Status of *In Vitro* Conservation of Cassava *(Manihot Esculenta* Spp.*)* Genetic Resources at CSIR Plant Genetic Resources Research Institute (CSIR-PGGRI).

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Abstract
Cassava (Manihot esculenta spp.) contributes significantly to global food security and serves as a major carbohydrate source in Ghana. Climate change and natural habitat destruction among others, cause loss of genetic diversity in cassava. Crop diversity is important for food security. Conservation of cassava genetic resources will ensure that their diversity is maintained for crop improvement and future use. Cassava genetic resources are conserved ex situ in field genebanks and under in vitro slow-growth conditions as tissue cultures or by cryopreservation. This paper highlights the in vitro conservation activities carried out on cassava genetic resources at CSIR - PGRRI. Cassava tissue cultures were initiated from nodal cuttings excised from young shoots and inoculated on Murashige and Skoog's medium supplemented with 3% sucrose, 0.8% bacteriological agar, 0.05 mg/l BAP and 0.01mg/l NAA. In vitro cultures were incubated at 25 ± 1 °C and 16/8 hours day/dark photoperiod provided by florescent light. The in vitro cassava collection was maintained by periodic sub-culturing at a minimum of six months intervals. Presently 241 cassava accessions are kept as tissue culture plantlets in the in vitro genebank of PGRRI.

Key words: Cassava, conservation, genetic resources, in vitro, tissue culture

État de in vitro des ressources génétiques du manioc (Manihot Esculenta Spp.) À CSIR - Institut de recherche sur les ressources phytogénétiques.

Résumé
Le manioc (Manihot esculenta spp.) Contribue de manière significative à la sécurité alimentaire mondiale et constitue une source majeure d'hydrates de carbone au Ghana. Le changement climatique et la destruction de l'habitat naturel, entre autres, entraînent une perte de la diversité génétique du manioc. La diversité des cultures est importante pour la sécurité alimentaire. La conservation des ressources génétiques du manioc assurera que leur diversité est maintenue pour l'amélioration des cultures et leur utilisation future. Les ressources génétiques du manioc sont conservées ex situ dans des banques de gènes et dans des conditions de croissance lente in vitro comme des cultures tissulaires ou par conservation. Cet article souligne les activités de conservation in vitro menées sur les ressources génétiques du manioc à Conseil pour la recherche scientifique et industrielle-Institut de Recherch sur les ressources phytogénétiques (CSIR-PGRRI) au Ghana. Des cultures tissulaires de manioc ont été initiées à partir de boutures nodales excisées de jeunes

pousses et inoculées sur milieu de Murashige et Skoog additionné de 3% de saccharose, 0,8% d'agar bactériologique, 0,05 mg/l de BAP et 0,01 mg/l de NAA. Les cultures in vitro sont incubées à 25 ± 1 °C et 16/8 heures de photopériode de jour/obscurité fournies par la lumière fluorescente. La collection de manioc in vitro a été maintenue par culture périodique à intervalles d'au moins six mois. Actuellement, 241 accessions de manioc sont conservées en tant que plantules de culture tissulaire dans la banque de gènes in vitro de CSIR-PGRRI.

Mots-clés: manioc, conservation, ressources génétiques, in vitro, culture tissulaire

Introduction

Plant genetic resources (PGR) are important for the development of agriculture because they contain useful characters of actual and potential value. They therefore need to be conserved to enhance food security. PGR can be conserved in their natural habitats (in situ), outside their natural habitat (ex situ) or in a combination of both methods (Jaramillo *et al.,* 2002). In vitro conservation, a type of ex situ conservation is useful for conservation of recalcitrant seeds, vegetatively propagated species and biotechnology products (Engelmann, 2011). *In vitro* conservation of plant genetic resources complements field conservation and provides a space saving and phytosanitary method for germplasm maintenance and exchange.

Cassava (*Manihot esculenta* spp.) is a root crop which contributes significantly to global food security and serves as a major carbohydrate source in the tropics. In some parts of Africa, the leaves are eaten as a green vegetable which provide a cheap source of protein and vitamins (Umuhozariho *et al.,* 2014). It is also used as livestock feed and raw material for the cassava industry. Cassava genetic resources, like other root and tuber crops are conserved ex situ in field genebanks and under in vitro slow-growth conditions as tissue cultures or by cryopreservation (Kameswara, 2004). Cassava has a narrow genetic base for crop improvement because it is clonally propagated. Climate change and natural habitat destruction among others, can lead to loss of genetic diversity in cassava. Conservation of cassava genetic resources, therefore, will ensure that their diversity is captured for crop improvement and future use. The objective of this study was to place and maintain cassava accessions under field conservation under *in vitro* conservation as tissue culture plantlets. This paper highlights the *in vitro* conservation activities carried out on cassava genetic resources at the CSIR-PGRRI, Ghana.

Materials and Methods

Medium Murashige MS and Skoog (1962), supplemented with 3% sucrose, 0.8% agar 0.05 mg/l Benzylaminopurine (BAP) and 0.01 mg/l Naphthalene acetic acid (NAA) was used for the conservation of cassava accessions throughout the germplasm introduction and maintenance stages. Medium pH was adjusted to 5.8 and sterilized by autoclaving at 15 psi for 10 -15 minutes.

Introduction of new germplasm: Twenty centimeters long stem cuttings from cassava accessions conserved in the PGRRI field gene bank were soaked in 20 g/l carbendazim for 5 minutes and planted in the screen house. Young shoots were collected and washed with detergent under running tap water for 5 minutes and soaked in 6 g/l carbendazim solution for 20 minutes. Explants were surface sterilized in a laminar air flow chamber first with 35% ethanol for 10 minutes, then with 10% commercial bleach containing 5% sodium hypochlorite for 10

minutes and rinsed with sterile distilled water until there was no trace of bleach. Nodal cuttings with an axillary bud was excised and inoculated on the culture medium. The cultures were labeled with their accession numbers and culture date, sealed and incubated at 25 ± 1°C under 16/8 hour day/dark photoperiod provided by fluorescent light with an intensity of 2000 lux. Regenerated plantlets were cut into nodal portions and subcultured onto fresh medium in the multiplication stage.

Maintenance of the in vitro cassava collection: Cassava accessions in the *in vitro* collection were subcultured at a minimum of six months intervals. Average of seven nodal cultures per accession was prepared with each subculture cycle. Weekly checks were carried out to track cultures that were contaminated, necrotic stunted growth. Accessions with less than five cultures were selected for subculture and lost accessions are reintroduced into the genebank.

Results and Discussions

The CSIR-PGRRI maintains the cassava genetic resources collection in Ghana which consists of accessions acquired from collection missions carried out across the country and some released varieties in a field genebank and in vitro as tissue cultures. The culture medium used in this study induced simultaneous development of shoot and roots in all cassava accessions. The concentration of BAP and NAA used wase same as that of medium used for *in vitro* cassava conservation at International Institute of Tropical Agriculture (IITA), Nigeria (IITA cassava gene bank manual, 2007). Optimal surface sterilization for plantlet regeneration with nodal explants of cassava was achieved with 35% ethanol and instead of 70% ethanol used for the same time. Washa *et al*. (2014) reported of successful regeneration of Dalbergia melanoxylon after sterilization with 35% ethanol for 20 minutes and 2.6% sodium hypochlorite solution for 20 minutes.

Table 1 shows the status of cassava accessions conserved *in vitro* from 2013 to 2015. Two hundred and seventy eight cassava accessions were conserved in the *in vitro* genebank as at December 2015. One hundred and fifteen new cassava accessions were introduced into the *in vitro* genebank from 2013 to 2015. Fifty-six accessions were added to the collection in 2013, 41 accessions in 2014 and 18 accessions in 2015. One hundred and three cassava accessions were lost from culture within this period. The highest loss of cassava accessions (54) comprising 113 cultures (5%) was recorded in 2015.

Loss of accessions in conservation was due to microbial contamination, necrosis, hyperhydricity of cultures and poor physiological performance. This could be due to frequent power cuts which resulted in changes in growth room temperature, relative

Table 1. Status of cassava accessions placed under *in vitro* conservation from 2013 to 2015

Status of accessions	Year		
	2013	2014	2015
Accessions introduced *in vitro*	56	41	18
Accessions lost from *in vitro* collection	32	17	54
Accessions re-introduced *in vitro*	16	11	54
Accessions available in culture at the end of year	225	260	278

humidity and lightening regimes. Changes in physical condition of growth room such as temperature, relative humidity and lighting could lead to loss of in vitro plantlets (De Klerk, 2007, Mafla *et al.,* 2009). Eighty one (81) of the lost in vitro accessions have been replaced or reintroduced. Current inventory of two hundred and forty-one (241) cassava accessions comprising 232 landraces and 9 released varieties are under *in vitro* conservation.

Conclusion

Cassava genetic resources have been placed under in vitro conservation as plantlets using MS medium amended with 3% sucrose, 0.8% agar, 0.05 mg/l BAP and 0.01 mg/l NAA in the CSIR-PGRRI. Frequent changes in the physical condition of growth room such as temperature, relative humidity and lighting during the period of the study played a major role in the loss of cassava accessions. A constant supply of electricity is essential to stabilize growth room conditions and prevent such losses.

Acknowledgements

The authors are grateful to all staff of the PGRRI Tissue Section and all staff who contributed to the daily management of the In vitro genebank.

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