

***In vitro* Micropropagation of Cassava Through Low Cost Tissue Culture**

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Abstract: Adoption of drought-tolerant crops such as cassava can help alleviate food insecurity in sub-Saharan Africa. However, production is constrained by lack of disease-free planting materials. This can be circumvented through tissue culture but the technology is costly limiting its adoption. There is therefore, need to put in place interventions that will reduce the cost of production hence making tissue culture products affordable. In this research, a low cost protocol for cassava tissue culture was developed and used to regenerate two farmer-preferred cassava varieties, KME 1 and Muchericheri. Easygro[®] vegetative fertilizer, a locally available foliar feed was used as an alternative source for conventional MS salts. Nodal explants were initiated on a low cost medium containing 2 g/L of Easygro[®] vegetative fertilizer supplemented with 30 g/L of table sugar and 9 g/L of agar and conventional medium containing MS salts supplemented with 30 g/L of sucrose and 9 g/L of agar. The conventional MS medium was used as the control. The number of leaves, nodes, roots and average plant heights for the resultant plantlets were determined and compared. The variety Muchericheri had a significantly higher regeneration index compared to KME 1 having produced a mean of 6.8 nodes on the low cost medium and 5.6 nodes on the conventional medium compared to KME 1 which had a mean of 5.6 nodes on the low cost medium and 4.5 nodes on the conventional medium. This is an indication that Muchericheri had a better regeneration efficiency compared to KME 1.

Key words: Foliar feed, low cost medium, nodal explants, regeneration efficiency

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a major staple crop for millions of people in East and Central Africa, mostly in the rural areas and it is the second most important staple crop in Africa after maize. Because of its importance in food security and poverty alleviation, cassava has been prioritized by the New Partnership for Africa's Development (NEPAD) as a 'poverty fighter' which will spur industrial development in Africa (Whingwiri, 2004). Acedo and Labana (2008) reported that the demand for cassava has been on the rise in the recent past because of its potential use in biofuel industry. The importance of cassava has triggered concerted efforts in breeding programs to develop better varieties. Genetic modification techniques are now widely employed in cassava improvement. According to Taylor *et al.* (2004) transgenic technologies have allowed transfer of important genes from one cassava cultivar to another and from wild relatives to domesticated cassava. In spite of

the strides made in cassava improvement, farmers continue to face shortage of quality planting materials.

Tissue Culture (TC) technology can be used to produce high quality seedlings instead of the traditionally used cuttings. It has a high fecundity, producing thousands of propagules unlike conventional techniques. Plant tissue culture technology has been extensively used to propagate cassava all over the world. This is because it has been found to be the best method of producing cassava planting materials. There is absence of flowering in some cassava genotypes and low production of seeds often associated with irregular germination (Mussio *et al.*, 1998). Therefore, cassava is multiplied mainly by stem cuttings which is a slow process compared to grain crops (Santana *et al.*, 2009). Diseases also often accumulate in the stem cuttings resulting in infected plants and low yields. Small-scale farmers acquire planting materials from neighbours, during travel or as volunteer plants left in fallow (Mutegi, 2009). This contributes to pest and disease accumulation and dissemination. Other challenges

with the cuttings include high perishability since they dry up within a few days, high handling and transport costs and inconvenient weight and bulk of the material (Escobar *et al.*, 2006). This makes tissue culture an important technology in setting up cassava seed systems. Tissue culture has been effectively applied in elimination of viruses and other systemic diseases from cassava vegetative materials allowing exchange and conservation of rejuvenated propagation materials with higher yields (Jorge *et al.*, 2000). However, the cost of *in vitro* plant production is an obstacle for its access to farmers. Thus, efforts to develop low-cost technologies are necessary (Thro *et al.*, 1999). This research sought to establish a cost-efficient protocol for cassava micropropagation using locally available materials.

MATERIALS AND METHODS

Plant material: Two characterized cassava varieties, KME 1 and Muchericheri were obtained from the Kenya Agricultural Research Institute (KARI) and maintained in a shade net at Kenyatta University.

Media preparation: The low cost medium developed contained 2 g/L of Easygro® vegetative fertilizer (27:10:16+Trace elements) supplemented with 30 g/L of table sugar and 9 g/L of agar. Easygro® vegetative fertilizer was used as an alternative source for MS basal salts. The conventional MS salts (Murashige and Skoog, 1962) supplemented with 30 g/L of sucrose and 3 g/L of gelrite was used as the control.

Preparation of explants: Healthy and vigorous plants were collected, leaves excised and the stems cut into about 10 cm long. They were washed thoroughly in running tap water for ten minutes to remove soil debris. The stem sections were then cut at the internodes to produce nodal cuttings of about 2-3 cm and transferred into a laminar hood. The cassava nodal cuttings were sterilized with 70% ethanol for 2 min followed by % Jik® (commercial bleach) 15 min. They were then washed 4 times in sterile distilled water.

Culturing of explants: Using a sharp sterile scalpel a slant cut was made on the damaged parts of each explant. Each explant was then carefully cultured. The cultures were incubated in the growth room at 28°C under an illumination of 2000 lux white light and a photoperiod of 16 h light and 8 h darkness. There were 9 replicates for each variety for both media. The numbers of nodes, leaves, roots and plant height were recorded at 7 days interval for 5 weeks. The experiment was repeated twice.

Experimental design and data analysis: Completely randomized block design with two treatments replicated

Table 1: Cost analysis of alternative nutrient sources for the low cost medium in comparison with the conventional MS medium nutrient source

Conventional TC nutrient	Low cost substitute	Cost in 1 L of the medium (Kshs.)		Cost reduction (%)
		Conventional	Low cost	
Macronutrient				
CaCl ₂	Easygro	1.6		
KH ₂ PO ₄	vegetative	0.6		
KNO ₃	fertilizer	6.8		
MgSO ₄		1.0		
NH ₄ NO ₃		10		
Sub-total		20		
Micronutrients				
CoCl ₂ ·6H ₂ O		0.0052		
CuSO ₄ ·5H ₂ O		0.0045		
Na ₂ EDTA		0.1540	1.6	
FeSO ₄ ·7H ₂ O		0.0780		
H ₃ BO ₃		0.1020		
KI		0.0700		
MnSO ₄ ·4H ₂ O		0.0920		
Na ₂ MoO ₄ ·2H ₂ O		0.0078		
ZnSO ₄ ·7H ₂ O		0.0160		
Sub-total		0.5295		
Total		20.5295	1.6	92.2
Carbon source				
Sucrose	Table sugar	105	3.0	97.1
Total		125.5295	4.6	96.3

nine times was used. Data collected was subjected to Analysis of Variance (ANOVA) using STATA® statistical computer program version 11. Means were separated using Tukey's test at 5% level.

RESULTS

Cost efficiency: The use of Easygro vegetative fertilizer as an alternative source of both macronutrients and micronutrients in the Low Cost Medium (LCM) reduced the cost by 92.2% (Table 1). The use of table sugar as an alternative source for carbon reduced the cost by 97.1%. This led to savings of 96.3% in the cost of nutrients and carbon source used in preparing a litre of the medium.

Number of nodes: There were significant differences ($p < 0.05$) in the number of nodes produced by the two cassava varieties on both the Low Cost Medium (LCM) and the Conventional Medium (CM). The highest number of nodes was recorded on the low cost medium for both varieties (Table 2). A significant difference ($p < 0.05$) in the number of nodes produced by the two cassava varieties was recorded with nodal production in the variety KME 1 being lower than that of Muchericheri.

Number of leaves: During initiation, a significantly high number of leaves were produced in the conventional medium as compared to the low cost medium. However, this changed during the first and second subcultures with more leaves being produced on the low cost medium as

Table 2: Mean numbers of nodes for cassava varieties KME 1 and Muchericheri during initiation and two subsequent subcultures (Mean number of nodes ^a)

Medium	KME 1				Muchericheri			
	Initiation	1 st subculture	2 nd subculture	Mean	Initiation	1 st subculture	2 nd subculture	Mean
LCM	5.6±0.400	5.9±0.26	15.4±0.324	5.6±0.145	7.0±0.316	6.9±0.295	6.6±0.369	6.8±0.120
CM	4.6±0.528	4.6±0.419	4.3±0.366	4.5±0.100	6.2±0.477	5.6±0.419	4.9±0.549	5.6±0.418

^a: Values are expressed as means±standard error of the mean

Table 3: Mean numbers of leaves for cassava varieties KME 1 and Muchericheri during initiation and two subsequent subcultures (Mean number of leaves ^a)

Medium	KME 1				Muchericheri			
	Initiation	1 st subculture	2 nd subculture	Mean	Initiation	1 st subculture	2 nd subculture	Mean
LCM	4.8±0.800	6.1±0.459	6.0±0.378	5.6±0.418	5.6±0.400	7.4±0.461	7.4±0.481	6.8±0.600
CM	5.1±0.553	5.3±0.453	4.9±0.295	5.1±0.115	7.3±0.333	6.4±0.498	5.8±0.366	6.5±0.436

^a: Values are expressed as means±standard error of the mean

Table 4: Mean numbers of roots for cassava varieties KME 1 and Muchericheri during initiation and two subsequent subcultures (Mean number of roots ^a)

Medium	KME 1				Muchericheri			
	Initiation	1 st subculture	2 nd subculture	Mean	Initiation	1 st subculture	2 nd subculture	Mean
LCM	4.0±0.316	3.6±0.297	3.8±0.250	3.8±0.115	4.8±0.200	4.1±0.351	3.7±0.286	4.2±0.321
CM	3.6±0.528	3.4±0.324	2.8±0.250	3.3±0.240	4.8±0.167	4.5±0.189	3.8±0.366	4.4±0.296

^a: Values are expressed as means±standard error of the mean

Table 5: Mean plant heights for cassava varieties KME 1 and Muchericheri during initiation and two subsequent subcultures (Mean plant height ^a)

Medium	KME 1				Muchericheri			
	Initiation	1 st subculture	2 nd subculture	Mean	Initiation	1 st subculture	2 nd subculture	Mean
LCM	4.7±0.403	4.6±0.462	3.7±0.286	4.3±0.318	5.3±0.244	4.9±0.295	5.3±0.286	5.2±0.133
CM	3.2±0.468	3.7±0.381	3.8±0.256	3.6±0.186	4.8±0.425	4.9±0.349	4.8±0.313	4.8±0.033

^a: Values are expressed as means±standard error of the mean

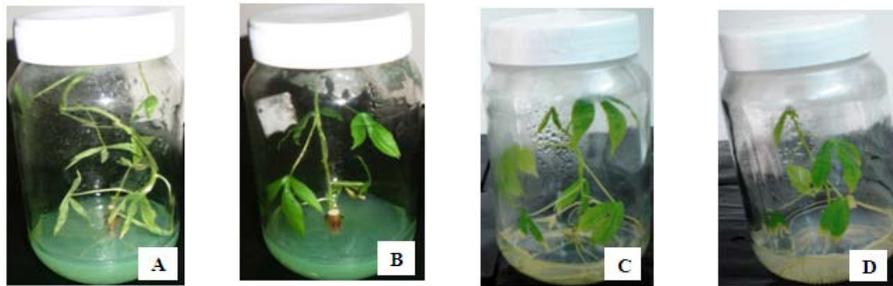


Fig. 1: Regeneration of cassava varieties, Muchericheri (A) and KME 1 (B) in the low cost medium and the conventional medium (Muchericheri (C), KME 1 (D))

compared to the conventional medium. There was a significant difference in the number of leaves produced between the two varieties with Muchericheri having more leaves compared to KME 1 in both media (Table 3).

Number of roots: Plants cultured on the low cost medium developed a significantly high number of roots compared to those cultured on the conventional medium for the variety KME 1. Muchericheri had the same mean number of roots on both media during initiation. This variety produced more roots on the conventional medium compared to the low cost medium during the first subculture. In the second subculture, no significant

difference was recorded in the number of roots produced by Muchericheri in both media. There was a significant difference ($p < 0.05$) in the number of roots produced by the two varieties with Muchericheri having higher mean number of roots compared to KME 1 in all the two media (Table 4).

Plant height: Plants cultured on the low cost medium were significantly taller compared to those cultured on the conventional medium for both varieties during initiation (Fig. 1). In the first subculture, KME 1 had significantly taller plants on the low cost medium compared to the conventional medium while in the second subculture the

plantlets were not significantly different in height. For the variety Muchericheri, plantlets had the same mean plant height in both media during the first subculture but in the second subculture significantly taller plants were produced on the low cost medium. There were significant differences between plantlets of the two varieties with Muchericheri producing taller plants (Table 5).

DISCUSSION

Tissue culture is indispensable as a tool for production of disease-free planting material and germplasm conservation especially in vegetative crops. Tissue culture technology offers an alternative for enhanced rates of cassava multiplication. The technology is, however, costly hence the low adoption rates in developing countries. The design and adoption of cost-efficient TC protocols is therefore paramount in the adoption of this technology.

This study has shown that it is possible to use locally available salts as low cost sources of tissue culture nutrients. This is in agreement with study done by Santana *et al.* (2009) who used different concentrations of a locally available fertilizer to micropropagate cassava. Escobar *et al.* (2006) also tried different kinds of fertilizers at different concentrations and realized a cost reduction of 24.4% for the medium prepared. Micropropagation by nodal cuttings used here promotes the development of a pre-existent morphological structure and the nutritional condition of the medium breaks the dormancy of the axillary bud promoting its rapid development (Rolando *et al.*, 1992).

The two cassava varieties evaluated during this study responded positively to the low cost medium. However, Muchericheri had a better regeneration efficiency compared to KME 1 producing significantly high number of nodes, leaves, roots and taller plantlets compared to KME 1. This may be due to genetic variation between the two cassava varieties. The cassava regeneration index observed in this study was 4-7 nodes per plantlet. This is comparable to previous findings by Santana *et al.* (2009) in which a regeneration index of 3-7 nodes per cycle was achieved.

The number of nodes per plantlet is of prime importance since these are regions for shoot development (Mutegi, 2009). *In vitro* multiplication of cassava by direct organogenesis is through nodal cuttings hence the higher the number of nodes the higher the number of plantlets. The branching habit of cassava is a stable morphological trait that has been shown to be of adaptive, agronomic and market importance (Gulick *et al.*, 1983). Cassava forms one or more axillary buds on the stem upon sprouting. These buds develop and sequentially form nodal units consisting of a node, a bud, a palmate leaf blade subtended by a long petiole and an inter-node whose length and mass depends on genotype, age of the

plant and environment (El-Sharkawy, 2003). Therefore a good culture medium should be able to supply enough nutrients for maximum node formation.

The mean number of leaves during initiation and the two subsequent subcultures was significantly high in the developed low cost medium compared to the conventional MS medium making this medium suitable for cassava tissue culture. This may be due to the fact that easygro[®] vegetative fertilizer used as the alternative source for MS salts also contains amino acids which may have added to the supply of nitrogen in the medium. Leaves are a major site of food production for the plant and a well-developed leaf-system is important in survival of TC plantlets during acclimatization. Plantlets with a high number of well-developed leaves are more efficient photosynthetically and therefore adapt quickly to natural environment as compared to those with smaller and fewer leaves.

Cassava variety Muchericheri produced more roots in both media as compared to KME 1. This may be due to genotypic differences between the two varieties and also indicates that Muchericheri has a better regeneration efficiency compared to KME 1. Roots have an essential role and function in plant life and development, supplying water and nutrients to the plant from the environment (Schiefelbein *et al.*, 1997). Regeneration of roots from nodal explants occurred easily without inclusion of a rooting hormone in the medium. This is consistent with Yona *et al.* (2010) who reported that cassava explants can naturally form roots without addition of auxins.

Muchericheri had taller plants in the two media compared to KME 1. This was a desirable quality during multiplication since plantlets that were tall had higher number of nodes resulting into more plantlets. The ideal TC medium should produce tall plants since more nodal cuttings can be obtained hence a higher multiplication rate.

CONCLUSION

This research has shown that it is possible to reduce the cost of plantlet production during tissue culture. This can be achieved through the use of alternative sources of MS nutrients that are available locally. The low cost medium evaluated here can be adopted easily in the production of cassava planting material. This will greatly enhance availability of cassava planting materials at an affordable cost which will boost its production.

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