

Full Length Research Paper

# Embryo rescue as a method to develop and multiply a backcross population of cassava (*Manihot esculenta* Crantz) from an interspecific cross of *Manihot esculenta* ssp. *flabellifolia*

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Cassava is one of the most planted tuber crops in the tropical world. The importance of cassava is growing as a food security crop in sub Saharan Africa, where malnutrition is a menace. However, a major hindrance in the fast improvement of the crop is the long generational cycle of cassava and poor germination of the seeds, and the low multiplication rate of the stem cuttings. *In vitro* germination of 495 seeds from backcross population was done. Each genotype was multiplied for sufficient planting material, hardened in the green house and transplanted to the field. Percentage germination of the seeds in embryo culture was high (66%). Raising plantlets in the green house was found to be useful to select healthy plants and thus obtain a uniform stand in the field. The genotypes were planted in a single row trial and harvested eight months after planting. Transplanted plantlets gave 98.89% establishment. Yield-related traits were significantly high compare to the result from the past experiments. The high percentage of plant recovery from seed through to the field is a means of overcoming some problems associated with the traditional method of cassava breeding through direct seed planting to generate planting materials.

**Key words:** Cassava, backcross population, interspecific, flabellifolia, embryonic axes.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz), a member of the family Euphorbiaceae, is a major source of calories and is rapidly becoming a critical factor in economic development in tropical lowland, and to some extent in tropical highland areas (Fregene et al., 1999). Cassava is the most important tropical root and tuber crop. The entire global production of cassava originates from the developing countries. About 70 million people obtain more than 500 cal per day from cassava roots. The crop is fundamental for food security of the poor communities (Cock, 1985; Kawano et al., 1998). As demand for cassava root quality improvement is intensified, cassava

breeders need to overcome the problem of low seed set, poor germination rates and long growth cycles, which still cause bottlenecks in the population development of cassava (Jennings, 1963; Bryne, 1984). In different clones, various degrees of sterility are common and low seed yield per pollination and seed sterility have frequently been reported as another major problem in cassava cross breeding (Hahn et al., 1979; Kawano, 1980).

*M. esculenta* ssp. *flabellifolia* occupies an important place within the Euphorbiaceae family with the high protein content found in its roots (CIAT, 2002; Ojulong et al., 2008). In a research project directed at increasing protein content in the root, tolerance to diseases and pests, attention has been concentrated on the utilisation of genetic resources of wild species through interspecific hybridisation (Fregene and Morante, 2002). Various types of barriers of crossability and obtaining viable

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hybrid seeds cause a bottleneck.

Improvement of germination rate holds the greatest promise for resolving some of the compelling problems of cassava population development. Germination of cassava seeds has been enhanced by various procedures including sacharification, treatment by heat and/or acid, exposing seeds to red light and more recently by embryo culture of mature and immature seeds (Nartey et al., 1974; Kawano et al., 1978; Biggs et al., 1986; Roca et al., 1988; Ng, 1989; Fregene et al., 1999). Embryo culture provides a simple technique for breaking seed dormancy and ensuring a fairly uniform germination rate (Biggs et al., 1986).

Raising cassava plantlets in a green house to harden them was found to be useful to select healthy plants and obtain a uniform stand and high establishment rate in the field (Akinbo 2008). The objective of this study was to use embryo rescue to generate sufficient planting material and reduce the time it will take for replicating trials of the protein multi-location and mapping population experiment.

## MATERIALS AND METHODS

### Source of seeds

Selected F<sub>1</sub> progeny (the selection criteria used was the percentage protein mean and standard deviation of the F<sub>1</sub> family and individual hybrids as discussed in Akinbo (2008) were re-evaluated for protein content. Individuals having a high protein content from families with low standard deviation and high average protein content were selected and used as parents for backcrossing to generate the seeds.

Parents for crossing were planted in the crossing block at CIAT, Palmira. Entries were planted in single rows of 1 m between plants and 2 m between rows, to facilitate movement. Genotypes were monitored for start of flowering. At the onset of flowering, daily visits to the plots were made. Each morning, plants were inspected for flowers about to open, and promising flowers enclosed with transparent bags, to prevent contact with stray pollen on opening. Pollen was collected in plastic bottles (perforated), from MTAI 8 male parents. At around 11.00 am when flowers open, the transparent bags were removed, and pollen from the MTAI 8 parent dusted on the stigma. All non-mature flowers were removed from the flower batch. The flower batch was tagged with a label containing the pedigree, number of female flowers pollinated, and date of pollination. The bag was removed to allow the fruit to develop freely. Four weeks after pollination, fruits were covered with netting bags to collect the fruits that explode at maturity (Jennings and Iglesias, 2002). Seed was collected from the field after 60 days. They were cleaned, and viable seeds identified and germinated *in vitro*.

### Excision and culture of embryonic axes

Seeds from the backcross (671) were tested for viability by soaking in water. After the viability test, embryos excised from the 328 viable seeds were cultured *in vitro* using a 17N culture medium. The culture medium was supplemented with 0.01 mg l<sup>-1</sup> 1-naphthaleneacetic acid (NAA), 0.01 mg l<sup>-1</sup> GA3, 1.0 mg l<sup>-1</sup> thiamine-HCl, 100 mg l<sup>-1</sup> inositol, 2% sucrose, 0.7% agar (Sigma Co.) and 25

mg l<sup>-1</sup> of a commercial fertilizer containing NPK (10:52:10). The medium had a pH of 5.7-5.8 (Roca, 1984).

Embryo culture was done at the tissue culture laboratory of cassava genetics of International Centre for Tropical Agriculture (CIAT), Cali, Colombia in January 2005 as follows: mature seeds were treated with concentrated sulphuric acid for 50 min, then washed thoroughly and rinsed with water, before soaking in water for 30 min. Seeds were surface-sterilized by immersion in 70% alcohol for 5 min followed by immersion in 5 % sodium hypochlorite and tween for 20 min, then rinsed three times with sterilized water. Under aseptic conditions, the seeds were split along the longitudinal axis and embryos removed by means of sterile forceps and scalpel. Excised embryos were placed radicle down in the prepared 17 N medium. The embryo cultures were then incubated in darkness for three days to promote radicle growth and then transferred to growth chambers with a 12 h photoperiod. Plantlets remained in the growth chamber for six weeks before they were transferred to the green house for intensive post flask management.

### Seedling growth in the greenhouse

For the post-flask management, plants were transferred from test tubes to polythene bags under protection from direct sun and insects. Optimal conditions were achieved using 7 to 10 cm polythene bags containing a sterilized substratum mixture of three parts soil with one part of fine sand. In the green house, to reduce the shock, the plants were covered with polythene bag for seven days with regular watering and addition of micro and macro-nutrient at intervals. Spraying of the leaves against fungi was also done intermittently for four weeks. After this time, the plants were hardened and then transplanted in the field in Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Palmira in November 2005 and March 2006.

### Field evaluation

The backcross family (B<sub>1</sub>P<sub>2</sub>) was planted twice to guarantee material from those genotypes that may not survive the hardening and field transplanting in November 2005. This first round of planting was to generate the stakes for the first replicated trials. The field layout was 1.6m x 0.8m between and within rows for the first single row trial (SRT).

The plantlets were arranged genotype by genotype on the field. Plantlets were soaked in "Terravite" (fertilizer in solution) to increase recovery after hardening. In both fields, regular irrigation was done during the first three weeks, after which rainfall served as source of water supply. Hand weeding was done around each plantlet and herbicide was applied to control weeds. Immediately after planting, both foliar and soil nitrogen fertiliser was applied around plants to boost them after hardening. The CIAT system was adopted to name the new genotypes from the tissue culture, with the first plantlet assuming number one and the rest subsequent numbers.

### Data collection

Before harvesting, there was flooding in the first field which made data recording impossible from that location. In the second field, each genotype was harvested; the harvestable biomass was divided into storage roots, and vegetative biomass, comprising leaves and stems. Roots were weighed to obtain fresh root yield. Roots which pass for sale in the local supermarkets were selected and counted to give number of commercial roots. Whitefly infestation was scored on a scale of 1 to 5 (when the plant is clean, it is scored 1 and when it is heavily infested, it is scored 5). Harvest

**Table 1a.** Seed generated and resulting plantlets from the B<sub>1</sub>P<sub>2</sub> family.

Cross	Pedigree	Seed generated	Viable seeds	<i>In vitro</i> plants	Percentage germination
B <sub>1</sub> P <sub>2</sub>	CW 198 – 11 X MTAI 8	671	495	328	66

**Table 1b.** Resulting plantlets from the *in vitro* to the field phase.

Cross	Pedigree	<i>In vitro</i> plantlets	Plantlets in the field	% survival
B <sub>1</sub> P <sub>2</sub>	CW 198 – 11 X MTAI 8	2117	1811	85.55

**Table 1c.** Establishment of the plantlets in the field.

Cross	Pedigree	Plantlets in the field	Plants at harvest	Percentage establishment
B <sub>1</sub> P <sub>2</sub>	CW 198 – 11 X MTAI 8	1811	1791	98.89

index was calculated by dividing fresh root yield by total biomass. Percentage DMC (dry matter content) of the roots was estimated using the standard CIAT procedure (Kawano et al., 1987; Jaramillo et al., 2005).

$$\%DMC = 158.3 \times [W_a / (W_a - W_w)] - 142$$

Dry yield was derived as a product of fresh root yield and DMC. Simple statistics were performed using the Excel programme (Nelson, 2000; Microsoft, 2003; Cach et al., 2006). Data obtained was subjected to simple analysis (SAS, 2002).

## RESULTS

A total of 671 seeds from the crosses between CW 198–11 and MTAI 8 were produced, of which 495 (73.77%) passed the water floating test. Embryonic axes of these seeds were excised and cultured. Three hundred and twenty-eight embryos (66%) germinated after four days. The B<sub>1</sub>P<sub>2</sub> account of percent germination of embryonic axes from the seeds is provided in Table 1a.

Due to heterozygosity of cassava, each seed is a different genotype. Depending upon the development of cultures, at least one single node cutting was obtained from each shoot, providing a multiplication rate of 1:4 after four weeks of culture. Growth and development rate of the plantlets were high (85.55%); the rate of establishment on the field was equally high (98.89%) which resulted in vigorous plants that produced sufficient material for replicated planting. The percentage survival and establishment is provided in Table 1b - c.

Table 2 describes the simple statistics of agronomic variables evaluated in B<sub>1</sub>P<sub>2</sub> at harvest in CORPOICA, 2007. The highest yield was recorded in B<sub>1</sub>P<sub>2</sub>–11 (25.00 tha<sup>-1</sup>) followed by B<sub>1</sub>P<sub>2</sub>–189 (24.21 tha<sup>-1</sup>), while highest dry root yield was recorded from B<sub>1</sub>P<sub>2</sub>–189 (7.82 tha<sup>-1</sup>).

Harvest index estimates ranged from 0.017 (B<sub>1</sub>P<sub>2</sub>–89) to 0.875 (B<sub>1</sub>P<sub>2</sub>–220). Dry matter content (%) ranged from 17.22 in B<sub>1</sub>P<sub>2</sub>–292 to 70.28 in B<sub>1</sub>P<sub>2</sub>–317.

The highest root weight was recorded for B<sub>1</sub>P<sub>2</sub>–11 (22.4kg). The average commercial root number was 4.5 with genotype B<sub>1</sub>P<sub>2</sub>–189 having the highest number of nine roots. There were a relatively high number of roots per plant, on average 3.90, with genotype B<sub>1</sub>P<sub>2</sub>–289 having the highest number of 10.

Table 3 showed that commercial roots were significantly correlated ( $P \leq 0.0001$ ) with root weight, roots per plant, fresh root yield, and dry root yield but it had no association with harvest index. Root weight was significantly correlated ( $P \leq 0.0001$ ) with fresh root yield and dry root yield. Root per plant was highly correlated ( $P \leq 0.0001$ ) with harvest index, fresh root yield, dry root yield but not significantly correlated with dry matter content. Harvest index was significantly correlated ( $P \leq 0.0001$ ) with fresh root yield and dry root yield. Fresh root yield was significantly ( $P \leq 0.0001$ ) correlated with dry root yield and dry root yield.

## DISCUSSION

The multiplication of interspecific cassava hybrids through embryo axes can be considered a very productive method of reducing multiplication time, reducing seed dormancy, increasing viability, and reducing the breeding and selection cycle time, which is a major bottleneck in cassava breeding. Selection for uniform, healthy plants to be transferred from the *in vitro* phase to the field is possible at this stage, to give good planting material for replicated trials.

The rate of establishment per genotype is usually high. For cultivated cassava, Fregene et al. (1999) demonstrated that, of 47 seeds germinated from mature fruits by culture, 91% germination was recorded after two days from improved cultivars, but for interspecific hybrids recording a 66% germination rate is a huge success considering the level of seed inhibition from seed germination. In addition, Fregene et al. (1999) demonstrated

**Table 2.** Simple statistics of agronomic variables evaluated in the BC<sub>1</sub> in CORPOICA, Palmira March, 2007.

Variables	Minimum	Maximum	Average	Standard deviation
ComRt <sup>a</sup>	0.00	4.50	0.909	0.824
Rtw <sup>b</sup>	0.04	0.62	0.212	0.095
Rtplt <sup>c</sup>	0.60	10.00	3.900	1.989
HI <sup>d</sup>	0.07	0.87	0.303	0.140
FRY <sup>e</sup>	0.39	25.00	6.707	4.892
DMC <sup>f</sup>	17.22	70.28	27.066	6.138
DRY <sup>g</sup>	0.07	7.82	1.840	1.440

<sup>a</sup>Commercial roots; <sup>b</sup>Root weight (kg); <sup>c</sup>Root per plant; <sup>d</sup>Harvest index; <sup>e</sup>Fresh root yield (tha<sup>-1</sup>); <sup>f</sup>Dry matter content (%); <sup>g</sup>Dry root yield (tha<sup>-1</sup>)

**Table 3.** Simple phenotypic correlations of yield related traits and pests evaluated on BC<sub>1</sub> population in 2007 at Corpoica, Palmira, Colombia.

	Whfly <sup>a</sup>	ComRt	Rtw <sup>c</sup>	Rtplt	HI	FRY	DMC
ComRt <sup>b</sup>	-0.0419						
Rtw <sup>c</sup>	0.0006	0.63**					
Rtplt <sup>d</sup>	-0.0522	0.66**	0.15ns				
HI <sup>e</sup>	-0.0506	0.34ns	0.26ns	0.52**			
FRY <sup>f</sup>	-0.0620	0.87**	0.67**	0.77**	0.49**		
DMC <sup>g</sup>	-0.1744	0.23ns	0.17ns	0.28ns	0.13ns	0.28ns	
DRY <sup>h</sup>	-0.0859	0.85**	0.65**	0.76**	0.46**	0.98**	0.40**

Whitefly (1-5); <sup>b</sup>Commercial root; <sup>c</sup>Root weight (kg); <sup>d</sup>Root per plant; <sup>e</sup>Harvest index; <sup>f</sup>Fresh root yield (tha<sup>-1</sup>); <sup>g</sup>Dry matter content (%); <sup>h</sup>Dry root yield (tha<sup>-1</sup>), \*\* P ≤ 0.01.

that depending upon the development of cultures, at least one single node cutting was obtained from each shoot, providing a multiplication rate of 1:3 after four weeks which agreed with our finding of 1:4 ratio after four weeks.

The range of dry matter content recorded in this evaluation was higher (70.0%) than that reported by Ojulong (2006), of 69.1%, Rajendran and Hrishi (1982), of 66.4% and Magoon et al. (1973), of 47.2%. There was no association between fresh root yield and dry matter content, which Kawano et al. (1998) also observed at earlier stages of selection. They came to the conclusion that fresh root yield and dry matter content can be handled largely as independent characters. This lack of association between fresh root yield and dry matter content is in agreement with findings reported by Ojulong (2006). The usefulness of embryo culture, notably the rescue of inter-specific hybrids by culture of immature embryos (Raghavan, 1985; Mejia-Jimenez et al., 1994) and mature embryos (Fregene et al., 1999) has been emphasized. It has been established from this study that this can be extended to the establishment of cassava populations, where low germination rate, that prevents rapid establishment of a population trial, is a bottleneck in cassava, can be overcome.

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