduce yields.

The fact that only a negligible proportion (less than 2%) of the exotic foreign pests and diseases have yet successfully infiltrated and established themselves in Nigeria constitutes the greatest testimony to the effectiveness of the Plant Quarantine Service. Although the entry of plant materials through quarantine and their subsequent maintenance under quarantine conditions are extremely time-consuming and costly, the rewards more than justify any costs. Through joint efforts with the public and private sector in importation programming and the strict adherence to plant quarantine regulation requirements, the service will continue to play an essential role in an agricultural economy striving toward self-sufficiency in the production of food, fiber, and industrial raw materials.

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# 4.3

# *Kenyan Plant Quarantine Service: Its Role and Responsibilities in the East African Region*

D.M. Okioga

Although some plant diseases and pests have become common in many countries, many are blocked from spreading freely by such natural barriers as deserts, mountains and oceans. Experience has shown that when they do reach new countries, however, by natural or artificial means, they are frequently far more damaging than the the pests and diseases that have been present in a country for a long period, because:

- the introduced organism may have arrived without the predators or pathogens that kept it in check in its original environment;
- a pest, weed or disease that may have been relatively unimportant in its original environment may find another country's environment more to its liking;
- when plants in new areas grow for long periods away from their natural pathogens, pests and predators, the genetic divergence which evolves may produce plants that have lost their resistances;
- the same pests or pathogens may remain minor under certain cropping systems or cultural practices, while under others they become more dangerous.

Although pathogens may be carried to new areas by insects and fungus spores in air currents, the spread is usually a result of the importation of infected plant material.

## THE KENYAN PLANT QUARANTINE SYSTEM

### Administration

Agriculture is the single biggest industry in Kenya and the largest employer. The overall objective of the Plant Quarantine Station (PQS) at Muguga is to protect the country's

agriculture from economically harmful diseases and pests. The PQS is legally under the Ministry of Agriculture, which is responsible for enforcing Kenya's Plant Protection Act. The Director of Agriculture is charged with the responsibility of implementing the Act, under which quarantine operates. The Officer in Charge of the PQS, and all other inspectors in the Ministry of Agriculture, regulate plant imports and exports on behalf of the Director of Agriculture. The Director appoints members of the Kenya Standing Technical Committee on Plant Imports and Exports (KSTCIE) to advise him/her on technical areas related to quarantine services. The committee is composed of plant pathologists, entomologists, horticulturalists, agronomists, plant breeders and other experts. The Officer in Charge of the PQS is secretary to this committee and is also responsible for supervising activities at the station and for the maintenance and upkeep of equipment. The Officer's decision on plant importation matters, based on whether a pathogen represents a risk or hazard to agriculture in Kenya, is guided by the regulations contained in 'The Plant Protection (Importation) Order', which is periodically revised by the KSTCIE.

The PQS is the only institution in Kenya charged with detecting and identifying fungi, bacteria, nematodes and viruses or virus-like pathogens on plant materials imported from abroad. It is responsible for establishing the best techniques for detecting such pathogens, relying mainly upon existing knowledge of the distribution and biology of plant pests and pathogens and techniques for their detection and control. Quarantine measures at the station are guided by biological principles and never dictated by economic or political pressure.

## Import regulations

Import regulations may be classified into five categories:

*1. Prohibited* — The plant is totally banned from entering the country, except for research purposes and only with the approval of the Director of Agriculture. The risks involved in importing the plants, in the context of Kenyan agriculture, are too high.

*2. Quarantine* — Significant and destructive diseases may be borne in or on the plant material. Laboratory tests are inadequate and the only safe approach is to limit the quantity of material which may be imported, apply a chemical treatment and grow the imported plant material in isolation at the PQS. Only after a plant pathologist has checked the material will it be released to the importer.

*3. On permit* — The risks involved in importing the plant are relatively low, and they enter the country on an Import Permit. Most seeds are imported on such a permit because they have few seedborne diseases or the diseases that they may carry are already in Kenya and are of little economic significance. On arrival, the seeds are inspected at the port of entry and may be treated before release, or destroyed if found to be infected with a dangerous pest, weed or pathogen. The Plant Inspector stationed at Mombasa port is responsible for inspecting large quantities of food grains, plants and plant products entering the country

through the port and for ensuring that garbage containing or contaminated by scraps or other plant waste is not thrown overboard into Kenya's territorial waters.

*4. Endangered and/or rare species* These species are imported only on the approval of the government of the exporting country and in accordance with the Convention on International Trade in Endangered Species of Wild Fauna and Flora. Restrictions are necessary in order to prevent the extinction of endangered species.

*5. Biocontrol agents* Potential biocontrol agents intended for importation into Kenya must first be established in pure culture at source. They are then shipped to the intermediary CIBC in the UK or at IITA, Ibadan, where they are further screened and reared through several generations under controlled conditions to ensure that the species have been correctly identified and the biocontrol agents are not accompanied by any new pest species or pathogens. The importation must therefore carry phytosanitary certificates from the country of origin and from the UK or IITA, as well as an import permit which specifies the requirements to be met before the shipment is made.

**Issue of import permit**

For materials under categories 1, 2, 4 and 5 above, an import permit is issued by the PQS. However, for certain in plants under category 4 an import permit is issued by Wild Life Conservation and Management Department of the Ministry of Tourism and Wildlife. For materials under category 3, an import permit is issued by either the National Agricultural Laboratories or the PQS.

The main features of import regulations can be summarized as follows:

1. All intending importers wishing to bring into the country plant materials or any article or class of articles that is likely to be injurious to any crop or forest trees must obtain an import permit prior to shipment, regardless of the purpose for which the materials are being imported. The import permit specifies the requirements for plant health, indicating prohibitions, restricted quarantine importations and additional declarations with regard to pre-shipment treatment. The original permit must therefore reach the plant health authorities in the country of origin so that Kenya's permit requirements can be strictly adhered to.

2. Any plant consignment arriving in Kenya should be accompanied by a copy of the import permit and a phytosanitary certificate which adheres to the specifications set out in the import permit.

3. Plant materials arriving in Kenya without authority and the correct accompanying documents are not allowed entry and may be destroyed or shipped back to the owner at the owner's expense.

4. All visitors arriving in Kenya must declare all plant materials in their possession on arrival. It is illegal and punishable by law (fine or imprisonment) to import plants into Kenya without proper authorization from the Ministry of Agriculture.

5. Legal authority is provided to the Plant Inspector to allow treatment or destruction of infested or infected plants or plant products imported into Kenya, regardless of whether they are accompanied by correct documents.

**Processing imported plant materials**

The regular checks made at the PQS on imported seed are as follows:

*Dry inspection* — The imported seed is examined directly by magnifying lens for impurities in the seed, uniformity in seed size, discoloration on the seed surface or presence of weed seeds. Only clean healthy seeds are selected for processing.

*Agar seedling method* — The seeds are planted (after surface sterilization) or agar incubated under near-ultra-violet (NUV) light, alternating 12 hours NUV and 12 hours total darkness, at a temperature of 21-22°C for 8 days. The seedlings are examined for fungal sporulation and bacterial lesions. Infected seedlings are discarded and healthy seedlings are transferred to sterilized soil in the glasshouses for virus indexing. Only seeds from disease-free healthy plants will be released to interested parties.

The checks made by the PQS on vegetative propagating materials involve treating them with insecticidal or bacterial solutions before they are planted in sterilized soil in glasshouses for propagation or for grafting for eventual indexing against virus diseases.

*Tissue culture* — Importation of germplasm, particularly of root and the tuber crops, through the tissue culture method is gaining prominence, since it has proved to be economical and convenient. The germplasm can be handled easily in view of the decreased bulk and the elimination of most diseases. There is, therefore, a positive trend toward promoting the international exchange of germplasm by shipping plantlets in the form of tissue culture. On arrival, the plantlets in test-tubes are kept under fluorescent light in a temperature-controlled room (22°C ± 1°C) for one week, then transferred to pots under indirect light and covered for the first 1-2 weeks by glass or plastic covers before they can gradually be released to ambient humidity. On hardening, the plants are transferred to glasshouses where they will eventually be indexed for virus diseases.

*Virus indexing* — Virus indexing may be defined as transmitting, by grafting or other means, the juices from a plant suspected of being infected

with a particular virus to another plant known to be susceptible to that virus in order to determine whether or not the suspect plant is actually infected. Virus indexing of plants imported as seeds, cuttings or tissue culture is routinely carried out at the PQS. The station relies mainly on virus indexing procedures using indicator plant methods. A large collection of indicator plants is maintained at the PQS. Inoculation of indicator plants is mainly by grafting or sap transmission, but insect vectors are also used. Other methods of virus indexing include microprecipitation tests, latex tests, enzyme-linked immuno-sorbent assay (ELISA) tests and nucleic spot hybridization (NASH) tests. The NASH tests are done in collaboration with the Centro Internacional de la Papa (CIP).

### Release of plants from quarantine

From time to time, release reports are sent to the Director of Research, the National Research Station, national universities and other interested persons indicating what plants materials have been processed through post-entry quarantine and released. Usually, 50% of the released plant materials is sent to the consignee (importer), 10% remains at the PQS and the remaining 40% is supplied to interested parties on request.

### Domestic quarantine

Under the Plant Protection Act, the PQS is responsible for regulating domestic quarantine for pests and diseases which may spread to other parts of the country. Domestic quarantines have been imposed on:

- greater grain borer, *Prostephanus truncatus*, now confined to south-western parts of Kenya (Taita/Laveta areas);
- serpentine leaf miner, *Liriomyza trifolii*, confined to eastern parts of Kenya (Kibwezi and Voi areas);
- cassava bacterial blight, *Xanthomonas manihotis*, confined to western Kenya;
- banana nematode, *Radopholus similis*, confined to central Kenya (Thika, Muranga).

## THE ROLE OF THE PQS IN GERMPLASM CONSERVATION AND SEED TECHNOLOGY

The PQS now maintains over 20,000 accessions of germplasm, primarily improved germplasm from foreign sources. A few collections comprise landraces and unimproved germplasm. All materials are available without charge to plant scientists in Kenya. In addition, materials at PQS are exchanged with foreign countries for germplasm needed by Kenyan scientists. In

providing germplasm to users, domestic or foreign, only a portion of a given accession leaves the system. A given accession is never exhausted; it is maintained and increased as necessary. New accessions of germplasm are added to the system at the rate of 2,000 per year. Approximately 90% of these are acquired directly through foreign sources; the rest come through exchange with other countries.

The major activities of PQS related to germplasm conservation are: introduction; maintenance; monitoring viability; retrieval; and regeneration.

*Germplasm introduction*

The PQS is the focal point for the acquisition and exchange of plant germplasm. It catalogues all incoming accessions and assigns plant introduction numbers (EAI). It provides import permits to facilitate shipments of plant materials to Kenya. To ensure that the introduced crop germplasm is pest- and disease-free, the PQS undertakes elaborate steps to prevent the introduction of new plant pests and diseases into the country. The germplasm screened at the PQS is distributed as indicated above. Some 10% of the germplasm is retained at the PQS.

*Germplasm maintenance*

*Ex situ* conservation of germplasm is practised at PQS as seed conservation, *in vitro* conservation and field genebank maintenance.

One of the most important phases of conservation of germplasm is seed storage, the aim of which is to preserve or maintain the genetic integrity of the germplasm throughout the storage period. Since all seeds begin to deteriorate as soon as the stage of physiological maturity is reached, the function of storage management is critical. At the PQS, over 15,000 accessions of crop germplasm are conserved as seed in a cold store fully operational at -4°C. Originally, the seeds were kept in brown paper envelopes, but efforts are now being made to transfer the seeds to hermetically sealed aluminum containers after the moisture content of the seed has been reduced to about 6%.

The application of *in vitro* conservation as an integral part of plant germplasm conservation has been restricted to potato, *Solanum tuberosum*, cassava and sweet potato. More than 70 clones of potatoes are conserved as *in vitro* plantlets at the PQS.

For crops that do not produce orthodox seed or do not produce seed at all, conservation in a field genebank is essential. Other plants, such as cassava, produce seed but it is essential that they are also maintained as clones. For this reason, the PQS has established a 20-acre field genebank. As of September 1988, a total of 947 accessions were being maintained on in the genebank, comprising 541 accessions of sugarcane, 240 accessions of pasture grasses, *Brachiaria* species, 62 clones of cassava, 50 accessions of temperate fruits, 36 accessions of sweet potatoes and 18 clones of banana.

*Monitoring viability*

For the purpose of maintaining seed of high viability during storage, it is essential that regeneration be carried out whenever the viability falls below 95%. Stored seeds are checked for viability after a period of storage of 3-5 years.

*Regeneration*

In 1983, it was observed that viability of soybean, *Glycine max*, and beans, *Phaseolus vulgaris*, had fallen below 90%; as a result, regeneration of bean accessions was started. In 1985, a total of 435 bean accessions were planted under field conditions for regeneration. Unfortunately, 138 accessions were found not to be viable since no germination of the entire accessions was recorded.

In the same year, 85 accessions of *Sorghum* species were regenerated under the field conditions. The regeneration experiment did not register any loss of accessions, although within accessions very low germination of seed was detected. Regeneration of other accessions is in progress.

*Retrieval*

Retrieval of plant germplasm from various research stations remains one of the principal activities of the PQS. A retrieval mission for recovery of some elite sorghum material was undertaken and a total of 46 accessions were retrieved from the Western Agricultural Research Station in Kenya.

## THE NATIONAL GENEBANK

The National Genebank set up by the German Technical Aid Agency (GTZ) at the premises of the PQS is a crucial facility and Kenya's only long-term (100 years or more) seed storage facility. The facility will maintain plant germplasm as a base collection for Kenya and will be a backup base collection for many crops in support of the national network of genetic resources centers. It will stock basic plant introductions made through the PQS, obsolete varieties, open-pollinated parental lines and genetic stocks.

The National Genebank base collection is not intended to meet the day-to-day needs of plant breeders and other plant scientists but rather to serve as a reserve stock to prevent loss of germplasm and erosion of genetic diversity. Seed samples in the base collection are distributed from the National Genebank only when they are unavailable from another source.

Base collection samples are for indefinite storage with regrowing as infrequently as possible so that genetic changes through repeated seed increase do not occur. However, the seed is regrown often enough to prevent loss of viability.

The PQS will continue to maintain plant germplasm as an active (working) collection to meet the research needs of breeders, geneticists, pathologists, entomologists, horticulturists and other users. Active collections include foreign acquisitions, wild relatives of crop species, domestic flora and cultivars, and some advanced lines. Officers at the PQS who maintain the active collection have a responsibility to maintain, protect, control access to and distribute specific plant germplasm. Like the officers in charge of the base collection, they are responsible for maintaining the collection under good storage conditions and for carrying out seed rejuvenation as required. They protect and manage field genebanks (plant repositories) in the case of clonally propagated species, keep an inventory of accessions in the collections and make reasonable amounts of the germplasm under their care available, at no charge, to research scientists and institutions.

# 4.4

# *Quarantine Aspects of the International Transfer of Genetic Resources from the International Institute of Tropical Agriculture (IITA)*

H. W. ROSSEL, G. THOTTAPPILLY, S. Y. C. NG and G. L. HARTMAN

The seed health and quarantine implications of international and adaptive testing of plant genetic resources represent a major concern to international agricultural research centers (IARCs) and others institutions involved in such efforts. In recent years, these implications also have attracted the attention of a number of bilateral and multilateral organizations, including the Food and Agriculture Organization of the United Nations (FAO), the Consultative Group on International Agricultural Research (CGIAR) and the International Board for Plant Genetic Resouces (IBPGR). These and other organizations are actively involved in genetic improvement of the world's major food crops in a number of less developed countries.

In this paper, we will discuss briefly the work being done in connection with the international transfer of such resources to and from the International Institute of Tropical Agriculture (IITA). Quarantine regulations governing the import and export of plant materials usually do not allow the presence of any pathogen. Therefore, such adequate elimination procedures as chemotherapy, fumigation, hot water treatment and shoot-tip culture, in conjunction with reliable detection (indexing) procedures, are prerequisites to the safe and efficient transfer of plant genetic resources. Obviously, the first step toward reliable sanitation is understanding the etiology of diseases. It is one of the important aspects of plant protection research at IITA, involving studies of diseases caused by bacteria, fungi, nematodes and viruses.

Many viruses are seedborne but usually at a low rate (less than 1%). Some, however, such as soybean mosaic virus (SMV), cowpea aphid-borne mosaic virus (CAbMV) and cucumber mosaic virus (cowpea strain) (CuMV) are known to be carried in legume seeds at considerably higher rates (10% or more).

Viruses occurring in legumes are notorious for their common seedborne nature; in vegetatively propagated crops, viruses, because of their highly systemic nature, are usually

passed on to all progeny plants. As a consequence, in the international transfer of germplasm, viruses are generally the most difficult to detect and control, and thus constitute potential quarantine hazards. Serious outbreaks of new virus diseases in regions where they had not formerly occurred are, however, infrequent, possibly because of more-or-less effective ecological barriers. This is somewhat surprising, considering the large volume of unofficial, international movement of untested and uninspected plant materials and seeds of various kinds and for various purposes.

Sudden outbreaks of so-called new virus diseases appear often to have been a conspicuous occurrence of an indigenous virus in an exotic, and mostly highly susceptible, genotype. In the traditional varieties, on the other hand, few virus outbreaks occur, probably because of a high degree of co-adaptation with the indigenous diseases. Often changes in agricultural practices cause different ecological and epidemiological conditions which favor the outbreak of seemingly new, though in fact indigenous, virus diseases.

## PEST RISK STATUS AND QUARANTINE SIGNIFICANCE

A 'pest risk analysis', according to Kahn (1979), regarding virus diseases of legumes such as cowpea and soybean for which IITA holds the CGIAR mandate, reveals that viruses in these crops have generally been well studied. The seedborne nature of some of the viruses involved is well documented. Most of these are known to have a worldwide distribution.

From an epidemiological point of view, virus diseases in crops propagated through true seed are disseminated differently than in vegetatively propagated crops. In the latter, infection incidence of any newly introduced virus is likely to increase constantly until it reaches 100% incidence, even in the presence of an inefficient vector system. In seed-propagated crops, virus incidence is erratic and is likely to vary greatly from season to season and from year to year. In such cases it may disappear when no ecological niche is found (no survival value). In fact, newly introduced (seedborne) viruses, or new virus strains, may become established only if the following conditions are met:

- an efficient vector is present;
- indigenous varieties of the crop are susceptible to the newly introduced virus or strain thereof;
- a potentially effective alternative host is encountered;
- seed transmission occurs in the indigenous varieties.

In principle, breeders' nurseries sent for testing in other countries are screened for resistance to the relevant viruses. Although some accessions may not be completely resistant to the prevailing virus diseases, in the field such moderately resistant accessions usually show only a low infection incidence. Thus, the chances of contamination by seedborne viruses of seed harvested from such nurseries is generally remote.

Fortunately, the virus spectrum of the major food crops in Nigeria is very similar to the virus spectrum found in the same crops elsewhere in Africa. We make this conclusion on the basis of what has been reported in the literature and as a result of our own studies and observations. In certain cases, Africa may be considered a phyto-pathologically homogene-

ous zone. In fact, the Inter-African Phytosanitary Council has already adopted a more liberal approach toward the international transfer within this continent of germplasm of some crops (for example, cassava and sweet potato). It is imperative that, with regard to viruses that have a known, worldwide distribution, more realistic and more liberal regulations be drawn up by the parties involved in the international transfer of germplasm of various kinds.

Moreover, in terms of food security, the importance of crops differs greatly from region to region. For example, in Africa cowpea, soybean and sweet potato are far less important as staple foods than such crops as cassava and maize.

Generally, the scientists concerned agree that the potential benefits to be reaped from international, adaptive testing programs involving a great variety of ecosystems generally greatly outweigh potential quarantine risks inevitably resulting from such activities. We all agree that genetic improvement of cowpea and soybean would not have been nearly as effective as it has been if unrealistically strict quarantine regulations had made effective execution of such multilocational and international adaptive testing programs impossible.

## IITA's sanitary procedures pertaining to viruses

With regard to sanitation, export and international testing of improved germplasm of crops propagated through true seed, IITA carries out the following procedures:

- As a first priority, it establishes which viruses prevail in the crop(s) being considered in the area of origin (multiplication) of such materials, and which of these viruses are potentially seedborne.

- Plants that are infected during the active growing period of such crops, up till flowering, are eliminated as far as possible (rogueing). In the case of soybean in particular, this seems to result in excellent control of SMV, as infection pressure of this virus results almost entirely from 'in-crop' (seedborne) sources of infection.

- Accessions that are susceptible to the prevailing virus diseases and have commonly become infected, or that have shown seed transmission in realistically designed testing methods, are not selected for further international testing; they are considered unsuitable because of their 'in-built, epidemic potential' pertaining to such viruses.

- Actual seed transmission incidence is monitored in the seed stocks of the various accessions harvested. Seed transmission rates are determined in grow-out tests performed under insect-proof conditions. The number of seeds tested per accession is of the same order as the number of seeds of such accessions sent to collaborators for international, adaptive testing. Accessions that show any seedborne-infected seedlings in these tests are, in principle, omitted from nurseries intended for international testing.

- Seed increase usually takes place in comparatively strict isolation. In general, it is recommended that all materials for international testing be increased at a location where germplasm rejuvenation is not simultaneously pursued, in order to prevent possible infection by viruses that might be prevalent among the numerous germplasm accessions grown out for rejuvenation.

- A plant germplasm health statement from the breeding program concerned, stating which seed health testing procedures have been applied to the seed lots sent to interested parties, and giving the outcome of such tests, is being to all seed consignments.

- Seed increase usually takes place in the most suitable season with the lowest expected vector activity and/or infection pressure of the viruses concerned.

Thus, it follows that the emphasis at IITA is placed primarily on sanitary procedures aimed at preventing seedborne infection. Moreover, seed transmission rates in any outgoing nurseries are carefully monitored. Accessions showing levels of seed transmission which are considered unacceptable for a particular virus are not exported.

Seedborne pathogens of other categories, such as fungi and bacteria, are, of course, also important in the dispersal and epidemiology of some diseases. Control measures, which in these cases may be of either a preventive or a curative nature, must eliminate or reduce bacterial and fungal pathogens below threshold levels. Preventive practices at IITA include chemical and cultural methods to reduce the incidence of such pathogens. Fungicides such as Demosan or Benlate are used routinely as a seed dressing and applied to any seed exported from the IITA Genetic Resources Unit (GRU), as well as from the respective breeding programs. In addition, seed increases of germplasm collections is done under dry conditions in the greenhouse and/or in the field at a time when pods mature at the end of a rainy season or under irrigation in the dry season. These chemical and cultural control methods are examples of how IITA helps reduce the risk of contamination and distribution of pathogens.

We should like to stress, however, that sanitary procedures of this kind are relevant to the production of true seeds only. They are not relevant where quarantine level ('zero tolerance') testing is required, as in the germplasm of vegetatively propagated crops. For this germplasm, an entirely different approach is required because all viruses present are carried on to every newly established plant; in general, a typical zero tolerance quarantine approach is indicated in such cases. The role *in vitro* culture techniques are playing in this respect is discussed here.

## Application of tissue culture techniques at IITA

Since Morel and Martin (1952) first used meristem-tip culture to produce virus-free dahlias, this technique has been widely applied to many vegetatively propagated crops in order to obtain virus-free plants from originally diseased materials. Only through a sound understanding of disease etiology and the availability of reliable virus indexing methods may the regenerated plants obtained from meristem-tip culture be declared free from viruses, particularly as some viruses may be latent. The method has greatly facilitated the international distribution of vegetative materials, as shipment in this form circumvents most quarantine restrictions. Through meristem-tip culture, virus-free clones have been regenerated from a wide range of economically important crops (Quak, 1977; Walkey, 1980). One of the important features of using meristem-tip culture is that the regenerated plants usually retain the genetic integrity of the parent plants. This is probably because of the more uniformly diploid nature of the meristematic cells (Murashige, 1974).

At IITA, meristem culture technique has been used to produce virus-free clonal germplasm of root crops for international distribution. A combination of thermotherapy and meristem culture has been successfully applied to eliminate cassava mosaic virus from cassava (IITA, 1979, 1980; Ng and Hahn, 1985) and yam mosaic virus from white yam (Ng, 1989). Meristem

culture alone was found to be effective in eliminating sweet potato virus disease complex from sweet potato (Frison and Ng, 1981; Ng and Hahn, 1985; Ng, 1986). Research is also conducted to investigate the effects of ribavirin on virus elimination in yam (IITA, 1988).

The procedure for obtaining virus-free plants using meristem-tip culture will be briefly described here. Obviously, it is important to know what kind of virus or viruses are present in the crops concerned, in order to assess the disease status of regenerated plants. Usually meristem-tips of approximately 0.3-0.5 mm in diameter are excised from surface-disinfected apical buds of cassava or apical and axillary buds of yam and sweet potato. The tips consist of the meristematic dome and two leaf primordia. They are then placed onto an appropriate culture medium under aseptic condition. Culture medium formulations, such as that formulated by Murashige and Skoog (1962), are commonly used, with slight modifications depending on the crop species treated. The presence of growth substances such as auxin, cytokinin and gibberellic acid are important in the regeneration of the meristem-tips; details of surface disinfection, explant removal and medium composition for root crops have been given by Quak (1977), Ng and Hahn (1985) and Ng (1986). Cultures are then incubated under controlled temperature conditions (25°-30°C) in a culture room.

Plants regenerated from meristem-tip cultures are not automatically free from pathogens and viruses. Regenerated plants must be indexed for freedom from virus infection. This is sometimes the most time-consuming stage in a disease elimination scheme. The regenerated plants are transplanted from tubes to sterile soil in pots and kept in an isolation room for further growth and monitoring for disease expression. Virus indexing methods vary, depending on the type of virus(es) involved and on available facilities. Ideally, regenerated plants should be tested for viruses by various methods, and these tests should be repeated several times. Virus indexing may take up to one year.

Methods used for indexing root crops plant materials are:

- monitoring for possible occurrence of symptoms over a long period of time under a condition which favors symptom expression (for non-latent, so-called self-indexing diseases);
- sap inoculation to test plants, for example, *N. benthamiana* (for cassava and yam);
- grafting to suitable indicator plants, *I. setosa* and sweet potato test clones (for sweet potato);
- inspection of materials under the electron microscope for possible presence of virus particles by means of the simple 'leaf crush' or 'leaf dip' method;
- immuno-sorbent electron microscopy (ISEM); for this procedure, antiserum is added to the electron microscopic carrier films to absorb more virus particles so that sensitivity will be increased; after this, virus particles may be 'decorated' with a specific antibody in order to identify the virus under investigation;
- enzyme-linked immuno-sorbent assay (ELISA).

In addition to the highly sensitive, serological techniques such as ELISA and ISEM, IITA, in collaboration with advanced institutions, is looking into the use of monoclonal antibody and cDNA technology for indexing meristem-regenerated plants.

Virus-tested and certified plants are multiplied *in vitro* and prepared for international distribution. Any fungus- or bacteria-contaminated cultures are discarded.

### Recommendations for improvement

As to further streamlining of present procedures, the following suggestions may be considered:

- More surveys should be made in countries to which nurseries are shipped, in order to determine whether the viruses, or the particular strains that might come with the seed, are already present in those countries. It is hoped that this kind of information will lead to a further rationalization/liberalization of quarantine regulations.

- Absence of seed transmission should be considered as a selection criterion in breeding. Its merits lie not only in the prevention of the spread of seedborne viruses, but also in the possibility that success might make seed testing redundant.

- We support the recommendation made by Kahn (1982) that a seed health specialist be appointed, or a committee or unit be formed within IITA, to promote the general health status of all breeders' materials grown for seed increase for international testing and for the Genetic Resources Unit.

- We should like to further recommend, however, that a position of this kind be created within the CGIAR to supervise and coordinate these activities at the CGIAR level in all the IARCs concerned. A similar recommendation, proposing that FAO take the lead in such activities, was made by Kahn (1982).

Although aphid control, thought to limit the secondary spread of viruses from primary (seedborne) sources, has been recommended by various virologists, the expected effect may not necessarily be obtained from such measures. In the case of cowpea, for instance, abundant sources of infection with relevant viruses, as well as aphid vectors, are generally present in the environment.

## QUARANTINE ASPECTS OF SEED TRANSFER FROM GERMPLASM COLLECTIONS

### Crop species propagated through true seed

Most of the considerations relevant to the adaptive testing of breeders' germplasm apply only partly, or not at all, to the management of germplasm collections. There are two different perspectives from which quarantine-related problems may be approached.

The first pertains to the introduction, at quarantine level, of collections of virus-tested accessions indexed for infection with relevant viruses. Only those viruses would be considered which occur in the area where evaluation and/or rejuvenation of germplasm takes place. In this case, there would be no qualitative differences between the general procedures

followed in the distribution of breeders'seeds and materials emanating from germplasm. There are major quantitative differences, however, which are a direct result of the following:

- the number of seeds of each individual accession available for international distribution, is generally much smaller than in the case of breeders materials;

- the number of different accessions is generally much greater than in the case of breeders' nurseries.

These two facts have practical implications: they make the sanitary testing of seed lots of each individual accession, as well as the rogueing of them in the field, as practised with breeders' seed, virtually impossible. This is because seed-testing procedures are, in principle, destructive, whereas rogueing would inevitably lead to the loss of many of the virus-susceptible accessions.

On the other hand, the chances of the GRU nurseries carrying seedborne viruses are much greater than in the case of breeders' nurseries. This is because of the largely unselected nature of such collections. Seed transmission seems to be a highly heritable character, against which selection takes place during breeding and selection. Inevitably, endemic viruses will occur in germplasm collections in the field during evaluation and rejuvenation, and these will spread rapidly, especially through the most susceptible accessions. Indeed, the chances are high that, as a result of the significantly higher infection pressure resulting from early infection in highly susceptible accessions, all susceptible accessions will become uniformly infected during their vegetative growth phase, thus greatly increasing the chances for seed transmission to occur. However, the differences with breeders' nurseries in this case being primarily quantitative, the same phytosanitary principles apply because, in this model, one needs to consider only the locally occurring, seedborne viruses.

The second approach assumes that during the assembling of the germplasm collection, new viruses or strains hitherto unknown in the environment where the collection is being maintained have come along and may already have spread through the entire collection. This probably represents a more realistic picture of the actual situation and, in this case, only the enforcement of zero-tolerance quarantine procedures would be acceptable. Thus the following approach has been proposed:

- All incoming collections, irrespective of their certification status, will pass through a post-entry quarantine stage in which only seed produced on visually inspected and, where appropriate, virus-tested plants, grown in isolation, is entered into the collection for storage. Plants showing virus symptoms and/or found to be infected will be discarded. Thus, only seed harvested from plants likely to be virus-free is entered into the collection. The chances of new viruses or virus strains spreading through the entire collection are thereby greatly reduced.

- The existing germplasm collection, which probably contains seedborne viruses or virus strains alien to the environment in which the collection is being maintained, will need to be cleaned up. This could be done in the same manner as that proposed above for new, incoming materials.

- Until the existing germplasm collection has been cleaned up, all outgoing GRU materials will need to go through a similar pre-export, or post-entry, quarantine stage. This means

that for the shipment of germplasm accessions to interested parties, a 3-4 month delay must be allowed for.

In this way, reliable and much needed information will be obtained as to alien viruses or virus strains possibly occurring in the IITA germplasm collection. This will show us which viruses are present in, or absent from, the existing collections and tell us more accurately what to test for when handling GRU resources in the case of import as well as export.

Once the germplasm collections concerned have been cleaned up, outgoing seed from the collections may be treated as breeders' seed, provided the proposed post-entry quarantine procedures are being adhered to with regard to new germplasm. It would then, in our opinion, no longer need to pass through the proposed pre-export or post-entry quarantine stage.

## Vegetatively propagated crop species

When discussing quarantine issues, the special status of crops that are propagated vegetatively is evident. Because of their zero-tolerance quarantine status in general, meristem-tip culturing followed by indexing for all known viruses occurring in such crops in the area of origin, and maintenance and shipment of the virus-tested materials in *in vitro* form, are at the basis of the procedures governing the international transfer of germplasm resources of this nature. Obviously, the international transfer of germplasm of such crops should not be considered when virus or virus-like diseases of unknown or uncertain etiology are known to occur in the region of origin of such materials.

Several virus diseases in such crops as cassava or sweet potato, both of which are of prime concern to IITA, are known to have a restricted distribution. Depending on indexing status and/or quarantine/pest relevance, their international transfer to and from Africa is subject to specific regulations drawn up by national or regional plant quarantine services and other parties involved. Alternative transfer procedures, such as shipments by way of an independent, high-standard research laboratory, may be agreed upon in mutual consultation with national or regional plant quarantine services.

IITA has been closely involved in activities relating to intermediate quarantine. For over a decade, we have been drawing on the services offered by the Research Institute for Plant Protection (IPO), Wageningen, The Netherlands, within the framework of long-standing cooperation between IITA and IPO in the field of plant virology. This cooperation has provided for extra safeguards during germplasm transfer, as in the case of the transfer of sweet potato germplasm, where such assistance was highly appropriate in view of the difficulties encountered in detecting viruses in this crop (Rossel and Thottappilly, 1988a, 1988b).

However, germplasm transfer appears to be less regulated in other parts of the world than in Africa, a situation which deserves increased attention. For example, in various parts of the world (Nigeria, Peru, the USA and Taiwan), germplasm collections of sweet potato are being constituted, but, to prevent the international spread of virus diseases of this crop, the exchange of germplasm between these collections needs to be properly regulated and further research must be conducted into sweet potato virus diseases. On the other hand, also in the case of sweet potato, a realistic approach, not attaching too high a quarantine relevance to viruses known to occur throughout the continent or worldwide, is desirable. In such cases, as with seedborne viruses in leguminous crops, agreement on how to treat the issues and how to adjust quarantine regulations to fit reality must be reached between all parties concerned.

## Seedborne viruses in IITA's mandated legume crops

Kahn (1981, 1982), in a consultation for FAO on plant germplasm health issues pertaining to six IARCs, including IITA, reviewed the procedures, safeguards and policies associated with the international transfer of genetic resources by the IARCs in cooperation with national quarantine services.

Of the four general recommendations made during a consultation between IBPGR, FAO and the IARCs following Kahn's review, IITA had already implemented three that are directly applicable to IARCs. Two of these are:

- The use of a plant germplasm health statement which, in addition to the phytosanitary certificate, is attached to each consignment of cowpea seed exported, stating in detail the procedures followed in assessing the actual rates of seed transmission of the viruses mentioned;

- Informing the receiving party (local quarantine service) of the pest-risk status of the viruses involved, and those possibly present at a very low levels in the cowpea nurseries received from IITA.

The receiving party is provided with a brief explanation of the possible quarantine implications of growing such nurseries, and is asked to omit from their field tests those lines received in which some seed transmission of specified virus(es) has been observed if the quarantine service in their country would require such action, or if they would feel more comfortable doing so. However, in the same letter it is pointed out that the viruses involved represent pathogens of possible worldwide occurrence which probaby occur in their country already and/or are of minor importance. Recently, a new multipurpose plant germplasm health statement has been drafted. It is attached to all germplasm shipments originating from IITA. With reference to materials originating from IITA's germplasm collection of cowpea, the current policy at IITA is that the receiving party is made fully aware of the likelihood of occurrence of various unspecified, seedborne viruses in the seed stocks received.

The third recommendation arrived at during the consultation was that a plant health unit, committee or post be created within the IARCs to deal specifically with plant germplasm health issues. However, no funds have been made available for establishing such a unit at IITA, although it is worth pointing out that Kahn (1981) considered that the IITA Virology Unit was handling viruses adequately. It needs to be stressed in this context that viruses constitute the bulk of sanitary problems.

The message that we hope we have been able to convey is that breeders' seeds of leguminous crops and the genetic resources of these crops emanating from germplasm banks are not comparable as far as quarantine requirements is concerned. We are of the opinion that for breeders' seed only a 'sanitation approach' applies, according to which seed infection rates are kept down to certain maximum acceptable levels ('tolerances'). A low level of seedborne infection by known viruses is considered acceptable when no significant quarantine relevance is attached to the viruses concerned. The materials in the GRU, in particular incoming seed, generally represent a higher risk group. We had, therefore, earlier recommended that a post-entry quarantine approach be followed for this category of materials.

As to the role of seedborne viruses, we should like to stress that in breeding programs such as those of mandated legume crops at IITA, there is no question of seed increase of 'finished'

products for large-scale farmers' production. What in fact applies is increase of seed of promising breeding lines, selected from (segregating) populations in field experiments at IITA or in IITA's testing sites elsewhere in Nigeria. Selection of such lines for further international testing is based on their performance in Nigeria, including a high degree of resistance to the relevant virus diseases. Seed increase usually takes place immediately after this testing stage in order for those selected lines to be distributed among collaborating scientists for the sole purpose of evaluation, under various ecological stresses, in an experimental setting only.

As mentioned earlier, in accordance with the regulations, all selected materials are subjected during the seed increase stage in the field to sanitary practices and are inspected in the field by Nigerian Plant Quarantine Service officials, accompanied by an IITA virologist.

As to concerns about latency and the possibly unnoticed transmission of viruses to seed that may result from such latency, we should like to mention here that our experiments with cowpea and soybean have shown that materials screened and selected as resistant to the relevant viruses, in further back-tests, generally proved free from such viruses. Thus, the fear of unnoticed transmission of these viruses through the seed in breeding lines selected for resistance seems to be largely unfounded.

## CONCLUSION

We should like to stress that, as a first priority, realistic guidelines should be drawn up to address issues of sanitation with 'acceptable' tolerances versus 'zero' tolerance, before we can discuss effectively the issues of plant health and quarantine in relation to the international transfer of germplasm. We believe that this needs to be done as a matter of urgency because various and sometimes widely differing opinions are held on these issues. For this purpose, a permanent working group needs to be constituted from the relevant IARCs and other parties concerned, including IBPGR, FAO and regional and national quarantine services.

Each 'virus-crop-region' system should be assessed on its own pest risk characteristics, and conclusions and/or recommendations with regard to seed health issues should be reached in close consultation and preferably in formal agreement with all parties involved in the transfer of genetic resources.

As unknown viruses might occur in the GRU collections, the international movement of such genetic resources of crops propagated through true seed should, in certain cases, be subject to quarantine considerations comparable to those pertaining to vegetatively propagated crops. This means that, for this category of materials, under certain conditions a zero-tolerance approach, involving pre- or post-entry quarantine, should be followed.

Organizations such as FAO or IBPGR should take the lead in getting the parties concerned together to establish guidelines for what is acceptable in the international transfer of genetic resources in general and of seed-propagated crops such as cowpea and soybean in particular.

As a zero-tolerance approach is unworkable, and often unrealistic, the blanket regulation that all seed exported must be free from seedborne pathogens (viruses) requires rethinking and rephrasing. In general, a good understanding between IARCs and regional or national quarantine agencies is imperative to the smooth functioning of such research establishments. The ultimate goal is crop improvement, which is often attainable only by identifying and introducing new and superior germplasm. Certain quarantine risks may be justifiable in this respect. In fact, these risks are taken in any case, since only known diseases or disease agents

can be tested for, but no one would want to claim that everything is known. As long as the risks taken are *realistically* assessed (pest risk analysis) and quarantine authorities are involved in the process and are in agreement with the sanitary and/or testing procedures adhered to, there is little chance for misunderstanding should something go wrong, such as the outbreak of a new and hitherto unknown disease.

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# 4.5

# *Plant Health Research at the International Board for Plant Genetic Resources (IBPGR)*

E.A. FRISON

The mandate of the International Board for Plant Genetic Resources (IBPGR) covers different aspects of the conservation of genetic diversity in crop and forage species, including research in a number of areas related to genetic resources, one of which is plant pathology.

The transfer of germplasm, especially of vegetatively propagated crops, can involve serious quarantine hazards unless the necessary precautions are taken. It is generally recognized that this risk is greater when vegetative propagules, rather than seeds, are moved (Kahn, 1977).

IBPGR engages in two types of activity in plant pathology. First, it stimulates, catalyzes and carries out research, specifically in those areas that will improve the efficiency and safety of germplasm exchange. And second, it studies the safety of germplasm movement and makes recommendations for the improvement of existing procedures.

## FACTORS AFFECTING THE SAFE MOVEMENT OF GENETIC RESOURCES

A real potential for improvement in current methodology is evident from the study of the factors influencing the efficiency and safety of the movement of germplasm, especially considerinng Kahn's concept of 'risk analysis' (Kahn, 1979).

### Nature and quantity of material to be moved

IBPGR recommends that seeds, rather than vegetative propagules, be collected whenever possible. Seeds are both safer to move and easier to store. Even in the case of some vegetatively propagated crops, it is possible to collect seeds instead of vegetative propagules. With respect to the preservation of genetic diversity, seeds are satisfactory as, with the exception of only a few clones, it is more important to preserve 'genes' than 'genotypes'.

When germplasm must be moved in vegetative form, however, *in vitro* culture offers great advantages over other methods. The mass of material transferred is much smaller and many hazardous pests and pathogens are eliminated by the *in vitro* procedure *per se*. In addition, as long as the material is maintained as *in vitro* cultures, the risk to it is practically nonexistent.

A distinction should be made between 'plant introduction' for short- or medium-term breeding purposes and the movement of germplasm for conservation purposes. In the former case, careful selection of material to reduce the number of accessions to be introduced is always advisable. To be most useful in a breeding program, selected material will probably require certain characteristics (such as resistance to some important biotic or abiotic factor) and should be adapted to local conditions. But when germplasm is moved for conservation purposes, selection according to these criteria is neither practical nor appropriate.

### Availability and speed of indexing methods

The expeditious, yet safe, movement of germplasm of asexually propagated crops requires sensitive, reliable and rapid disease indexing methods. Research in this field is the core of IBPGR's plant pathology program. Most emphasis is given to virus and virus-like diseases of tropical crops, as adequate techniques for these crops are often lacking. Recent progress in biotechnology has provided rapid and sensitive molecular indexing methods for some viruses and virus-like diseases. IBPGR would like to see these techniques developed for diseases of tropical crops. Improvements on existing techniques are also required, as the tests that have been developed are not always appropriate for disease indexing related to quarantine procedures.

Available virus detection techniques have often been developed for diagnostic purposes and are therefore very specific, in order to differentiate between strains of a given virus. This is a handicap for quarantine indexing in the case of little-known diseases, when it is desirable to detect all possible strains. Broader spectrum tests can be developed at little expense if the researcher has quarantine indexing in mind at the development stage. When 'probes' are developed, be they nucleic acid hybridization probes or monoclonal antibodies, a number of clones are obtained from which a selection can be made. Some will be very specific, while others will have a broader spectrum.

Another area which needs urgent attention is the lack of rapid and simple virus-detection tests. Techniques requiring purification steps or sophisticated equipment can be satisfactorily applied in a well-equipped laboratory and when small numbers of samples are involved, but they are highly impractical for large-scale indexing. For example, $^{32}P$ labeled nucleic acid probes are valuable tools for research purposes, but their poor shelf life (about 2 weeks) makes it difficult to use them in remote areas or in a laboratory that does not have the ability to produce its own labeled probes.

### Availability and use of therapy methods

IBPGR recommends, especially for wild relatives of crops, the systematic application of therapy methods prior to final indexing as a means of reducing risks by eliminating the majority of diseases that might infect a plant. Therapy methods need to be improved, as some diseases do not respond to currently used methods.

The combination of *in vitro* therapy and *in vitro* indexing is very attractive, as it provides a contained quarantine system. IBPGR is encouraging research in this direction.

### Availability and capacity of quarantine facilities

When appropriate quarantine facilities are available, arrangements must be made with quarantine authorities regarding such issues as the number of accessions in question, the port of entry and required packaging and documentation, to ensure that material will be handled promptly upon arrival. When the appropriate facilities are not available or are unable to handle all of the material to be introduced, arrangements must be made for third-country quarantine. In this case, it is important that quarantine authorities mutually recognize indexing programs.

### Availability of information on diseases and pests

Little is known of the etiology and distribution of many of the diseases that affect tropical crops and thus it has not been possible to develop detection or therapy techniques for them. Further research on the etiology of economically important 'obscure' diseases is required. In addition, the compilation of existing information on pests and diseases would make such information more accessible and, hence, more readily usable. Pathologists in quarantine stations would benefit greatly from the development of a database on the geographical distribution of pests and diseases of quarantine concern.

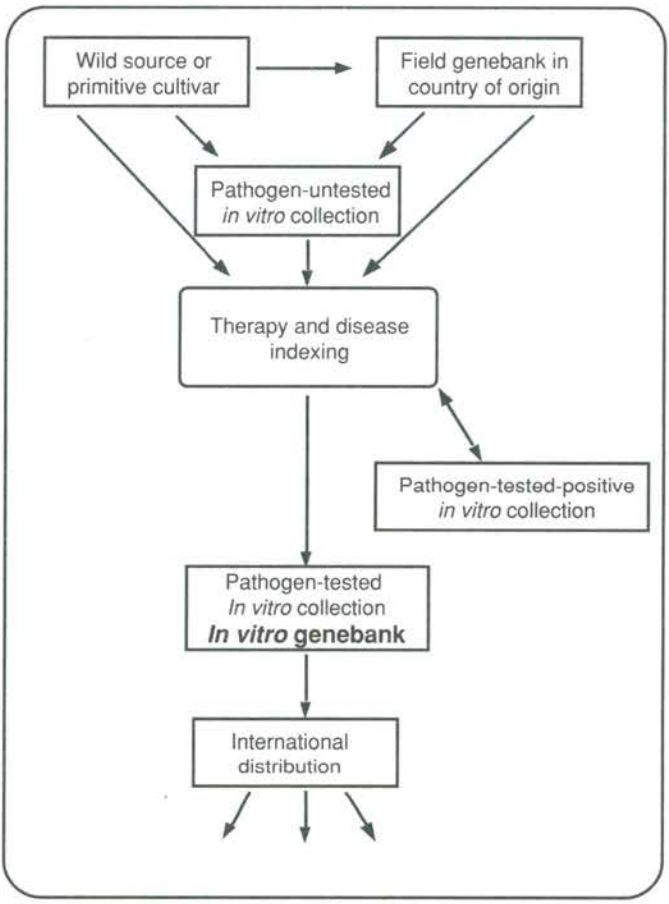
### Availability of information on indexing and therapy methods

Although scientists throughout the world are engaged in research on virology, it is often some time before their results become available to the people responsible for plant quarantine. There is an urgent need for the development of internationally accepted procedures for disease indexing and therapy. As a first step, a meeting of a Special Task Force, convened by IBPGR in 1975, resulted in the publication of book, with contributions from 31 specialists, on plant health and quarantine issues involved in the transfer of genetic resources (Hewitt and Chiarappa, 1977). A subsequent meeting (FAO, 1986) stressed the need for the development of crop-specific guidelines on indexing and therapy. This was also one of the major recommendations of a meeting, organized by IBPGR and held in North Carolina, USA, in 1987, on *in vitro* culture and disease aspects of the conservation and movement of vegetatively propagated crops (IBPGR, 1988). All this led IBPGR and FAO to initiate the joint program described below.

## FRAMEWORK AND GUIDELINES FOR THE MOVEMENT OF GERMPLASM

A conceptual framework for assessing the factors associated with the movement of vegetatively propagated crops and based on the assumption that *in vitro* methods are available (as is the case for many crops) was developed at the 1987 IBPGR-sponsored meeting in the USA (IBPGR, 1988). Figure 1 (*overleaf*) illustrates the major elements of this framework.

**FIGURE 1 Recommended framework for the movement of germplasm**




Ideally, wild material is 'cleaned' before being stored in an *in vitro* genebank. The cleaning operation generally includes therapy (meristem-tip culture, used alone or in combination with thermotherapy) to free the material from diseases and pests, and disease indexing to establish whether the material is indeed free of diseases, especially viruses. Once the material is considered to be healthy it can be multiplied *in vitro* for international movement.

In cases when material cannot be cleaned or tested before being stored, because adequate techniques are not yet available or have not yet been applied, it should be kept in a 'pathogen-untested *in vitro* collection'. If valuable material is tested and is shown to contain one or more pathogens, it can be kept in a 'pathogen-tested-positive *in vitro* collection'. Material in these two types of collections should not be released, but can be kept for conservation and research purposes under the supervision of a pathologist. The maintenance of such collections will allow the safe storage of material prior to therapy and indexing, and will accommodate anticipated improvements in indexing and therapy techniques. It will also provide the opportunity for selection of resistant or tolerant genotypes, the rapid development of

polycross nurseries under containment and the preservation of pathogens. International exchange of material in such collections, under the supervision of quarantine authorities, must be made possible in order to allow proper duplication of conservation collections.

FAO and IBPGR have initiated a program for the safe and expeditious international exchange of germplasm. The program, which was announced at the 5th International Congress of Plant Pathology (Frison and Putter, 1988), will generate a series of crop-specific protocols that describe indexing and other procedures to ensure phytosanitary safety when germplasm is moved. In close collaboration with specialized research institutions and international research centers, FAO and IBPGR will convene expert consultations to discuss specific crops and pathogens that pose a quarantine risk. The resulting protocols will be published as a series of crop-specific guidelines and will reflect the technical consensus of eminent crop specialists. Priority will be given to vegetatively propagated crops. The first crops for which guidelines have been produced are cocoa, bananas and plantains.

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# PART 5

# Biotechnology and Wide Crosses

# 5.1

# *The Implications of Biotechnology in Germplasm Conservation and Utilization*

A. CHARRIER, J. DEREUDDRE and F. ENGELMANN

The current preferred method of germplasm preservation is seed storage at low temperature. However, for vegetatively propagated plants and plants producing recalcitrant seeds, long-term seed storage is inappropriate or impossible. A new method under consideration in biotechnology for germplasm banks is *in vitro* culture. About 350 species can be cultivated by *in vitro* culture, in the form of excised shoot-tips or meristems, adventitious budding from callus or cell-suspension culture and somatic embryogenesis from an original explant. It is possible to initiate cultures from immature anthers, ovules, protoplasts and inflorescences. Germplasm storage may be short term (with frequent periodical subcultures), medium term (with reduced growth rate) or long term in liquid nitrogen. *In vitro* storage appears to be more suitable for species that produce recalcitrant seeds (Withers and Williams, 1982) and for vegetatively propagated plants.

## CONSERVATION OF GENETIC RESOURCES

### Plants producing recalcitrant seeds

Usually, seeds are used for storage. Seeds may be either orthodox or recalcitrant, according to their ability to tolerate desiccation. Most temperate species produce orthodox seeds. Recalcitrant seeds, which are produced by many perennial species, are unable to withstand desiccation and freezing (Roberts, 1973), and such seeds pose considerable problems with respect to long-term preservation (*see* Table 1 *overleaf*).

### Vegetatively propagated plants

Genetic erosion of many economically important crop plants, including potato, cassava, yam and sweet potato, has been taking place in several ways: replacement of native cultivars by

**TABLE 1 Dessication-sensitive seeds**

*Desiccation-sensitive seeds are produced by:*

1 Species from aquatic habitats (such as wild rice)

2 Large-seeded woody plants (such as rubber, cacao, coconut, mango, avocado, jackfruit and mangosteen)

3 Herbaceous tropical perennials (such as plantain, cassava, sweet potato, yam, banana and sugarcane)

4 Trees of temperate latitudes (such as oak, chestnut, maple and walnut)

high-yielding varieties; destruction of natural habitats; and loss of collected material. In these cases germplasm preservation has been applied to wild species and primitive cultivated forms. While genebanks could be set up for the storage of seeds from wild species and fertile cultivars, conservation of sterile cultivars and clones would require a meristem-cryopreservation procedure.

Several international centers, such as the Centro Internacional de la Papa (CIP) in Peru, the Centro Internacional de Agricultura Tropical (CIAT) in Colombia and the International Institute for Tropical Agriculture (IITA) in Nigeria, maintain collections of these vegetatively propagated plants.

Two of the most problematical crops are coconut, *Cocos nucifera*, and oil palm, *Elaeis guineensis*. These species are heterozygous and the juvenile stage exceeds 6-7 years. Coconut seeds are recalcitrant and methods for vegetative propagation do not yet exist (Pannetier and Buffard-Morel, 1982). For oil palm, a method has been established which ensures continuous production of plantlets by means of theoretically indefinite adventitious embryogenesis (Pannetier et al., 1981).

## *IN VITRO* CULTURE FOR GERMPLASM PRESERVATION

Whereas asexual preservation is needed for selected clones, *in vitro* culture is an appropriate tool only for short- or medium-term storage (Dereuddre, 1985). There are several types of *in vitro* storage.

### Maintenance at normal growth

This method is useful for shoot-tips and indefinite somatic embryogenesis propagation; it is inappropriate for callus or cell suspension cultures, which often require the presence of 2.4-D. Indeed, repeated subcultures in the presence of auxins can lead to abnormal progeny and loss of totipotency. Calluses and cell cultures subcultured for an extended period have a tendency to undergo chromosomal variations and changes at the ploidy level (D'Amato,

1978). Haploid cultures, in particular, are known to be highly unstable and can revert in a few weeks or months to a diploid state (D'Amato, 1977). Maintenance of these cultures as haploids is highly desirable.

### Medium-term storage

Growth-limiting conditions offer the possibility of reducing the frequency of subcultures; culturing at reduced temperatures (4-10°C) and addition of mannitol or growth inhibitors, such as abscissic acid, to the growth medium (Westcott 1981a, 1981b) may be appropriate for many species originating from temperate regions. Low temperature and abscissic acid seem to be unsuitable for the majority of tropical species. Other techniques have been developed for tropical species, such as the use of low concentrations of sucrose in the culture medium (Kartha et al., 1981) or the addition of polyols.

The Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM) is developing the preservation of coffee germplasm by *in vitro* culture of immature zygotic embryos of various coffee species (Bertrand-Desbrunais, unpubl.).

For medium-term storage, hypoxia has been applied to several types of callus (Augereau et al., 1986). However, periodic subcultures (every 1 or 2 years) remain necessary, with regular controls of the culture's ability to initiate regrowth.

## *IN VITRO* CULTURE FOR THE MOVEMENT OF PLANT GERMPLASM

*In vitro* methods can be used to move plant germplasm in collecting missions and for exchange with other collections (Withers, 1986).

### *In vitro* collecting

The possibility of using collecting techniques based on *in vitro* methods has been explored in recent years for both vegetative material and recalcitrant seeds. A range of techniques have been developed from the use of a fully equipped local laboratory to the use of an outdoor working area. The most successful attempt has been the use of an outdoor working area to collect zygotic embryos of coconut in the field to solve the problems encountered in transporting and storing coconut seeds. This work, supported by the International Board for Plant Genetic Resources (IBPGR), will interface with *in vitro* methods for clonally propagating coconut carried out by the Office de la Recherche Scientifique et Technique d'Outre-Mer (ORSTOM)/Institut de Recherche sur les Huiles et Oléagineux ( IRHO). Direct inoculation into culture on media has been successfully carried out under field collecting conditions. An alternative method holds embryos in endosperm cores in a salt solution for brief periods before inoculation in a laboratory (Assy-Bah, 1986; Assy-Bah et al. 1987)

### *In vitro* exchange

This operation has been carried out successfully for several years by some of the IARCs. The IBPGR *in vitro* conservation databases show a steady increase in reports of the distribution

of culture. In addition, *in vitro* culture can help to prevent phytosanitary, quarantine and related problems. According to Withers (1988), *in vitro* movement of plant germplasm has enormous advantages but the apparent cleanliness of cultures could promote a false sense of security.

## CRYOPRESERVATION AS A TOOL FOR LONG-TERM STORAGE

Cryopreservation in liquid nitrogen is the most convenient technique for long-term storage and is now applied to a wide range of species. This technology can be applied to protoplasts, cell suspensions, calluses, meristems and shoot-tips, as well as to somatic, pollinic and zygotic embryos. Seeds may also be stored in liquid nitrogen.

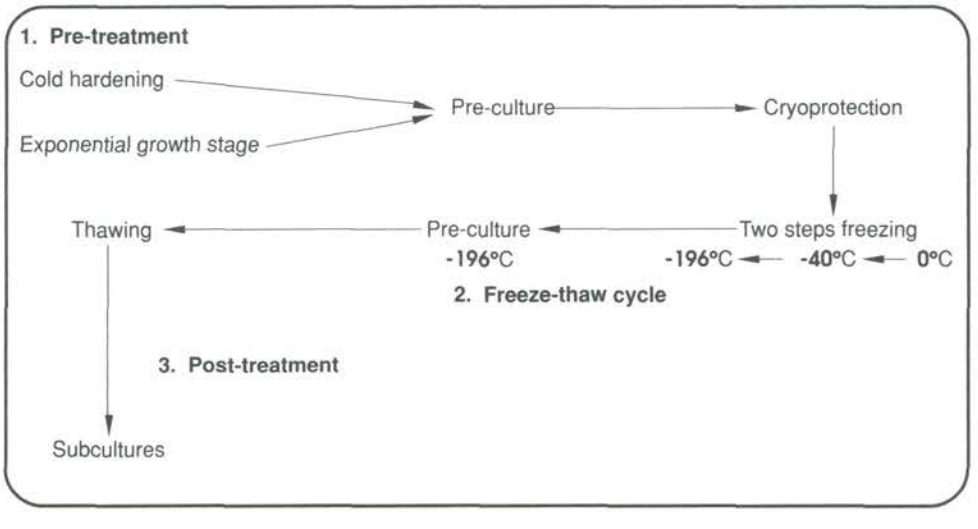
Cryopreservation offers several advantages as a method for storage. Stability for a hypothetically infinite period without deterioration is ensured; indeed, at the temperature of liquid nitrogen, cells no longer divide and all metabolic processes come to a halt. The material investment is relatively low if *in vitro* techniques are routinely used in the laboratory. The liquid nitrogen storage containers are compact and require only periodic filling.

### Principles of cryopreservation

The cryopreservation process includes three main steps: pre-treatment, which prepares the cells and organs for resistance to the second step, the freeze-thaw cycle, and post-treatment, which may increase the growth recovery rate (*see* Figure 1).

The example of oil palm will serve to illustrate the process (Engelmann et al., 1986). The pre-treatment includes two or three successive steps. Cells or organs may be in a fast-growing

**FIGURE 1 Cryopreservation process**



stage (exponential growing phase). Pre-culture is carried out at room temperature or under the same conditions as in the routinely cultured cells, callus or donor plants. The cryoprotective mixture is generally added progressively at 0°C. Cooling is achieved in two steps: after a first slow cooling to -40°C, the samples are directly immersed in liquid nitrogen. Freezing is generally induced artificially a few degrees below the freezing point of the cryoprotective medium. For thawing, the cryobiological ampoules are transferred directly from liquid nitrogen into a water bath at 40°C. Post-treatment involves several subcultures in gradually reduced concentrations of cryoprotectant, and finally on standard medium. We have registered survival rates up to 30%. We have recently obtained plantlet regeneration after the freeze-thaw cycle for *Coffea arabica* (Bertrand-Desbrunais et al., 1988).

## Cryopreservation of meristems

With cell suspensions, growth recovery occurs only if a certain proportion of cells survives to the freeze-thaw cycle. In the case of organized structures, such as meristems and embryos, entire organs must be preserved.

Cryopreservation of meristems and shoot tips is recommended for long-term storage of plants micropropagated by *in vitro* culture. It appears to be the main technique for preservation of genetic stability and generation of true-to-type progenies. However, it is necessary to avoid adventitious organogenesis and the possible genetic changes that might occur if morphogenetic calluses are allowed to proliferate from the explant after storage. The explants used for cryopreservation are apical or lateral shoot-tips. They include the apical dome and one to three leaf primordia; they measure about 0.5 mm in length, and are thus larger than those utilized to obtain virus-free plants.

Cryopreservation of meristems should permit the storage of five important types of material (Kartha, 1982):

- disease-free micropropagated plants;
- germplasm from recalcitrant seeds;
- sterile hybrids;
- vegetatively propagated plants;
- plants producing seeds contaminated by pathogens.

Thirteen species have been cryopreserved in liquid nitrogen in the form of meristems. They include: *Arachis hypogaea, Brassica napus, B. oleracea, Cicer arietinum, Lycopersicum esculentum, Manihot esculenta, Pisum sativum, Solanum etuberosum, S. goniocalyx* and *S. tuberosum*. The results depend very much on the variety (Bajaj, 1981; Towill, 1984).

## Cryopreservation of embryos

Embryo storage is another important tool for the conservation of genetic resources. It can be used for:

- storage of pollinic embryos, which could allow regular plantlet production, until now impossible because of the seasonal production of such structures;
- conservation of genetic resources of plants producing recalcitrant seeds, when micropropagation by means of somatic embryogenesis or meristem culture does not exist;
- storage of embryogenic lines;
- storage of hybrid embryos which abort at early developmental stages.

Cryopreservation of embryos appears more critical than cryopreservation of meristems. Since it implies survival of the two apical meristems and of hypocotyl tissues, cryopreservation of embryos requires the use of material at early stages of development (globular or early heart-shaped embryos).

Survival rates differ according to the type of embryo. Direct regrowth (that is, without intermediary callus formation) has been observed in only a few cases: zygotic embryos of *Brassica napus* and oil palm (Grout et al., 1983); somatic embryos of oil palm, via somatic embryogenesis (Engelmann et al., 1985); and pollinic embryos of *Brassica napus* (unpubl.). In other cases, intermediary callus formation occurred (Bajaj, 1984). Embryos successfully cryopreserved in liquid nitrogen are listed in Table 2.

**Table 2 Species successfully cryopreserved as somatic, pollinic and zygotic embryos**

| Somatic embryos | Pollinic embryos | Zygotic embryos |
|---|---|---|
| *Elaesis guineensis* | *Arachis hypogea* | *Brassica napus* |
| | *Brassica campestris* | *Cocos nucifera* |
| | *Gossypium arboreum* | *Elaeis guineensis* |
| | *Nicotiana tabacum* | *Oryza sativa* |
| | *Oryza sativa* | *Zea mays* |
| | | *Gossypium arboreum* |

Generally, younger (globular) embryos give rise directly to plantlets, whereas older embryos (late-heart or torpedo stage) survive only partially, because of their size, and form calluses. In most cases, auxins such as 2.4-D are still added to the post-treatment medium in order to ensure regrowth of the frozen-thawed material (Bajaj, 1984).

## STABILITY

Organogenesis is preserved after long-term storage in liquid nitrogen, as noted for somatic embryogenesis in carrot cell suspensions. Oil palm somatic embryos reproliferate normally after 7-18 months of storage in liquid nitrogen without any loss of the resumption rate (Engelmann, 1986), giving rise to plantlets, the development of which in the nursery has so far been comparable to that of non-frozen controls.

Regeneration of plantlets after storage is also maintained in date palm (Tisserat et al., 1981) and alfalfa (Finkle et al., 1985). No differences in isoenzyme patterns were found between plantlets produced by unfrozen calluses and those produced by cryopreserved calluses (Ulrich et al., 1982). Microtuberization in potato plants regenerated from freeze-preserved meristems is maintained even after 2-4 years of storage (Bajaj, 1985).

## CONCLUSION

The increasing number of species propagated *in vitro*, which include many tropical crop species with recalcitrant seeds, emphasizes the problem of storage. Much experience has been gained in several international centers, including CIP, CIAT and IITA, concerning the handling of *in vitro* collections and germplasm exchange. However, in the case of slow growth, used for medium-term storage, genetic stability must be assured, and a renewal process must be devised which permits medium-term storage (Withers, 1986).

The development of cryopreservation has been considerable during the past decade. Nevertheless, although cryopreservation of cell suspension cultures can be used routinely without major problems, difficulties still remain when freezing organized and/or macroscopic structures. These difficulties will be resolved both by improving cryopreservation technical processes and by developing more fundamental research.

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# 5.2

# *Taxonomy and Wide Crosses of Pulse Crops, with Special Reference to* Phaseolus *and* Vigna

J. P. BAUDOIN and R. MARECHAL

Food legumes are potentially the most valuable sources of plant protein for the tropics and play a very important role in tropical farming systems. Nevertheless, their yields and levels of resistance remain low in many parts of the world.

To achieve better productivity and adaptation, breeders are constantly drawing on the resources of germplasm collections. Priority has been placed on landraces and wild conspecific materials of the major food legume crops. More attention is now being given to the utilization of the alien germplasm composed of related taxa. The latter represents an additional source of genetic variability and provides an important reservoir of genes which could be useful for the breeder. Interspecific hybridization may be utilized to increase resistance or tolerance to pests and diseases or to stressful environments, to upgrade seed quality, to improve plant architecture, to alter modes of reproduction or even to develop cytoplasmic sterility.

In spite of the rich diversity in this alien germplasm, most plant breeders are still using it only as a last resort. There are several reasons for this. First, plant breeders are rarely familiar with close relatives of crops and are often misled by confused or incomplete taxonomy. Second, collections are not representative of the accurate genetic diversity of the related species and evaluation data on useful morphoagronomic traits are very scanty. Finally, with interspecific crosses, more breeding efforts are required to develop lines with agronomically acceptable attributes.

The literature on this subject is enormous and it continues to grow, but instead of presenting a review of attempts which have been made in the area of interspecific hybridization in genetic improvement, we have preferred to select some examples with which we ourselves have experimented in two genera, *Phaseolus* and *Vigna*. For this purpose, biosystematic and genepool considerations will first be outlined; we will then discuss obtaining interspecific crosses, the obstacles to hybridization and various approaches used to breed the resulting materials.

## TAXONOMY OF PULSE CROPS

In food legumes, recent activities in the search for germplasm in centers of diversity have revealed some interesting biotaxonomic information that contributes to a better understanding of the total diversity available for the improvement of the crop. We take, as an example, the taxonomy of Phaseolinae which has been previously studied by Verdcourt (1970), Lackey (1977) and Maréchal et al. (1978, 1981).

The latter, using numerical methods, integrated as many criteria as possible (organographical, cytological, palynological and seedling characters) in a very large sample of taxa. The results of these investigations allowed a more precise delimitation of natural groups at the generic and subgeneric level. In particular, the various characters, evaluated without *a priori* weighting, provided a neat boundary between the two most important genera, *Phaseolus* and *Vigna*. *Phaseolus* appears to be a very homogeneous, well-isolated genus of neotropical. origin, while *Vigna* apears to be a large, heterogeneous group of pantropical distribution. The broad conception of *Vigna*, divided into subgenera and sections, is justified by the continuity of the group and the close phyletic affinities linking its members (Maréchal, 1982). This study, however, is far from being completed.

Systematic exploration in sites of origin and of maximum diversity have been or are still being organized. The first data from these collecting missions have already provided much more precise information and have shown convincingly that the natural diversity in the two genera is much larger than suspected. No doubt, in-depth studies on the materials collected will improve the classification suggested by Maréchal et al. (1978). Modifications will concern mainly the specific and infraspecific levels. To contribute efficiently to the genetic improvement of food legumes, it is now essential to determine the relationships between the different natural populations and to see how they can be used most profitably in breeding programs.

## THE GENEPOOL OF THE MAJOR CULTIGENS IN *VIGNA* AND *PHASEOLUS*

A good taxonomic scheme of a germplasm group contributes to an efficient crossing program aimed at improving the main cultigens. Reciprocally, results of hybridizations and cross-compatibilities will provide solid arguments for improving plant classification. This approach will also permit the ranking of the germplasm available for hybridization according to the genepool concept of Harlan and De Wet (1971). This concept labels germplasm resources as primary, secondary or tertiary.

The primary genepool corresponds to the biological species and involves both the cultivated forms and the wild conspecific ancestors. The secondary and the tertiary genepools comprise all the taxa among which gene flow is possible through interspecific hybridization but with increasing degrees of difficulty. The boundary between secondary and tertiary genepools may not always be very well defined. When a cross between a cultigen and a donor species is possible but the $F_1$ hybrid is sterile or presents some meiotic irregularities because of marked structural differentiation between the two genomes, then the donor species is considered to belong to the tertiary genepool of the cultigen. When the $F_1$ hybrid is more fertile or presents moderate structural heterology, the donor species will then be considered to belong to the secondary genepool. In this case, normal chromosome pairing may be observed at meiosis.

The range of the total genepool varies with each crop, but in food legumes the working range of exploitable genetic resources generally does not extend beyond the genus for a specific crop (Smartt, 1980). In addition, the isolation mechanisms operating between species in legumes are generally strong, early and remarkably effective. Introgression will often require considerable and continuous effort throughout the breeding process. Although studies of experimental hybridizations have still to be carried out, given the newly collected forms, it is interesting to examine the actual genepool situation with the main crops of *Vigna* and *Phaseolus*.

With *Phaseolus*, the numerical analysis by Maréchal et al. (1978) has shown a sequential organization of the genus between two extremes representing the two most important cultivated species: *P. vulgaris* L. (the common bean), at the one end, and *P. lunatus* L. (the lima bean), at the other end. Table 1 gives the secondary and tertiary genepools of these two taxa; this is the picture that emerges from Baudoin and Maréchal (1985), Belivanis and Dore (1986) and Baudoin (1988). In the genetic improvement of the common bean through interspecific hybridizations, *P. coccineus* L. and *P. polyanthus* Greenm. appear as the most useful and exploited donor species; numerous crosses have been reported among the three taxa. The other four species successfully crossed with *P. vulgaris* belong to the tertiary genepool. *P. acutifolius* A. Gray, *P. filiformis* Bentham and *P. angustissimus* A. Gray seem to occupy an intermediate position in the genus, while *P. ritensis* Jones shows a closer affinity with *P. lunatus*. For the latter, no secondary genepool has been recognized, but seven wild species (two not yet botanically identified) could serve as tertiary genepools. They have been successfully crossed with lima bean in our greenhouses at Gembloux. Interspecific hybridization appears, therefore, to be very promising for the genetic improvement of lima bean. It is important to note that in *Phaseolus* the classification of its taxa based on pollen grain structure constitutes a good guide for determining the phylogenic distances between the various members of the genus (Le Marchand and Maréchal, 1977).

With *Vigna*, successful interspecific hybridizations have been claimed only in the subgenus *Ceratotropis*, a clear-cut, homogeneous and very specialized group of Asiatic origin. All the typical *Vigna* characters are here represented with a high degree of expression.

**TABLE 1** **Secondary and tertiary genepools of *Phaseolus vulgaris* and *P. lunatus***

| Species | Secondary genepool | Tertiary genepool |
|---|---|---|
| *P. vulgaris* L. | *P. coccineus* L.<br>*P. polyanthus* Greenman | *P. acutifolius* A. Gray<br>*P. filiformis* Bentham<br>*P. ritensis* Jones<br>*P. angustissimus* A. Gray |
| *P. lunatus* L. | | *P. maculatus* Scheele<br>*P. ritensis* Jones<br>*P. polystachyus* B. S. and P.<br>*P. jaliscanus* Piper<br>*P. salicifolius* Piper<br>NI 402[1] and NI 702[1] |

1 Introduction number in Gembloux collection of Phaseolinae.

*Ceratotropis* encompasses five cultigens. Table 2 gives their secondary and tertiary genepools based on reviews or investigations made by Ahn and Hartmann (1978), Jain and Mehra (1980), Lukoki (1980), Chen et al. (1983) and Smartt (1985). The results suggest the presence of three rather isolated groups: *V. radiata* (L.) Wilczsek-*V. mungo* (L.) Hepper; *V. umbellata* (Thunb.) Ohwi and Ohashi-*V. angularis* (Willd.) Ohwi and Ohashi; and *V. aconitifolia* (Jacq.) Maréchal-*V. trilobata* (L.) Verdc. Other hybrids have, however, been obtained successfully between members of these three different groups.

One interesting but little studied species of the *Ceratotropis* subgenus should be mentioned — *Vigna glabrescens* M. M. and S. It is the only known case of amphidiploidy (2n = 44) in the whole Phaseolinae subtribe and probably arose from a natural cross between *V. radiata* and *V. umbellata* (Krishnan and De, 1968a, 1968b). Its tetraploid level gives it vigorous growth, a strong root system and great potential for a high yield of good-quality fodder. Unfortunately, this taxon remains represented by extremely little diversity in the current live collections, making any genetic progress difficult. It is a typical illustration of plant resources insufficiently exploited because no collecting missions have been carried it out in its center of diversity, the Indochinese peninsula (Baudoin and Maréchal, 1988).

**TABLE 2 Secondary and tertiary genepools of the cultivated *Ceratotropis Vigna***

| Species | Secondary genepool | Tertiary genepool |
|---|---|---|
| *V. radiata* (L.) | *V. mungo* | *V. umbellata, V. angularis*<br>*V. glabrescens* M. M. and S.<br>*V. trilobata* (L.) Verdcourt |
| *V. mungo* (L.) Hepper | *V. radiata* | *V. umbellata, V. angularis*<br>*V. glabrescens, V. trilobata* |
| *V. umbellata* (Thunb.) Ohwi and Ohashi | *V. angularis* | *V. radiata, V. mungo* |
| *V. angularis* (Willd.) Ohwi and Ohashi | *V. umbellata* | *V. radiata, V. mungo* |
| *V. aconitifolia* (Jacq.) Maréchal | *V. trilobata* | — |

In the largest group of the genus — the subgenus *Vigna* — no interspecific crosses have been reported with the two cultigens *V. unguiculata* (L.) Walp., (cowpea) and *V. subterranea* (L.) Verdc. (Bambara groundnut). In terms of combining abilities, these two species are much more constrained than the other species in the Phaseolinae subtribe. In the case of cowpea, the section *Catiang*, to which it belongs, appears to be rather neatly isolated from the other sections of the subgenus. *Catiang* appears to contain only one other species, *V. nervosa* Mark. Initial attempts by Mithen (1987) to cross the two taxa have not yet succeeded but should not be abandoned. In the present state of biotaxonomic research, *V. nervosa* may be considered the only known possibility for a secondary genepool of cowpea. One should point out,

however, that intraspecific diversity of *V. unguiculata* is very large, providing substantial hope for genetic improvement within the primary genepool. For instance, it is worth mentioning the very hairy forms discovered among the natural populations of southern and south-eastern areas of Africa, the main centers of diversity for the wild forms. In the framework of a biotaxonomic study, it is important to attempt crosses between the numerous different wild forms of cowpea to detect any eventual incompatibility barriers (for example between the large-flowered perennial forms, the small-flowered annual forms, the pubescent forms and the numerous other forms found in Southern Africa).

It seems highly improbable with the present techniques of hybridization that crosses will succeed between the section *Catiang* and the other groups of *Vigna*. Those *Vigna* species that have the highest morphological similarity with *Catiang* (for instance, in the sections *Liebrechtsia* and *Membranacea*) are characterized by a different chromosome number (2n = 20 instead of 2n = 22, as commonly found in *Vigna*). This may hinder considerably the gene flow in combinations (Baudoin and Maréchal, 1985).

Another wild, semi-domesticated species which has attracted some interest is *V. vexillata* (L.) A. Rich. Its seeds are characterized by a high content of a dipeptide para-aminophenylalanine (Dardenne et al., 1972), and this compound is claimed to be an important component of resistance against two major bruchid pests of *Vigna* and *Phaseolus* (Birch et al., 1986). *V. vexillata* belongs to the subgenus *Plectotropis* and hybridizations between members of that subgenus and *V. unguiculata* are likely to be unsuccessful using conventional methods. Perhaps new approaches in genetic manipulation may produce a viable hybrid between such distantly related species. However, from past investigations it appears that legumes are very difficult materials for *in vitro* cultivation, protoplast fusion and chromosome engineering. A breakthrough may come from basic research (in embryogenesis, for example) and from the full exploitation of the potential of the wild forms.

The second cultigen of the *Vigna* subgenus, *V. subterranea*, accumulates all the attributes defined for the section *Vigna*. It represents a geocarpic species of importance in the dry or subhumid savanna of Africa and shows the highest similarities with the amphicarpic *V. hosei* (Craib.) Backer, of the same section. This wild species may be assigned to the secondary or tertiary genepools of *V. subterranea*. No attempts have yet been made to combine the two taxa.

## INTERSPECIFIC HYBRIDIZATION

Introducing alien germplasm into the finely balanced genetic system of any cultivated species requires constant effort from the first step (making the cross) to the last one (developing a breeding line which combines the highest expression of the introgressed characters with acceptable agronomic attributes). Many reviews of interspecific hybridization in different crops, including food legumes, have been published in the past few years (Harlan, 1976; Knott and Dvorak, 1976; Stalker, 1980; Smartt, 1984; Hucl and Scoles, 1985; Ladizinsky et al., 1988; Sullivan, 1988). They describe a wide range of problems and the various degrees of facility with which interspecific gene transfers are made. There is no doubt that the polyploid complex constitutes an ideal material in that respect; the duplication of genetic background in two or more different genomes offers a sort of buffering system that can withstand alien transfer more easily. This is the case, for example, in *Gossypium*, *Nicotiana* and *Triticum*. In the Phaseolinae, which is composed mainly of diploid species, the situation

is more difficult but similar behavior can be observed, depending upon the phyletic distances between the two parental genotypes. We will illustrate these problems and the methods to overcome them by drawing on our work and other investigations concerning *Phaseolus* and *Vigna*.

## Obtaining interspecific hybrids

When two species show close affinity and little heterogenetic structural differentiation between their chromosomes, interspecific crosses with partial fertility are generally obtained with much difficulty through repeated hand pollinations. However, success rates may vary considerably according to ecological conditions, parental combinations and direction of the crosses. This last factor is probably the most obvious, demonstrating in many cases a nuclear-cytoplasm interaction. Such unilateral incompatibilities are found in the crosses *V. radiata* x *V. mungo*, *P. vulgaris* x *P. coccineus* and *P. vulgaris* x *P. polyanthus*. In this paper, success is reported only when the former parent of each combination is used as the female (Smartt, 1970; Lukoki, 1980; Camarena and Baudoin, 1987).

Our own investigations with *Phaseolus* and *Vigna* have also shown that unilateral incompatibilities may be much stronger with parental cultivars than with wild forms. For instance, in our greenhouse at Gembloux, crosses between *P. coccineus* (O) and *P. vulgaris* have been obtained by hand pollinations only when wild forms of *P. coccineus* have been used (Baudoin et al., 1985). In addition, the wild forms may act also as a bridge between the cultivated forms of the two species involved; they represent an excellent tool to favor exchange between genetic materials. We have been able to identify some very compatible wild cytoplasms of *P. coccineus*, which have been used to enhance introgression from many cultivated *P. coccineus* and *P. polyanthus* donor parents into *P. vulgaris*.

When the distance between the two parental species becomes larger, additional efforts are required. Results depend upon the degree of divergence between the two genomes. For example, in *Phaseolus* combinations, when the number of univalents (a measure of the lack of structural homology between two sets of chromosomes) is moderate (varying from one to six), hybrids are obtained by conventional means, but only after numerous attempts at artificial pollination. This has been well demonstrated in the combinations between *P. lunatus* and such wild species as *P. maculatus*, *P. ritensis*, *P. jaliscanus* and *P. salicifolius* (Baudoin, 1981; Katanga and Baudoin, 1987). When the number of univalents is higher (varying from seven to ten), possibilities of obtaining hybrids are often much more difficult when using conventional methods. Success may still be possible by resorting to a wide range of genotypes of both parents and by repeated crossings in both directions. However, in this case, embryo culture appears to be of considerable help in increasing the rates of successful hybridization or in obtaining specific requested combinations between selected parents. Embryo rescue has, for instance, been used in the crosses between *P. vulgaris*, on the one hand, and *P. acutifolius* or *P. filiformis*, on the other (Prendota et al., 1982; Weilenmann de Tau et al., 1986).

As a general rule, in both *Phaseolus* and *Vigna* intensive attempts at wide crossing have made possible the identification of genotypes within one species with better cross-compatibility than others (Baudoin, 1981; Parker and Michaels, 1986). The differential intraspecific reactions in combining ability are thought to be controlled by some genetic factor and this might be related to the geographic origin of genepools. The true nature of this incompatibility

system needs further investigations (for example, in the fields of biochemistry or molecular genetics). Some authors (Chen et al., 1983; Thomas and Waines, 1984) also suggest that obtaining hybrid seeds might be facilitated when using intraspecific hybrids as parental genotypes. This may be so in some interspecific combinations, but it has yet to be confirmed.

In the Papilionaceae, post-mating barriers to hybridizations usually represent the greatest handicap (Gritton and Vierzbicka, 1975; Le Marchand et al., 1983; Ladizinsky et al., 1988). This has also been confirmed by our own studies on *Phaseolus* and *Vigna*. Even in crosses between distantly related species, pollen can germinate on the foreign stigma, pollen tube growth can reach the ovule, and fertilization takes place, followed by the first divisions of the oosphere. Such observations were made in *P. vulgaris* x *P. filiformis* combinations (Weilenmann de Tau et al., 1987). In this hybrid and others we attempted to produce, the embryo starved out at an early stage because of the lack of formation of the triploid albuminic tissue. Abortion of fertilized ovules may also result from a reduced development rate of the embryo or cotyledon abnormalities. However, no in-depth studies (in these as well as in other food legumes) have been undertaken to discover the primary causes of embryo abortion. A better understanding of the relationships between embryo, cotyledons, albumen and maternal tissues in needed.

## Viability, fertility and general behavior of interspecific hybrids

In any generations following the crosses, a wide range of growth reaction can be observed, ranging from non-germinating seeds to vigorous flowering and productive plants. The most conspicuous feature of the interspecific populations is the presence of unbalanced genotypes, commonly designated as hybrid breakdown. These plants are characterized by various degrees of abnormalities, such as stunted growth with very short internodes, crinkled leaves, seedling senescence, lack or malformation of flowering buds and reduced or total sterility. The hybrid breakdown can occur whatever the phyletic distances separating the two intercrossed species. For instance, in our interspecific materials, we have observed it in crosses between closely related species (*P. vulgaris* x *P. coccineus*, *V. radiata* x *V. mungo*) as well as in crosses between distantly related species (*P. vulgaris* x *P. acutifolius*, *P. vulgaris* x *P. filiformis*). The same observation has been reported in other similar hybrids of *Vigna* and *Phaseolus* (Bemis and Kedar, 1961; Chen et al., 1983; Parker and Michaels, 1986; Waines et al., 1988). Often, the incidence of unbalanced genotypes is strongly influenced by the parents involved in the combination and is likely to be under nuclear genetic control. Other factors are lack of chromosome homology, chromosomal rearrangements or heterozygosity from chromosomal interchanges.

In general, the introduction of alien germplasm into the genetic background of the recurrent species causes a major disturbance. Chiasma formation is reduced and crossing over may not occur regularly (Maréchal, 1971). Thus, it is not surprising that in the segregating populations difficulties in breaking linkages between useful and deleterious characters have often been reported. Another consequence of this lack of recombinants between the two genomes involved is a strong tendency in the progenies to the return to one or other of the parental forms. We will return to this factor later when discussing the breeding methods which are applied to interspecific hybrids. It must also be noted that genotypic combinations leading to unbalanced individuals, even at the $F_1$ stage, have been observed at the intraspecific level —for example, in the case of *P. vulgaris* (Singh and Gutierrez, 1984). Such behavior has been

related the origin of the two parents and can be interpreted as the beginning of speciation (Gepts and Bliss, 1985).

In terms of fertility, interspecific crosses usually show a lower seed productivity and a poorer pollen stainability than do the intraspecific crosses. However, fertility may be quite satisfactory in hybrids involving closely related species. When the parental species are more remote, the gametic fertility of the hybrids can be strongly reduced. In some combinations (for example, *P. lunatus* x *P. ritensis*, *P. lunatus* x *P. maculatus*, *P. lunatus* x *P. salicifolius*, *P. lunatus* x *P. jaliscanus*), the remaining fertility is sufficient to continue an introgressive selection using, initially, a certain number of backcrosses (Baudoin, 1981; Katanga and Baudoin, 1987). This procedure may, however, contribute to hastening the rejection of the donor genes in the segregating populations. If the distance between the two parents is so large that meiotic pairing shows more than six univalents, the hybrids are generally completely autosterile. Fertility restoration will then require chromosome doubling through colchicine application. Using this method, we have succeeded in restoring the fertility of our *P. vulgaris* x *P. acutifolius* and *P. vulgaris* x *P. filiformis* combinations (Prendota et al., 1982; Weilenmann de Tau et al., 1986).

## Breeding procedures

The exploitation of interspecific hybrids for plant improvement is always complicated by the presence of some types of incompatibility barriers which act as a hindrance to heterogenetic recombinations. The degree of difficulty will depend on the botanical group and on the phyletic distance between the parental species.

In legumes, the incompatibility barriers between species may be considered as relatively severe. In the best cases, interspecific crosses may be used in medium-term breeding programs, but it will then be necessary to adapt special breeding methods. We will now discuss ways of handling such populations according to the degree of parental affinities.

### *Hybrids between closely related species*

The cross between *P. vulgaris* and members of the *P. coccineus-P. polyanthus* complex constitutes a good illustration of this case. Although chromosomes pair well in these combinations, incompatibility barriers do occur and cannot be overcome by simply following a conventional pedigree selection.

Several tendencies have been observed in the advanced generations:

- the distribution of genotypes is strongly skewed toward the parental types; this shift is considerably hastened when backcrossing is made with the recurrent parent;
- true intermediates are rare and generally have low fertility; this suggests a gradual elimination of heterozygotes and thus a gradual reduction of recombinations;
- selection for restoring high yields will lead very rapidly to an almost complete return to the recurrent parent; maintaining the useful characters of the donor parent will lead, on the other hand, to unbalanced forms and poor fertility.

Gene-cytoplasm interaction, genetic incompatibility, sterility and undesirable linkages are all probable explanations for this general behavior. One way to slow down this evolution and to facilitate introgression is to search for more compatible cytoplasm to act as a bridge between the two intercrossed species. This has been verified in indirect crosses between cultivated forms of *P. vulgaris* and *P. coccineus* involving cytoplasm of wild *P. coccineus* (Baudoin et al., 1985).

The breeding methodology will, of course, be influenced by the number and inheritance of characters to transfer. Obviously, it is easier to introduce a single useful character of simple monogenic or paucigenic inheritance from the donor to the recurrent species. This has been done in the past to introduce a disease resistance character from *P. coccineus* into *P. vulgaris* and to introduce the determinate bush growth habit of *P. vulgaris* or *P. coccineus* (Freytag et al., 1982; Bannerot, 1983). In such examples, the breeding methodology has consisted mainly of repeated backcrosses with the recurrent parent, while maintaining a strong selection pressure for the desired attribute. The greater the population size, the higher the possibility of recovering desirable genotypes after each backcrossing cycle. It has still to be discovered, however, whether or not the most useful genes have been lost during the introgression process, although it seems likely that adopting such a procedure would reinforce the selection against gametes segregating for the donor parent. The development of agronomically acceptable lines still remains the ultimate challenge.

Attempts to introduce several characters from the donor species, rather than just a single one, or characters of polygenic inheritance are far more complicated. Pedigree or backcrossing selections are seldom, if ever, effective in achieving this objective. One of the main reasons is the marked restriction in character recombinations and in break-up of deleterious linkages. The most appropriate procedure in this case is cumulative or recurrent selection. This method is designed to maintain, over several generations, a state of heterozygosy, thus increasing the probability of crossing between linked genes. The greatest deterrent to using the cumulative method is the necessity of making a large number of intercrosses among selected genotypes. In legumes, hybridization is often tedious and time-consuming, particularly with the twisted or tiny flowers of some species. It is therefore highly advantageous to exploit the presence of stable forms of genetic male sterility, as found in cowpea (Rachie et al., 1975). Provided that two conditions are met — a sufficiently high number of intermatings and an intensive selection pressure for the most useful characters of the donor parent — we can reasonably expect at each cycle of the breeding process a gradual upgrading of the level of desirability in the populations to be sampled and the development of genotypes with the most favorable alleles. These genotypes will then be used as mother plants in a conventional pedigree selection.

### *Hybrids between less closely related species*

If the phyletic distance between the two species is greater (this is often reflected by the presence of an average of two to four univalents at meiosis), the fertility of the $F_1$ hybrids may not be sufficient to provide enough materials for the breeding process. Emphasis should first be placed on ways to increase the seed production of the initial crosses. This can be achieved chiefly through manual selfings or through backcrosses using pollen from one of the two parents (in most cases, from the recurrent parent). In this way we have obtained sufficiently large populations in combinations between *P. lunatus* and some wild related species (such

as *P. maculatus* and *P. ritensis*). However, our experience has highlighted the danger of the backcrossing procedure compared with the selfing procedure. Indeed, backcrosses entail a profound disturbance in the pattern of segregation by hastening considerably the shift toward one of the parental forms. It is not advisable to go beyond the second cycle of backcrossing and, in any case, selfings should be recommended as a better way of keeping the useful genes from the donor parent. Once we have obtained a fair amount of seed to be tested, the resulting populations will be bred using the same cumulative selections mentioned above.

### *Hybrids between distantly related species*

Interspecific hybrids combining species which are phylogenically more distant will show incomplete chromosome pairing at meiosis, leading to extremely low gametic fertility. The hybrid is then completely autosterile. This is the case for the following combinations in the Phaseolinae: *P. vulgaris* x *P. filiformis*; *P. acutifolius* x *P. vulgaris*; *P. lunatus* x *P. polystachyus*; and *Vigna radiata* x *V. umbellata*.

In attempts to exploit such combinations for introgression breeding, two pathways may be considered: the simple backcross method and the allotetraploid method.

### The simple backcross method

The autosterile hybrid has almost no viable pollen. Since castration of the flowers is not necessary, it is possible to pollinate large numbers of flowers with pollen from the recurrent parent. As usual in plants, female gametic fertility is always superior to male gametic fertility. After numerous backcrosses, it is possible to collect a small number of seeds from the hybrid plants. In some recent experiments (Thomas and Waines, 1984; Weilenmann de Tau and Baudoin, 1988) the seeds obtained from such backcrosses have produced plants morphologically identical to the recurrent parent. It is possible that the only fertile female gametes in the hybrids are those issued from megasporocytes where, by chance, all or at least most of the chromosomes from one species have been separated at anaphase from those of the other species; however, the probability of such an event is slight. The possibility of heterogenetic exchange during the meiotic phase does exist, but again the probability of such an exchange is low because of the weak residual homology between the genomes.

The chances of obtaining genotypes introgressed with genetic material from the donor parent are reasonable only if the conditions are favorable for a large number of artificial pollinations. Success with this method has been reported by Thomas and Waines (1984), McElroy (1985) and Waines et al. (1988).

### The allotetraploid method

The initial objective of this method was to increase the chances of heterogenetic exchanges by multiplying, through several generations, the number of meiotic phases. For this, it is necessary to restore fertility of the hybrid by doubling its chromosome number using colchicine treatment. This approach has been successful in genera in which natural polyploidy has occurred though evolution — for instance in *Gossypium* (Louant et al., 1977),

*Triticum* (Sidikki, 1977), *Glycine* (Singh and Hymowitz, 1985) and *Arachis* (Smartt, 1986; Stalker and Moss, 1987). The method has also been applied for the genetic improvement of some diploid food legume crops, particularly with the following interspecific hybrids of *Phaseolus* and *Vigna*: *P. lunatis* x *P. polystachyus* (Fozdar, 1962); *P. vulgaris* x *P. ritensis* (Braak and Kooistra, 1975); *P. vulgaris* x *P. coccineus* (Haq et al., 1980); *P. acutifolius* x *P. vulgaris* (Prendota et al., 1982; Thomas and Waynes, 1984); *P. vulgaris* x *P. filiformis* (Weilenmann de Tau et al., 1986); *V. radiata* x *V. mungo* (Dana, 1966a); *V. radiata* x *V. trilobata* (Dana, 1966b); *V. umbellata* x *V. radiata* (Sawa, 1974; Biswas and Dana, 1975); and *V. aconitifolia* x *V. trilobata* (Biswas and Dana, 1976).

In allotetraploid combinations, it is generally expected that fertility will be inversely proportional to the affinities between the parental genomes. This is because important residual homology between chromosomes will favor multivalent chromosome associations at meiosis and cause irregularities at anaphase. When the parental genomes are sufficiently distant the allotetraploid should be fertile, and when a moderate residual homology does exist, there will be a moderate number of multivalent associations at meiosis. These associations are the sites where heterogenetic exchanges may occur. Multiplying the colchiploid generations (designated as $C_0$, $C_1$, $C_2$, etc.) should increase the possibility of these exchanges occurring. At a particular $C_n$ generation it would be expected that the chromosomes would have accumulated a certain number of heterogenetic inclusions. In the case of food legumes of natural diploidy (as in most *Phaseolus* and *Vigna* species) it would be necessary to return to this diploid level via a triploid phase issued from a backcross to the diploid recurrent parent.

The results so far, however, have been rather disappointing because of a particular behavioral trait of allotetraploids, which seems to be a general rule in many legumes. The above-mentioned experiences with *Phaseolus, Vigna, Glycine* and *Arachis* are concordant. The meiotic behavior of the allotetraploids is remarkably uniform, whatever the affinities of the parental genomes as revealed by chromosome pairing at meiosis of the diploid hybrid. Table 3 (*overleaf*) illustrates this trend for some interspecific *Phaseolus* and *Vigna* crosses. Allotetraploid hybrids combining closely related species such as *P. vulgaris* x *P. coccineus* or *V. radiata* x *V. mungo* with complete pairing in $F_1$ will behave in the same way as those combining species more distantly related with six univalents in $F_1$. The bivalent number is nearly identical in all the combinations quoted.

Moreover, the $C_1$ populations show good fertility restoration but little interplant variability, and this variability does not increase during the subsequent generations (Haq et al., 1980; Weilenmann de Tau and Baudoin, 1988). As pointed out by Haq et al. (1980) and Singh and Hymowitz (1985), the allotetraploids have a quasi-diploidlike behavior resembling that of natural amphidiploids. This means a strong preferential pairing between chromosomes of the same genome, which is a major hindrance to introgression breeding. The possibility of increasing heterogenetic exchanges during the C generations is thus very limited.

Diploi-like meiosis is a common occurrence in natural hexaploids of Graminae. As first demonstrated in *Triticum aestivum* L. by Riley and Chapman (1958), amphidiploid behavior in all investigated cases is under genetic control. In the *P.vulgaris* x *P. coccineus* combination, Haq et al. (1980) have observed that multivalent chromosome associations have decreased from $C_0$ to $C_4$. This may indicate that a positive selection for preferential pairing has occurred. The authors suggest, therefore, that a genetic system favoring more strictly homologous pairing leading to increased fertility is being established by this selection process.

**TABLE 3** **Meiotic pairing at Metaphase I of various diploid $F_1$ hybrids and their corresponding allotetraploids in *Phaseolus* and *Vigna***

| Combinations | F1 (diploid) | Co (allotetraploid) | References |
|---|---|---|---|
| *P. vulgaris* x *P. coccineus* | 0,73 I + 10,41 II + 0.05 III + 0.12 IV | 1.36 I + 21,04 II + + 0,14 IV | Haq et al., 1980 |
| *P. acutifolius* x *P. vulgaris* | 6 I + 8 II | 1,76 I + 19,3 II + 0,33 III + 0,6 IV | Prendota, 1984 |
| *P. vulgaris* x *P. filiformis* | 5,69 I + 8,08 II + 0,04 III | 0.75 I + 2,83 II + 0,2 III + 3,8 IV | Weilenmann de Tau & Baudoin, 1988 |
| *P. lunatus* x *P. polystachyus* | 9,31 I + 6,34 II | 1,41 I + 20,17 II + 0,02 III + 0,53 IV | Fozdar, 1962 |
| *V. radiata* x *V. mungo* | 11 II | 0,73 I + 20,34 II + 0,64 IV | Dana, 1966a |
| *V. radiata* x *V. umbellata* | 6 I + 8 II or 8 I + 7 II | 0,81 I + 21,53 II + 0,03 IV | Biswas & Dana, 1975 |
| *V. aconitifolia* x *V. trilobata* | 2 to 6 I + 8 to 10 II | 1,0 I + 21,3 II + 0,1 IV | Biswas & Dana, 1976 |

It is worth mentioning that the artificial doubling of the chromosome number with colchicine has also successfully produced autotetraploid plants in many legumes (Kumar, 1945; Sen and Chheda, 1958; Sen and Bhowal, 1960; Sen and Marimuthus, 1960; Monge and Moh, 1963; Biswas and Bhattacharyya, 1976; Goswami, 1979). Their meiotic behavior has been observed to be generally less irregular than expected, with a relatively low number of multivalent associations in some species. In this case, preferential pairing cannot be initiated.

For food legumes, at least those of natural diploidy (such as *Phaseolus* and *Vigna* species), the tetraploid level obtained through colchicine application may not be significant, although seed production can be improved through selection. The plant type is too vigorous, slow growing and late producing compared with the diploid crop plants, and may be better suited as fodder or cover crops. This has been well demonstrated with the unique case of a natural amphidiploidy in Phaseolinae — *Vigna glabrescens* from South-East Asia. This species displays all the qualities of an excellent fodder crop. Artificial allopolyploids, such as the one derived from the *P. vulgaris* x *P. filiformis* cross, should be evaluated for this purpose, particularly for use as fodder crops in regions with long dry periods.

## CONCLUSION

Until recently, one of the main problems in using secondary or tertiary genepools for the genetic improvement of pulses was the paucity of materials available in genebanks. This

problem is now being overcome as a result of the systematic explorations and collections undertaken by various international organizations in centers of diversity. However, to exploit these materials fully, a number of conditions must prevail. First, wild or related species should be properly seed-multiplied and maintained under long-term storage conditions; this requires a better understanding of the physiology and ecology of the materials. The germplasm collection should also be botanically identified and evaluated for the most useful morphoagronomic traits. A comprehensive knowledge of the taxonomy, the phyletic relationships and the genetic components of a botanical group not only gives an accurate idea of its potential but also greatly enhances the probability of successful interspecific breeding.

Once the crosses have been obtained, other difficulties will appear, particularly with regard to gene transfer from the donor parent to the recurrent one. It must be stressed here that the interspecific hybrids will always necessitate special breeding methods to achieve the final objective, namely the genetic improvment of the major food legume crops. One of the main constraints to the efficient utilization of interspecific hybrids is their low fertility, which often results in small populations and the inability to select desirable recombinants. Techniques which can be used to overcome some sterility barriers include the use of bridge species, intraspecific hybrids or compatible wild cytoplasm and colchiploidization. Once large populations have been developed from the initial crosses, some kind of recurrent or cumulative selection must be adopted, not only to incorporate the desirable genetic material into the recurrent genome but also to recover yield and quality standards requested by the grower and the consumer. Cumulative selection remains the most appropriate breeding procedure for these purposes, especially as many desired traits are multigenic.

For the reasons mentioned above, introgression breeding from interspecific hybrids cannot be inserted into short-term programs with insufficient means. Unfortunately, for the same reasons, such materials have often been disregarded. If it is true that the primary genepool remains the main source of diversity for crop plant breeding, the related species (either wild or already domesticated) represent new possibilities for important genetic progress. It is hoped that technological advances will facilitate the procedure of introgressing the genes from a wide range of related species.

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# *Closing Statement*

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De même que le séminaire tenu récemment à Nairobi et financé par le PNUD, cette manifestation a une valeur inestimable pour sensibiliser les autorités africaines aux actions vitales permettant d'affronter la question perpétuelle du déficit alimentaire du continent. L'identification de marqueurs génétiques pourrait bien être d'une importance capitale parmi les techniques de biotechnologie et devrait retenir toute l'attention du chercheur africain d'aujourd'hui. Cependant, un des résultats essentiels de cet atelier devrait être la mise en place d'un réseau pour les ressources génétiques, financé et dirigé de manière appropriée afin qu'il puisse gagner la reconnaissance et le support des Gouvernements africains.

'Conservation programmes are scarce; so are the planning, training, education, research and other resources needed to make them work. International assistance and guidance available to developing countries wishing to start up such programmes are (at best) modest. And how can governments in developing regions, lacking the basic information needed to help plan practical genetic conservation measures, rally around an idea with which they are barely familiar?'

United Nations Environment Programme

Plant genetic resources constitute one of the most valuable assets of the cultural heritage of mankind. In Africa, knowledge of this heritage is not only inadequate but poorly understood. There have been very few activities aimed at harvesting this body of knowledge and translating it into scientific and pragmatic terms with a view to its preservation and potential utilization.

The workshop held recently in Nairobi and the current workshop at the International Institute of Tropical Agriculture, in Ibadan, are not only complementary to one another but also extremely useful for making the relevant authorities in Africa aware of a spectrum of activities that are crucial for increased food production on the continent. The workshop in Nairobi dealt with various aspects of plant genetics, including medicinal plants and a few food crops, while the current workshop is targeted mainly at the mandate crops of the International Institute of Tropical Agriculture (IITA), including cassava, maize, cowpeas,

rice, yam, plantain, sweet potato and soybean. These two workshops highlight the essence of international cooperation, as shown by the active participation of some United Nations agencies, such as the United Nations Environment Programme (UNEP) and the Food and Agriculture Organization (FAO), some international agricultural research centers and representatives of donors, such as the National Research Council (CNR) of Italy.

The first workshop on crop genetic resources in Africa was held here in Ibadan and organized by IITA and the Association for the Advancement of Agricultural Sciences in Africa (AAASA). AAASA was financially supported by the OAU and the United Stations Agency for International Development (USAID), but its fortunes have dwindled considerably because of the global economic depression. Like many organizations in Africa, it is no longer able to support this vital activity which it co-sponsored only a decade ago.

However, the request by the African Ministers of the Environment to establish a continental network for plant genetic resources conservation and evaluation is further evidence of the collective will of the African governments to address the perennial issue of food shortage.

I am particularly pleased to see the active participation of various scientists from the African countries. Most of the presentations which I have had the opportunity of hearing were descriptive and predominantly of an epidemiological nature. Basic research at the molecular level is still lacking. I hope that future workshops will be devoted to the presentation of research results, including topics such as the role of genetic markers of improved varieties. The identification of these markers may be of significance in biotechnological techniques which should command the attention of the modern researcher in Africa. The days of presenting national problems should be fast disappearing. It is high time we started hearing some results of research from African scientists engaged in the national agricultural research systems. If I may make an observation, African scientists with master's and doctoral degrees should not be engaged solely in collection and storage of cultivars and plants. Well-trained technicians and technologists will suffice for such basic tasks.

The Inter-African Phytosanitary Council (IAPSC) of the OAU, which is a subregional office of the OAU/STRC, has published a monograph on 'Major Crop Pests in Africa' in English and French. This office, located in Yaoundé, Cameroon, is technically responsible for the control of movements of germplasm between various African countries, and could play an important role in the development and establishment of the proposed plant genetic resources network. The Executive Committee of the IAPSC meets once every two years, and this initiative can be presented for discussion and approval in principle at its next meeting.

With so many countries in Africa — and nearly all of them having problems producing enough food for their populations — one must ask the following questions:

- What has gone wrong?
- How do you go about correcting or solving the problems?
- Are the faults or problems in us or in our stars (to paraphrase Shakespeare)?

Of course, there are many factors which have led to this situation. The Nairobi and Ibadan workshops have attempted to identify some of these factors. The major constraints to progress in Africa include the lack of finance, lack of expertise, lack of direction, and lack of adequate political will. The Lagos Plan of Action has identified some of these problems, but there has been a lack of comprehensive implementation.

I wish to recommend strongly that training be given top priority. The OAU would be willing to offer scholarships to deserving students in this field. There are two types of training to be considered — training field workers in plant genetic resources and training researchers and laboratory technicians. This should be pursued vigorously and national scientists should be involved in all the steps of genetic resources research, starting with collection, storage, characterization, and so on.

The importance of genetics as an individual discipline cannot be over-emphasized. In the field of human science, a lot of work has been carried out, particularly at the molecular and submolecular level. Research results have yielded a substantial amount of information which has contributed towards understanding pathogenesis and the natural history of some diseases. The prevention and control of such diseases have resulted in better life. In the animal and plant sciences, a considerable amount of work has also been done but there is an urgent need for more work to be done in these fields, particularly in Africa.

One of the projected results of this workshop should be the establishment of an active network which will be adequately funded and directed so that the governments of the African countries can recognize it and give it the support it deserves. What should be guarded against at these workshop is the compilation of a long shopping list which cannot be implemented. Duplication of efforts is fairly common in Africa, partly because of poor communication. Such duplication should be avoided at all levels. The greatest weakness in the productive sector of African agriculture is the lack of trained personnel and inadequate infrastructure for research and management. It is, therefore, abundantly clear that human resource development should be given high priority.

It is very encouraging to see that the IITA has already collected and stored over 35,000 genetic species of food crops which are being housed in a bank. It is also interesting to know that some international centers in Europe and the Americas are actively involved in similar or related activities. The proposed development of a genebank in Lusaka, Zambia, for the Southern African Development Coordination Conference (SADCC) countries, at a cost of US$ 20 million, is a welcome development, and will be given full support by the OAU. Similar development projects in other regions of Africa should also be encouraged because of their importance in ensuring food security on the continent. Plant quarantine facilities should be an integral part of this development. The interplay between genetics and environment, however, must not be overlooked in the activities of these networks.

I am sure that you have all listened and discussed the various papers eloquently presented during this workshop. It is my considered opinion that the concrete recommendations which you have adopted will be of interest to donors. The OAU/STRC which will present them to the OAU Council of Ministers for financing. The OAU has, in fact, encouraged and financed ethno-botanical surveys in the areas of medicinal plants. We shall certainly be interested in expanding our areas of interest by collaborating with the various institutions at the national and international levels in areas of agricultural activity that will lead to food security. The three major areas in which the OAU can play an important role are:

- training scholarships for technicians/technologists;
- movement of germplasm on the continent (facilitated by IAPSC);
- collaboration with institutions to ensure the success of the proposed network in genetic resources.

I wish to express my gratitude to the organizers and co-sponsors of this workshop. I want to thank Dr Stifel and Dr Ng for inviting me to this meeting, which has been organized very well. I look forward to our continued collaboration so that this initiative will grow and flower for the benefit of the peoples of Africa in particular and humanity in general.

On behalf of the Secretary General of the OAU, I thank all the donors and the research centers for the continued interest you continue to show in African agriculture but I would like to stress that although the countries of Africa needs your sustained interest and support, they should be independent not only in political terms but also in their ability to feed their populations. The gaps which have been identified in our knowledge should be addressed so that the long-term security and genetic diversity of our plant resources can be assured.

# *Recommendations*

## GROUP A: ROOT AND TUBER CROPS AND STARCHY BANANAS

The Group considered the recommendations of the Nairobi meeting and decided not to duplicate them. Our recommendations therefore do not touch on such issues as training or other forms of strengthening national programs.

The Group identified gaps in collections, national program capacity, diversity of species, long-term security, evaluation and documentation of plantains, starchy bananas, yams and cassava.

### Plantains

1. Comprehensive collections of plantains exist at IRA in Cameroon and at the International Institute of Tropical Agriculture (IITA). These collections, which can be considered duplicates, are fairly representative of available diversity. Areas in need of further exploration and collection are the Congo, Cameroon and Gabon. Collections of East African bananas are held at IRAZ in Burundi. Further collecting is needed in Tanzania and Uganda.

2. National programs should join existing international networks in conserving germplasm.

3. To ensure the long-term security of existing collections of plantains and bananas, the holdings should be conserved *in vitro*. Short-term and long-term field maintenance could also be used.

4. Attention should be given to the establishment of descriptors for plantain cultivars and cultivars of East African bananas.

### Yams

1. Priority for collecting should be given to species originating in Africa and to existing cultivated and related wild species. Fairly comprehensive collections exist in Togo and Côte d'Ivoire but further collecting is needed throughout the area of distribution in West and Central Africa. There should be more well-planned and thorough collecting missions. Collecting should be followed swiftly by characterization and evaluation so that duplicates would be eliminated.

2. Long-term conservation should be by various methods of *in vitro* technology. Field maintenance of the collections could also be employed for short-term and long-term storage.

3. Catalogues should be developed for characterizing within-species diversity; and available catalogues should be reviewed.

### Cassava

1. Cassava collections exist in some national programs but these are not representative of available diversity. Therefore national programs are to be encouraged to undertake collections within their countries.

2. Priority should be given to the cultivated *Manihot* species. Efforts should be made to compile a complete collection of existing wild *Manihot* species and to assemble drought-resistant South American cultigens. National programs should be encouraged to collect materials, characterize and evaluate them, and feed the information into a common databank.

3. The setting up of *in vitro* facilities for long-term conservation should be intensified. Genetic resources must also be conserved in the form of seeds under appropriate conditions.

4. National programs should be encouraged to set up *in vitro* facilities where they do not already exist.

## GROUP B: GRAIN LEGUMES AND CEREALS

The working group on grain legumes and cereals resolved to:

1. Identify gaps in the grain legume and cereal collection of cultivars (and their wild relatives when feasible) of IITA-mandated crops, and develop and implement procedures to fill the gaps.

   *Strategies*

   A. Each national germplasm resources program will complete the passport documentation of its accessions.
   B. Seed and documentation of any accessions of the national programs not already in the IITA base collection will be sent to IITA for seed storage and entry of documentation in the database.
   C. A complete list (in the appropriate language) of all IITA accessions in the base collection will be sent to each national program involved in that crop for review.
   D. A workshop of crop specialists for each crop will meet to develop plans for required collections and procedures to implement the collecting activities.

(i) Identify traits that are limiting factors in production (drought tolerance, resistance to specific insects and diseases) and seek ecological areas with strong selection pressures for this trait for collection.

(ii) Identify the geographical areas that have not been covered by collecting missions. Example of gaps in collections where further exploration and collection may be needed are:

Cowpea: Guinea, Liberia, Mauritania, Guinea-Bissau, Sierra Leone, Zaïre, Angola, Somalia, Ethiopia

Rice (*O. sativa*): Chad, Congo, Mauritania, Togo, Botswana

Rice (*O. glaberrima*): Central African Republic, Congo, Niger, Republic of Benin, Zaire

Bambara groundnut: Botswana, Côte d'Ivoire, Ethiopia, Kenya, South Africa, Sudan, Swaziland

2. Develop and implement procedures to complete the characterization and evaluation of each accession in the base collection of cultivated (and wild relatives when feasible) of IITA-mandated crops.

*Strategies*

A. Coordinate evaluations between national germplasm resources programs and IITA, using descriptor lists produced by the International Board for Plant Genetic Resources (IBPGR).
B. Enter all characterization and evaluation information into the IITA database.
C. Distribute the characterization and evaluation information (in the appropriate language) to all national programs involved in the crop so that their plant breeders can utilize this information to identify useful source materials.

3. Maximize the utilization of the wild relatives of the main cultivated crops in the IITA mandate. This will require collections covering all the genetic diversity available in the alien genepool, maintenance of the material, evaluation and biotaxonomic studies, and development and application of various techniques to transfer the most useful genes.

4. Develop centers for the base collection with a centralized database for important food legumes (such as bambara groundnut), cereals, vegetables and fruit trees not in the IITA mandate.

*Strategies*

A. Coordinate the collection, maintenance and evaluations among the national programs involved in the crop.
B. Enter all documentation into the centralized database.
C. Distribute the documentation from the central database to all national programs involved.

5. Strengthen national germplasm resources programs to provide the foundation of a coordinated African Plant Germplasm Resources System.

*Strategies*

A. Encourage commitment of the appropriate national Ministry and the germplasm resources staff to participate in the African Plant Germplasm Resources System.
B. Obtain funding for expanded training in all germplasm resources areas of national programs, including collection, preservation and evaluation (stability of staff in their positions after training is critical).
C. Improve storage facilities as needed in the national programs by ensuring distribution of all available research information and IBPGR recommendations in the appropriate languages (low seed moisture in sealed containers is essential to supplement cold storage).
D. Expanded staffing and funding of breeding activities in national research centers is needed to fully utilize materials in the expanded African Plant Germplasm Resources System

## GROUP C: PLANT QUARANTINE

The working group on plant quarantine recommended the following:

1. Encourage national quarantine services in Africa to implement quarantine recommendations of the Inter-African Phytosanitary Council (IAPSC); if lack of resources is a limiting factor, national services should avail themselves of regional quarantine services whose budgets should be supported adequately to provide such services.

2. Encourage the establishment of regional plant quarantine programs for countries surrounded by natural barriers (for example, Senegal to Cameroon surrounded by ocean, Sahel and rain forest) so as to obtain protection against the entry of exotic pests and pathogens using the 'ring', 'frontier' or 'perimeter' system of exclusion.
    (i) Update lists of exotic pests and pathogens whose likely entry along man-made pathways threatens the agriculture of the region (A1 pests).
    (ii) Update list of economic pests and pathogens that are of limited distribution within the region but whose further spread would affect agriculture (A2 pests).
    (iii) Conduct regional pest risk analyses using collected biological data about these pests.
    (iv) Survey for selected A2 pests to locate disease-free or pest-free areas to facilitate the export of germplasm from those areas.

3. Technical guidelines for handling designated A1 and A2 pests should be developed, based on data obtained under the activity of 2 (iii).

4. Safeguards or quarantine locations (including intermediate quarantine) should be suggested (for example, IAIC or its regional programs) that would facilitate the timely but safe entry of germplasm.

5. Donors provide support for intermediate or third-country quarantine activities for tropical crops in suitable locations as a service provided to national programs.

6. The resources of IAPSC should be strengthened to facilitate direct communication with national quarantine services and to enhance existing publication and technical support programs.

7. Endorse the FAO initiative to improve plant quarantine services as a means of facilitating the timely but safe transfer of genetic resources (for example, reference RAF87).

# *Recommandations*

## GROUPE A: PLANTES A RACINES ET TUBERCULES, ET BANANES FARINEUSES

Le groupe a examiné les recommandations de la réunion de Nairobi et décidé de ne pas les répéter. Ainsi, nos recommandations ne portent pas sur la formation ou d'autres formes de renforcement des programmes nationaux.

Le groupe a identifié des lacunes dans les collections, la capacité de programmes nationaux, la diversité des espèces, la sécurité à long terme, l'évaluation et la documentation des plantains, des bananes farineuses, des ignames et du manioc.

### Plantains

1. Il y a des collections importantes de plantains à l'IRA (Cameroun) et à l'IITA. Ces collections, que l'on peut considérer comme des duplicata, sont assez représentatives de la diversité disponible. Les zones où les collections doivent être enrichies sont le Congo, le Cameroun et le Gabon. Les collections de bananes de l'Afrique de l'Est sont conservées à l'IRAZ, au Burundi. Il est nécessaire d'intensifier la collecte en Tanzanie et en Ougandre.

2. Les programmes nationaux devraient s'associer aux réseaux internationaux existant pour la conservation du matériel génétique.

3. Pour assurer la sécurité à long terme des collections actuelles de plantains et de bananes, les accessions devraient être conservées *in vitro*. On pourrait aussi recourir à l'entretien à long et court terme en champ.

4. Il faudrait veiller à établir des descripteurs pour les cultivars de plantains et de bananes de l'Afrique de l'Est.

### Ignames

1. Il faudrait accorder la priorité en matière de collecte aux espèces originaires d'Afrique et aux espèces actuellement cultivées, ainsi qu'aux espèces sauvages apparentées. Des

collections relativement complètes existent au Togo et en Côte d'Ivoire mais il faut poursuivre la collecte sur l'ensemble de la zone de distribution en Afrique de l'Ouest et en Afrique centrale. Il faudrait prévoir davantage de missions de collecte bien organisées et systématiques. Après la collecte, il faudrait procéder sans retard à la caractérisation et à l'évaluation afin d'éliminer les duplicata.

2. La conservation à long terme devrait être assurée par diverses méthodes de technologie *in vitro*. L'entretien des collections en champ pourrait aussi servir au stockage à court et à long terme.

3. Des catalogues devraient être préparés pour caractériser la diversité des espèces et les catalogues disponibles devraient être révisés.

**Manioc**

1. Il existe des collections de manioc dans le cadre des programmes nationaux mais elles ne sont pas représentatives de la diversité disponible. Il faut, par conséquent, encourager les programmes nationaux à constituer des collections dans leurs pays.

2. La priorité devrait porter sur les espèces *Manihot* cultivées. Il faudrait s'efforcer d'obtenir une collection complète des espèces *Manihot* sauvages existantes et de réunir des cultigènes sud-américains résistants à la sécheresse. Les programmes nationaux devraient être encouragés à collecter, caractériser et évaluer leur matériel, et à transmettre les informations à une banque de données commune.

3. La création d'installations pour la conservation à long terme *in vitro* devrait être intensifiée. Les ressources génétiques doivent aussi être conservées dans de bonnes conditions sous forme de semences.

4. Les programmes nationaux devraient être encouragés à mettre en place des installations de conservation *in vitro* là où elles n'existent pas encore.

GROUPE B: LEGUMINEUSES A GRAINES ET CEREALES

Le groupe de travail sur les légumineuses à graines et les céréales a décidé:

1. D'identifier les lacunes dans la collection de légumineuses à graines et de céreales portant sur les cultivars (et les espèces sauvages apparentées, si possible) de cultures relevant de l'IITA, et d'élaborer et mettre en oeuvre des procédures visant à combler ces lacunes.

   *Stratégies*

   A. Chaque programme national de ressources génétiques établira le passeport de ses obtentions.
   B. Des semences et la documentation relative à toute accession des programmes nationaux ne figurant pas encore dans la collection de base de l'IITA lui seront

envoyées pour stocker les semences et introduire la documentation dans sa base de données.

C. Une liste complète (dans la langue appropriée) de toutes les accessions de l'IITA figurant dans sa collection de base sera envoyée à chaque programme national concernant cette culture pour examen.

D. Un atelier de spécialistes pour chaque culture sera organisé afin de planifier les collections et procédures nécessaires aux activités de collecte.

(i) Identifier les caractères qui entravent la production (tolérance à la sécheresse, résistance à des insectes spécifiques, et maladies) et de chercher les zones écologiques où les contraintes de sélection relatives à ces caractères se font beaucoup sentir.

(ii) Identifier les zones géographiques qui n'ont pas été couvertes par des missions de collecte.

Exemples de lacunes dans les collections pour lesquelles il sera peut-être nécessaire de poursuivre l'exploration et la collecte:

Niébé: Guinée, Libéria, Mauritanie, Guinée-Bissau, Sierra Leone, Zaïre, Angola, Somalie, Ethiopie

Riz (*O. sativa*): Congo, Mauritanie, Tchad, Togo, Botswana

Riz (*O. glaberrima*): République centrafricaine, Congo, Niger, République du Bénin, Zaïre

Pois bambara: Botswana, Côte d'Ivoire, Ethiopie, Kenya, Afrique du Sud, Swaziland

2. Elaborer et mettre en oeuvre des procédures permettant de compléter la caractérisation et l'évaluation de chaque accession dans la collection de base des plantes cultivées (et d'espèces sauvages apparentées, si possible) relevant de l'IITA.

*Stratégies*

A. Coordonner les évaluations entre les programmes nationaux de ressources génétiques et l'IITA en utilisant les listes de descripteurs préparées par le CIRPG.

B. Introduire toutes les informations relatives à la caractérisation et à l'évaluation dans la base de données de l'IITA.

C. Distribuer les informations relatives à la caractérisation et à l'évaluation (dans la langue appropriée) à tous les programmes nationaux concernant la culture afin que leurs sélectionneurs puissent utiliser ces données pour identifier des sources utiles de matériel.

3. Utiliser au maximum les espèces sauvages apparentées aux principales plantes cultivées qui relèvent du mandat de l'IITA. Ceci nécessitera des collections couvrant toute la diversité génétique disponible dans la réserve de gènes étrangers, l'entretien du matériel, des études d'évaluation et de biotaxonomie, la mise au point et l'application de diverses techniques permettant de transférer les gènes les plus utiles.

4. Etablir des centres pour la collection de base dotés d'une base de données centralisée pour les légumineuses alimentaires importantes (comme le pois bambara), les céréales, les légumes, les arbres fruitiers, etc. qui ne relèvent pas de l'IITA.

*Stratégies*

A. Coordonner la collecte, l'entretien et l'évaluation au niveau des programmes nationaux travaillant sur la culture.
B. Introduire toute la documentation dans la base de données centralisée.
C. Diffuser la documentation provenant de la base de données centrale à tous les programmes nationaux pertinents.

5. Renforcer les programmes nationaux de ressources génétiques pour établir les assises d'un système africain coordonné de ressources phytogénétiques.

*Stratégies*

A. Encourager l'engagement du ministère national intéressé et du personnel chargé des ressources génétiques à participer au Système de ressources phytogénétiques.
B. Obtenir que les programmes nationaux bénéficient d'une formation et d'un financement accrus dans tous les domaines intéressant les ressources génétiques, y compris la collecte, la préservation et l'évaluation (la stabilité du personnel en poste après formation est vitale).
C. Améliorer les installations de stockage en fonction des besoins du programme national en assurant la diffusion de toutes les informations issues de la recherche ainsi que les recommandations du CIRPG, dans les langues appropriées (par exemple, un faible taux d'humidité des semences conservées dans des récipients hermétiques est le complément indispensable au stockage réfrigéré).
D. Le renforcement du personnel et du financement affectés aux activités de sélection dans les centres nationaux de recherche est pleinement nécessaire pour utiliser le matériel dans le Système élargi des ressources génétiques africaines.

## GROUPE C: QUARANTAINE PHYTOSANITAIRE

Le groupe de travail sur la quarantaine phytosanitaire a formulé les recommandations suivantes:

1. Encourager les services nationaux de quarantaine en Afrique à mettre en oeuvre les recommandations sur les quarantaines de l'IAPSC; mais, si le manque de ressources constitue une contrainte pour ces services, ils devraient faire appel aux services régionaux de quarantaine dont les budgets devraient être suffisamment renforcés pour répondre à de telles demandes.

2. Encourager l'instauration de programmes régionaux de quarantaine phytosanitaire pour les pays entourés de barrières naturelles (par exemple les régions allant du Sénégal au Cameroun qui sont entourées par l'océan, le Sahel et la forêt tropicale) de façon à les protéger contre l'entrée de ravageurs et d'éléments pathogènes étrangers utilisant le système d'exclusion de 'l'enceinte', de 'la frontière' ou 'du périmètre'.
    (i) Mettre à jour les listes de ravageurs et d'éléments pathogènes étrangers dont l'entrée probable le long des voies de communication créées par l'homme menace l'agriculture de la région (ravageurs A1).

(ii) Mettre à jour les listes de ravageurs et éléments pathogènes économiques peu répandus dans la région mais dont la prolifération affecterait l'agriculture (ravageurs A2).

(iii) Effectuer des analyses sur les risques liés aux ravageurs à l'échelle régionale en utilisant les données biologiques recueillies sur ces ravageurs.

(iv) Enquête sur certains ravageurs A2 pour localiser les zones exemptes de ravageurs ou de maladies afin de faciliter l'exportation de matériel génétique de ces régions.

3. Il est recommandé d'établir des directives techniques pour agir sur les ravageurs classés A1 et A2 en s'appuyant sur les données obtenues dans le cadre des activités de 2 (iii).

4. Il est recommandé que des précautions ou des emplacements de quarantaine (y compris de quarantaine intermédiaire) soient suggérés (par exemple l'IAIC ou ses programmes régionaux) qui faciliteraient l'entrée en temps utile mais sans risque du matériel génétique.

5. Il est recommandé que les donateurs fournissent un soutien aux activités de quarantaine intermédiaire ou à celles menées dans un pays tiers à titre de service fourni aux programmes nationaux.

6. Il est recommandé que les ressources de l'IAPSC soient renforcées pour faciliter les communications directes avec les services nationaux de quarantaine et pour valoriser les publications existantes et les programmes de soutien technique.

7. Souscrire à l'initiative de la FAO visant à améliorer les services de quarantaine phytosanitaire dans le but de faciliter le transfert en temps utile mais sans risque des ressources génétiques (par exemple, voir RAF87).

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