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Cassava *in vitro* germplasm management at the International Institute of Tropical Agriculture

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Abstract/Résumé

In vitro techniques have been applied to the conservation and exchange of cassava germplasm at the International Institute of Tropical Agriculture (IITA). The initial explants, meristems or nodal cuttings, obtained from plants grown in the field/screenhouse, were cultured on modified Murashige and Skoog (MS) medium. Regenerated plantlets were subcultured to increase the number of cultures before they were transferred to lower incubation temperatures (18 to 24°C) for storage. Embryos from seeds of wild *Manihot* germplasm were cultured on half strength MS medium. Plantlets obtained from these embryos were subsequently cloned and then transferred to the reduced temperature conditions for storage, or transplanted to the field for evaluation. Using meristem/nodal culture technique, some cassava germplasm collections in national programs were successfully transferred to IITA for conservation. Currently, a total of 727 accessions of cassava and related *Manihot* species are maintained in reduced incubation temperature conditions. Cultures can be stored for 8–14 months. For dissemination of selected cassava germplasm to collaborators outside Nigeria, plantlets regenerated from meristem culture were transplanted and indexed for African cassava mosaic virus (ACMV). Plantlets regenerated from nodal cuttings obtained from these virus-tested plants were used for distribution upon request. NARS in more than forty countries in Africa received these certified virus-tested plantlets for evaluation.

Key words/Mots clés: *Manihot esculenta*, germplasm conservation, embryo culture, meristem culture

Gestion de matériel génétique de manioc *in vitro* a l'institut international d'agriculture tropicale

Les techniques *in vitro* ont été appliquées à la conservation et l'échange de matériel génétique de manioc à l'Institut international d'agriculture tropicale (IITA). Les explants initiaux, méristèmes ou boutures nodales, obtenus à partir de plants ayant poussé au champ/serre, ont été mis en culture sur un milieu de culture modifié de Murashige et Skoog (MS). Les plantules régénérées ont été repiquées pour augmenter le nombre de cultures avant d'être stockées à des températures d'incubation plus basses (18°C à 24°C). Les embryons issus de semences de matériel génétique sauvage de *Manihot* ont été mis en culture sur milieu MS dilué de moitié. Les plantules obtenues à partir de ces embryons ont été par la suite clonées et alors stockées à la température réduite ou transplantées au champ pour évaluation. Grâce à la technique de culture de méristèmes/noeuds, certaines collections de matériel génétique appartenant à des programmes nationaux ont été transférées avec succès pour conservation à l'IITA. A l'heure actuelle, un total de 727 accessions de manioc et espèces apparentées de *Manihot* sont conservées à des températures d'incubation réduites. Les cultures peuvent être stockées pendant 8-14 mois. A fin de distribuer le matériel génétique de manioc sélectionné aux collaborateurs en dehors du Nigeria, les plantules régénérées à partir de culture de méristèmes ont été transplantées et indexées pour le virus africain de la mosaïque du manioc (ACMV). Les plantules régénérées à partir des boutures nodales obtenues à partir de ces plants ayant subi le test du virus ont été distribuées sur demande. Les SNRA de plus de quarante pays africains ont reçu ces plantules ayant subi le test du virus pour évaluation.

Introduction

Cassava was introduced to Africa by the Portuguese during the late 16th century. It became one of the most important staple food crops grown in tropical Africa and plays a major role in efforts to alleviate the African food crisis (Hahn & Keyser 1985). Because of the importance of the crop and the range of habitats into which it was moved, apparently wide new genetic diversity evolved in this relatively short period of cultivation in the continent (Allem & Hahn 1991).

The International Institute of Tropical Agriculture (IITA) began collecting cassava germplasm in the early 1970s with the aim of establishing a genetic improvement program. The collection was assembled from the tropics and local cultivars from within Nigeria. Additional collections continue to be made in collaboration with National Agricultural Research Systems (NARS). The cassava germplasm collection at IITA comprises of mainly *Manihot esculenta* (1,863 accessions) and some wild *Manihot* (95 accessions of 24 species). This *Manihot esculenta* collection consists of African local cultivars, germplasm introduced from Latin America and the hybrids between Nigerian and Latin American germplasm (Ng et al. 1994). The *Manihot* germplasm collection is maintained as living collections and some of the selected germplasm are duplicated in *in vitro* genebank at IITA as a complimentary method for the conservation of the germplasm. This paper summarizes the applications of *in vitro* techniques in cassava germplasm conservation and exchange at IITA.

Germplasm Introduction. Cassava germplasm was introduced to IITA in the form of seeds and *in vitro* cultures, both meristem culture and nodal cutting cultures. Seeds introduced are normally germinated and planted directly for evaluation. Some of the seeds are also stored under medium term storage conditions. The introduced seeds of *Manihot* species were germinated using embryo culture technique and a portion of the seeds are also stored under medium term storage conditions.

Meristem/nodal Culture. Meristem culture technique/nodal cutting culture have been successfully used to transfer cassava germplasm from the field genebank at IITA, Ghana and

Republic of Benin to *in vitro* genebank at IITA. For meristem culture, apical buds of cassava obtained from field genebank or screenhouse were used. The buds were surface disinfected with 70% ethanol, followed by 7% sodium hypochlorite solution with Tween 20 for 20 minutes, they were rinsed with three changes of sterile distilled water. Meristems with one to two leaf primordia were excised from the buds and inoculated in Murashige and Skoog (MS) basal medium (Murashige & Skoog 1962) supplemented with 3% sugar, 80 mg/l adenine sulfate, 0.15 mg/l benzyl amino purine (BAP), 0.2 mg/l napthalene acetic acid (NAA), 0.04 mg/l gibberellic acid (GA_3) and 0.6% agar. For nodal cutting culture, nodes obtained from green young shoots were surface disinfected with 70% ethanol for 5 minutes, followed by 10% sodium hypochlorite solution with Tween 20 for 20 minutes, followed by 5% sodium hypochlorite solution for 10 minutes. The nodes were rinsed thoroughly with three changes of sterile distilled water and placed on MS medium supplemented with 3% sugar, 0.01 mg/l NAA, 0.05 mg/l BAP and 0.7% agar. Cultures were incubated in a culture room with temperature range from 28–30°C and 12 h photoperiod.

A simple collapsible portable transfer hood made with plywood and installed with a UV light was used for the transfer of germplasm in NARS where tissue culture facility is not available. Culture media in test tubes and sterile distilled water were prepared at IITA and transported to the location where the field genebank is located. Cultures were shipped to IITA within 10–14 days for incubation.

Embyro culture. Embryo culture technique has also been used to germinate embryos of wild *Manihot* seeds introduced from Latin America. The seeds were treated with concentrated sulfuric acid for 1 h, rinsed in continuous running tap water for 1 h. Seeds were surface disinfected with 70% ethanol for 5 minutes and 10% sodium hypochlorite solution with Tween 20 for 20 minutes. They were rinsed in three changes of sterile distilled water. Seeds were left overnight in the third change of sterile distilled water. Embryos were dissected and cultured on half strength MS medium supplemented with 3% sugar and 0.6% agar (Ng 1992). Plantlets were obtained within 4 to 6 weeks after culturing. The species that were successfully germinated through embryo culture were *M. flabellifolia*, *M. alutacea*, *M. peruviana*, *M. gabrielensis* and *M. quinquepartita*.

Germplasm conservation. A schematic representation on the in vitro genebank management is shown in Figure 1. This also shows the inter-relationships between the field and in vitro genebank as well as germplasm distribution of cassava. Germplasm that were introduced as in vitro cultures, the first quarantine inspection took place few days after arriving IITA. Contaminated cultures were destroyed. Plantlets obtained from meristem, nodal cutting or embryo culture were subcultured in the nodal cutting culture medium for initial increase. Ten tubes per accession were cultured. Cultures were incubated at 28-30°C with 12 h light for 3-4 weeks. They were checked for possible contamination before being transferred to lower incubation temperature, 18-24°C, and lower light intensity for storage. Cultures were checked periodically and those that showed deterioration were subcultured. Currently, a total of 727 accessions of Manihot germplasm are maintained in vitro under reduced growth condition (Table 1). Cultures can be kept for 8 to 14 months. However, some of the wild species are difficult to propagate. This suggests that there is a need to formulate culture medium targeting for some of these wild species. For field evaluation of introduced germplasm, plantlets were transplanted to an isolation room for virus indexing and quarantine inspection before transplanting to the field.

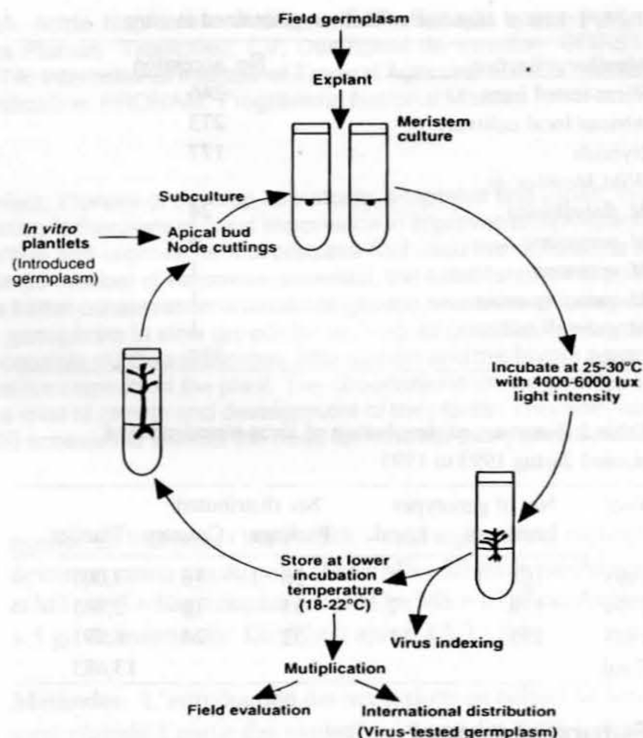

Figure 1. Procedures for *in vitro* reduced growth storage of cassava germplasm at IITA

Germplasm delivery. Meristem culture or combination of heat treatment and meristem culture have been successfully applied to eliminate African cassava mosaic virus (ACMV) infection from cassava (Ng 1991). The meristem culture regenerated plantlets after initial multiplication in vitro were transplanted to an insect-free isolation room for virus indexing. The plants were allowed to grow for at least four months in isolation room with lower temperature (25°C) which favours African cassava mosaic disease symptom expression. The symptomless plants were indexed by sap inoculation to a test plant, *Nicotiana benthamiana*, and enzyme linked immunosorbent assay using both polyclonal and monoclonal antibodies. The negatively indexed materials were then certified by the Nigerian Plant Quarantine Services. The ACMV-tested plants were then micropropagated *in vitro* and distributed to collaborators in Africa. A total of two hundred and forty-six selected cassava genotypes were cleaned from ACMV infection and are available for distribution upon request. These include two hundred and thirty-five improved genotypes and eleven local clones (Table 2). Over the past three years a total of over thirteen thousand and six hundred plantlets were distributed to research collaborators in NARS (Table 2).

Table 1. List of *Manihot* collection maintained *in vitro*

Manihot collection	No. accession
Virus-tested lines	246
African local cultivars	273
Hybrids	177
Wild *Manihot* sp.:	
M. flabellifolia	24
M. peruviana	4
M. eprinosa	1
M. quinquepartita	1
M. gabrielensis	1
Total	727

Table 2. Summary of distribution of virus-tested cassava planted during 1993 to 1995

Year	No. of genotypes		No. distributed:		
	Improved	Local	Package	Country	Plantlet
1993	104	2	26	18	3,007
1994	128	2	33	18	2,385
1995	235	11	32	24	8,291
Total					13,683

Future prospect

The conservation of cassava germplasm using reduced growth storage demands frequent checking of the cultures and subculture. Cryopreservation offers long term conservation of germplasm. Success in cryopreservation of cassava had been reported (Kartha et al. 1982) and the recovery rate from a range of genotypes has been improved (Escobar & Roca, personal communication). IITA is in the process of initiating research in cryopreservation of cassava.Different cryopreservation protocols will be tested.

The transfer of germplasm from the field to *in vitro* culture will be intensified and the micropropagation of some wild *Manihot* species which are difficult to propagate will be improved. The use of morphological and molecular markers in monitoring genetic stability of the conserved germplasm will be a high priority.

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Gestion d'une banque de genes de manioc: experience au Congo

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Abstract/Résumé

Le matériel génétique d'une variété qui disparaît est irreplaçable. Pour séléctionner améliorer ou développer de nouvelles souches adaptable et variées, cette information doit être préservée. La conservation du matériel génétique est donc d'une grande importance dans un programme d'amélioration ou de création variétale. Au Congo des 1975 le programme de sélection et d'amelioration du manioc a utilisé des collections vivantes sur le terrain à Loudima, Odziba et Kindamba. Ceci a abouti à la perte d'un grand nombre de matériel végétal. La mise en place d'une banque de gènes in vitro de manioc à été réalisée dans le but d'assurer une meilleure conservation des ressources génétiques de manioc disponibles. Des conditions de culture in vitro permettant de conserver le germoplasme de manioc en condition de vie ralentie aussi longtemps que possible ont été déterminées. Un milieu de culture a été notamment mis au point. Ce milieu comprend peu de saccharose et un peu de manitol et favorise une conservation des plantes jusq'au delà de six mois sans altérer la faculté de multiplication des plantes. Des observations sur des génotypes d'origines diverses (IITA, Zaïre, genotypes locaux) sont réalisées au niveau de la croissance et du développement des plantes. Ce travail a abouti à une meilluere gestion de la vitrothéque par le stockage de plus de 100 accessions dans avior recours au nécessaire renouvellement chaque six semaine connu au paravant.

Keywords/ Mots clés: banque de gene, manioc, culture *in vitro*