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IN-VITRO PLANT PROPAGATION: AN EFFECTIVE TOOL FOR GERMPLASM INTRODUCTION AND EXCHANGE FOR CASSAVA IMPROVEMENT

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ABSTRACT
More than 250 million Africans rely on the starchy root crop cassava (Manihot esculenta) as their staple source of calories. Cassava is vegetatively propagated; the planting materials are also source of spread of major cassava diseases and results from repeated use from season to season. In-vitro techniques can alleviate these problems. In-vitro techniques have been very useful in the transfer of exotic germplasm from latin America to Nigeria. Important advances have been obtained in research institutions for distribution and movement of germplasm especially for vegetatively propagated crops. However, the dissemination of these improved varieties is a huge challenge due to low multiplication in cassava. In-vitro technique is now being explored to disseminate these improved varieties by National Root Crops Research Institute (NRCRI), Umudike.We report update of results obtained with the use of in-vitro method in germplasm transfer and improvement of cassava at National Root Crops Research Institute (NRCRI), Umudike Nigeria. The paper also describes how this can be effectively utilized as a successful tool for germplasm development in crop improvement program.

Keywords: In-Vitro, Germination, Survival, Seeds, CIAT and NRCRI

Introduction

Cassava (*Manihot esculenta Crantz*) is a stable food for many people in large parts of tropical Africa, South America and Asia (Hasley *et al.*, 2008). In Africa, cassava is regarded as a food security crop because of the ability of the crop to be tolerant to poor soil and drought, and its high potential in preventing famine in times of civil unrest (Okogbenin *et al*., 2003). Apart from edibility of cassava by individuals, there is a growing demand of the root of this crop by the starch, food, animal feed and ethanol industries (Ceballos *et al.,* 2007). The Centro Internacional de Agricultura Tropicai (CIAT) maintains a collection of 6,000 cassava accessions. This consists of landraces from Latin America and Asia, elite clones selected by CIAT and the International Institute for Tropical Agriculture (IITA) Nigeria, and other several *Manihot* species. These genetic resources are distributed world worldwide for breeding programmes. Traditional breeding of cassava is constrained by a number of intrinsic factors, including high levels of genetic heterozygosity, variable fl owering patterns, and low seed set and germination (Jennings and Iglesias, 2002). Because of these difficulties, modern biotechnology and especially genetic modification through recombinant DNA methodology may be expected to play a significant—indeed, an essential—role in future improvements of cassava. Because of its role as a subsistence crop in tropical agriculture, and the vegetative propagation system typically used in cassava culture, research into the agronomy, genetics, and improvement of this important crop is often neglected by scientists in industrialized countries and by commercial entities. Instead, such research is frequently performed by public-sector scientists and research institutions in developing countries, who typically lack the resources that may be brought to bear on major temperate crops of industrialized countries, such as maize (Zea mays L.) and soybean [(Glycine

max L. (Merr.)]. The application of tissue culture techniques and development of molecular markers in crop improvement has efficiently facilitated gene pool development and breeding of superior varieties. (Fregene *et al*., 2000; Okogbenin *et al*., 2003; Akinbo *et al*., 2012). Cassava is propagated vegetatively by means of stem cuttings but these are bulky and very expensive to transport. Moreover, phytosanitory regulations prohibit the movement of cassava stem cuttings across international borders to prevent the spread of diseases and insects. Propagation of cassava by vegetative cuttings guarantees uniformity but at the same time cuttings generate relatively low multiplication rates as they can only be obtained from orthotropic branches (Akinbo *et al*., 2010). Multiplication by tissue culture techniques (In-vitro multiplication) permits the production of relatively uniform plants, on a massive scale in a shorter period and with a narrower genetic base (Vinod Kumar *et. al*., 2006). There is need for germplasm transfer in National Root Crops Research Institute Umudike, Nigeria because Africa is the secondary center of cassava diversity to capture the diversity existing in the center of origin for noble traits.

Materials and Methods

Introductions from sexual seeds

These consist of sexual seeds of drought tolerant breeding populations and obtained from Centro International de Agricultura Tropical (CIAT). They were screened and certified disease free before importation into National Root Crops Research Institute (NRCRI) Umudike. Viability test were conducted on these seeds. The viable seeds were planted in seed trays. Three weeks after planting, they were transplanted to the field. Data on the Survival count of these sexual seeds were collected before and after transplanting to the field.

Introductions from in-vitro plantlets

In-vitro plantlets of valuable traits low to post-harvest physiological deterioration (PPD), tolerant to drought, resistance to cassava mosaic disease (CMD), resistant to cassava green mite (CGM) and high protein content were received from CIAT to broaden the narrow genetic base of National Root crops Research Institute (NRCRI) Umudike. Plantlets were inspected; those found to be contaminated, broken or malformed were eliminated. Clean plantlets were kept in the culture room at room temperature 28 ± 2^0C. The photoperiod of the culture room is 16 hrs light and 8 hrs darkness.

In-vitro propagation and multiplication of the imported samples

Plantlets certified to be clean, healthy and growing vigorously were transferred to fresh Murashige and Skoog (MS) basal medium supplemented with 30 g/l sucrose and 1 mg/l vitamin solution. The culture were maintained at a temperature of 28 ± 2°C for a period of 4 weeks in a 16 and 8 hours light and dark regimen respectively. The materials were successively transferred to fresh medium of same composition every 7 to 8 weeks for clonal multiplication and for conservation of these materials.

Hardening

Vigorously growing plants with well-established roots were acclimatized first in the humidity chamber and eventually in the greenhouse before establishment in the field. Peat pellet and vermiculite were used as substrates for the acclimatization process in a combined ratio of 2:1 respectively. Prior to use the substrates, were soaked in water for a period of 2 hours to allow the penetration of fluid into the substrate and contained in a small transparent polybags locally constructed in the institute.

Plantlets were extracted from culture vessels by gently tapping the cultures to disintegrate the gelling agents. The roots of the extracted plants were gently washed with warm water very close to room temperature (28°C) and gently seated in a finger hole made in the substrate contained in the locally constructed polyethene bags. The bags are further filled with substrate to ensure the roots are properly covered. The transferred plantlets are placed in a humidity chamber, a locally constructed cone using a wide circumference board, very thick transparent bags, thumb tags and ropes. The plantlets were sprayed with water about 4 to 5 times to ensure the environment gets the desired humidity. The humidity chamber was sealed off and hung unto a vertical hold. Two days later the humidity chamber was pierced to allow slow expulsion of hot air from the chamber and allow the inflow of fresh air into the chamber. On the third day, the air passage was increased by

creating two small windows on the chamber for cross ventilation within the chamber. Two grams of high phosphate fertilizer (N:P:K: 9: 45: 15) dissolved in 1litre of distilled water was applied to the plantlets 5 days after planting to boost the growth of the plantlets. The plantlets were transplanted unto sterilized topsoil locally obtained within the Institute and contained in a nursery bag of dimension 75 + 50 x 180 mm when laid flat and of 50 micron in thickness and volume capacity of 0.75Lt. The topsoil was locally sterilized at temperatures above 121°C for 1 hour. After transplanting the plants were watered daily and treated with foliar fertilizer (NPK 20:20:20) 2 weeks after transplanting. The plants were evaluated in the greenhouse for a period of 6 weeks.

Results and Discussion

Germplasm with valuable target traits via in-vitro and seed introduction were exported in National Root Crops Research Institue (NRCRI) from Centro Internacional de Agricultura Tropicai (CIAT) (Table 1) for genetic improvement and to broaden the narrow genetic base of cassava in Africa being a secondary Centre of diversity. The number of seed germination recorded in this study was low when compared with the number of plantlets that was transplanted and survived in the field. (Tables 2 and 3). The low germination count observed in the germplasm via seed introduction might be attributed to the age of the seed which could result to loss of viability and eventually loss of genotype. The only high numbers of surviving genotypes from seed were the introgressed CW and SM series, although the multiplication process was slow. Demand for quality plants are rapidly increasing worldwide. Propagation of cassava by seeds requires large quantity of seeds. This is very expensive and lengthens the breeding cycle of cassava because the first year will be used to generate planting materials before proper evaluation. In-vitro plant propagation facilitates the introduction and dissemination of plants across quarantine zones, both domestic and international. It has aided in the development of genetic resources and with this, countries have been able to share and exchange germplasm without introducing diseases.

Conclusion

The study presented an update on *in-vitro* plant propagation as an effective tool for germplasm introduction and exchange for cassava improvement from other countries to Nigeria. Results show that majority of the seeds had little or no germination probably because of the age of the seed. There is therefore need for in-vitro plant propagation which is more efficient and effective in terms of dissemination and facilitation of plants across quarantine zones.

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Table 1: Germplasm introduced, mode of introduction and traits

S/No	Family code name	Mode of introduction	Traits
1	CW-	Seeds	Gene mining
2	OW-	Seeds	Gene minning
3	SM-	Seeds	Gene mining
4	AR-	In-vitro	Resistant to green mite and cmd
5	CPCR-	In-vitro	High protein content
6	CPDCR-	In-vitro	Tolerant to ppd
7	CTSCR-	In-vitro	Tolerant to drought
8	CTS-	In-vitro	Tolerant to drought
9	SITS-	In-vitro	Tolerant to drought
10	CR-	In-vitro	Resistant to cmd

Table 2: Seed germination of selected lines showing the top, intermediate and the least

S/No	Family code name	Numb. of seeds planted	Numb. germinated	% germ
1	CW-	1005	578	57.5
2	OW-	1740	30	0.02
3	SM-	63	39	61.9
5	AM-	5	0	0
6	CM-	33	7	21.21
7	GM-	14	2	14.28

Table 3: *In-vitro* survival on transplant

S/No	Family code name	Numb transplanted	Surv. on the field	% survival
1	CPDCR-	288	253	87.84
2	SITS-	33	31	93.93
3	CTSCR	70	68	97.14
4	CR-	60	52	86.66
5	CTS-	84	77	91.60