

*Full Length Research Paper*

# Cost-effective nutrient sources for tissue culture of cassava (*Manihot esculenta* Crantz)

Ogero K. O.<sup>1\*</sup>, Gitonga N. M.<sup>2</sup>, Mwangi M.<sup>1</sup>, Ombori O.<sup>3</sup> and Ngugi M.<sup>2</sup>

<sup>1</sup>Department of Agricultural Science and Technology, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya.

<sup>2</sup>Department of Agriculture, Meru University College of Science and Technology, P. O. Box, 972- 60200, Meru, Kenya.

<sup>3</sup>Department of Plant and Microbial Sciences, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya.

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**Application of tissue culture technology is constrained by high costs making seedlings unaffordable. The objective of this study was to evaluate the possibility of using locally available fertilizers as alternative nutrient sources for cassava micropropagation. A Low Cost Medium (LCM) whereby the conventional sources of four Murashige and Skoog (MS) macronutrients had been replaced with locally available fertilizers was developed. Stanes Iodized Microfood® from Osho Chemical Industries in Nairobi was used as the alternative source of micronutrients. Modified conventional MS medium was used as the control. Both media were supplemented with 30 g/l of table sugar and 3 g/l of gelrite. Two cassava varieties, Muchercheri and KME 1 were regenerated on the two media. Node, leaf and root formation patterns plus plant height were determined and compared. A reduction of 95.50% in nutrient cost was achieved. The two cassava varieties had a significantly ( $p < 0.05$ ) higher number of nodes on the conventional medium compared to LCM. There were no differences in node formation by the two varieties on the low cost medium during both initiation and multiplication. KME 1 produced significantly more leaves on the LCM compared to Muchercheri during both initiation and multiplication. Acclimatization of plantlets was successful; hence, this protocol can be adopted in cassava regeneration.**

**Key words:** Tissue culture, cassava, nutrient sources, micropropagation, low cost medium, acclimatization.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in the sub-Saharan Africa; hence it requires an efficient and affordable seedling production system. Cassava is a major source of calories to more than 250 million people in the sub-Saharan Africa and 600 million people globally (Obiero et al., 2007). Due to its ability to thrive well in marginal areas, cassava has become appropriate for food security and economic development in unfavored areas of the tropics (Mutegi, 2009). The crop ranks second after maize, as the most important staple crop in Africa (ASARECA, 2008).

In spite of its enormous opportunities in alleviating food insecurity, cassava production per hectare has been on

the decline in the sub-Saharan Africa (Nweke et al., 2002). The little growth noted in cassava production is majorly due to increase in hectareage under the crop, rather than increase in productivity (Hillocks et al., 2002). This decline can be attributed to a number of factors such as cassava mosaic disease (Pheneas and James, 2007). This situation is exacerbated by lack of healthy planting materials. Farmers often use cuttings sourced from old plants in propagation, and this contributes to the spread of diseases. This is often a channel for transmission of systemic infections from one generation to other, leading to poor yields in successive seasons (Roca and Mroginski, 1991). This is also a major limitation in germplasm maintenance and exchange of materials across borders. The multiplication rate of cuttings is also very low compared to grain crops, which are propagated by true seeds. *In vitro* propagation of cassava helps surmount these challenges by availing disease-free

\*Corresponding author. E-mail: [Ogero.ko@gmail.com](mailto:Ogero.ko@gmail.com). Tel: +254720314828. Fax: +254-20-4223600/631599.

planting materials in large numbers. Tissue culture techniques provide a great opportunity to produce healthy seedlings *en masse* (Ajithukumar and Seemi, 1998). This technology is faster and requires less space than that required for conventional methods of producing seedlings (Aladele and Kuta, 2008). However, adoption of tissue culture in many developing countries especially in the sub-Saharan Africa is hampered by high cost of production (Prakash et al., 2004). The process often requires sophisticated equipment, expensive chemicals and highly skilled personnel. Establishing and maintaining a tissue culture laboratory is quite expensive thus limiting its application to large institutions such as universities and a few farmers who have the required financial capacity.

Tissue culture techniques are requisite in the quest to improve cassava productivity in the sub-Saharan Africa and globally. Adoption of the technology in seedling production will improve production levels along with quality improvement. However, to optimize its application in cassava propagation, there is a need to identify and implement strategies that will contribute towards reducing the cost of plantlet production. This will enable resource-challenged farmers to access healthy planting materials at an affordable cost, which will in turn increase cassava production. Cost reduction in cassava tissue culture can be approached in a number of ways. For instance, the expensive and sophisticated equipment can be substituted with cheaper alternatives. In previous studies, the autoclave has been replaced with a pressure cooker, with no detectable contaminations (Gitonga et al., 2010). Another point of intervention is the use of alternative media nutrient sources that are available locally at an affordable cost. This study adopted locally available fertilizers as alternative nutrient sources for cassava tissue culture, with an aim of producing affordable healthy seedlings.

## MATERIALS AND METHODS

### Plants

Two farmer-preferred cassava varieties were used in this work. Cuttings of the two varieties (Muchercheri and KME 1) were obtained from the Kenya Agricultural Research Institute, Embu. The cuttings were grown in polythene pots to establish the mother stock plants at Kenyatta University. This study was carried out for eleven months from September 2010 to July 2011.

### Cost evaluation

The cost of each of the nutrient used to make one litre of medium was calculated based on the price of the amount bought. This was done for both the low cost and the modified conventional media, and differences in the costs were determined.

### Media preparation

Locally available fertilizers were used as alternative sources of

Murashige and Skoog (MS) salts.

The conventional sources of four MS macronutrients (potassium dihydrogen phosphate, ammonium nitrate, magnesium sulphate and potassium nitrate) were substituted with locally available fertilizers (Table 10). Stanes Iodized Microfood<sup>®</sup> (from Osho Chemical Industries, Nairobi) was used as the alternative source of micronutrients. Conventional source of laboratory sucrose was replaced with table sugar. Forty millilitres per litre (40 ml/l) of the macronutrients' stock solution, 0.20 g/l of Stanes Iodized Microfood<sup>®</sup>, 30 g/l of table sugar and 3 g/l of gelrite were used to prepare 1 L of the low cost culture medium. Modified conventional MS salts were supplemented with 30 g/l of table sugar and 3 g/l of gelrite, and this was used as the control. The media was dispensed into culture bottles and sterilized by pressurized steam at a temperature of 121°C and 15 pounds of pressure per square inch for 15 min, using a pressure cooker.

### Explants sterilization, initiation and multiplication

3 cm long nodal cuttings of each variety were obtained from the mother stock plants and washed in running tap water to get rid of the dust. They were surface sterilized in 70% ethanol for 2 min, followed by 1.5% sodium hypochlorite containing a drop of Tween 20<sup>®</sup> for 15 min.

The cuttings were then rinsed four times with sterile distilled water. The nodal cuttings were spliced at the damaged parts and cultured onto the initiation medium. The cultures were transferred into the growth room and incubated at a temperature of 28 ± 2°C and a photoperiod of 16/8 h of light/darkness. The cultures were monitored daily for growth and any contaminations over a period of five weeks. *In vitro* plantlets were subcultured after five weeks to increase the number of plantlets. The number of nodes, leaves, roots and plant heights were determined and recorded after five weeks of culture both during initiation and multiplication, and comparisons were made between the two media and the two varieties.

### Acclimatization of plantlets *ex vitro*

Acclimatization of regenerated plantlets was carried out using sterilized vermiculite, vermiculite mixed with red soil in the ratio 1:1 and red soil mixed with rice husks in the ratio 2:1. The three acclimatization media were dispensed into 20 L buckets up to the quarter-mark level.

The plantlets were then transplanted onto these media and the buckets were covered with transparent polythene sheets to retain moisture and to protect the plantlets from desiccation. Small holes were made on the polythene sheets after every four days to adapt the plants to the natural environment. After three weeks, the polythene sheets were completely removed and plantlets transplanted onto the soil contained in polythene bags, then put in a chamber made with transparent polythene sheet. The plantlets were sprayed with water regularly to avoid desiccation. Survival rate of plantlets on the three acclimatization media was determined by counting the number of plants that were alive on each medium after 21 days.

### Experimental design and data analysis

A completely randomized design with two treatments and nine replicates for each variety was used. Any difference between plantlets cultured on the two media was ascertained. Data was analyzed using analysis of variance with STATA<sup>®</sup> version 11. Tukey's test at 5% confidence level was used to separate the means.

**Table 1.** Comparisons of the costs of the developed low cost medium and the conventional MS medium.

Modified conventional MS medium (CM)	Low cost substitute (LCM)	Cost in 1 L of the medium (KShs.)		Cost reduction (%)
		Conventional	Low cost	
<b>Macronutrients</b>				
CaCl <sub>2</sub>	-	1.60	-	0
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium phosphate (MKP)	0.60	0.04	93.20
KNO <sub>3</sub>	Potassium fertilizer	6.80	0.34	94.90
MgSO <sub>4</sub>	Epsom salt	1	0.07	93.00
NH <sub>4</sub> NO <sub>3</sub>	Ammonium quarry salt	10	0.38	96.20
Sub-total		20	2.43	87.80
<b>Micronutrients</b>				
CoCl <sub>2</sub> .6H <sub>2</sub> O			0.005	
CuSO <sub>4</sub> .5H <sub>2</sub> O			0.005	
Na <sub>2</sub> EDTA			0.15	
FeSO <sub>4</sub> .7H <sub>2</sub> O			0.08	
H <sub>3</sub> BO <sub>3</sub>	Stanes iodized microfood <sup>®</sup>	0.10	0.24	
KI			0.07	
MnSO <sub>4</sub> .4H <sub>2</sub> O			0.09	
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O			0.008	
ZnSO <sub>4</sub> .7H <sub>2</sub> O			0.02	
Sub-total		0.538	0.24	28.90
<b>Carbon source</b>				
Sucrose	Table sugar	105	3	97.10
Total		125.538	5.67	95.50

## RESULTS

### Cost analysis

A significant reduction in the cost of plantlet production was achieved. Cost savings of 87.80% was recorded in the cost of macronutrients while 28.9% cost reduction was recorded in the cost of micronutrients when alternative sources of nutrients were used. In overall, the use of low cost alternative nutrient sources led to 95.50% cost reduction per litre of medium (Table 1).

### Initiation

Regeneration of plantlets of the two cassava varieties was successful on both the modified conventional medium (CM) and the low cost medium (LCM) (Figures 1 and 2).

#### ***Effect of the type of the media on the number of nodes formed during initiation***

The two cassava varieties produced significantly ( $p < 0.05$ ) higher number of nodes on modified conventional

medium compared to the low cost medium. Significant ( $p > 0.05$ ) differences were not detected on the number of nodes produced by the two varieties on the low cost medium. The variety KME 1 had a mean of 3.70 nodes per plantlet while Muchericheri had a mean of 3.50 nodes per plantlet on the low cost medium (Table 2). Muchericheri produced significantly ( $p < 0.05$ ) higher number of nodes on the modified conventional medium, with an average of 6.20 nodes per plantlet compared to KME 1 which had an average of 4.60 nodes per plantlet.

#### ***Effect of the type of the media on the number of leaves formed during initiation***

Leaf formation was better on the modified conventional medium (CM) compared to the low cost medium (LCM), with both varieties producing significantly ( $p < 0.05$ ) higher number of leaves on CM compared to LCM. KME 1 produced significantly higher number of leaves on the low cost medium with a mean of 3.40 leaves per plantlet compared to Muchericheri which had an average of 2.70 leaves per plantlet (Table 3). The variety Muchericheri had a mean of 7.30 leaves per plantlet on the modified conventional medium. This was significantly ( $p < 0.05$ ) higher compared to KME 1 which had a mean of 5.10



**Figure 1.** Plantlets of cassava varieties Muchericheri (A) and KME 1 (B) regenerated on the low cost medium after five weeks of culture.



**Figure 2.** Plantlets of cassava varieties Muchericheri (A) and KME 1 (B) regenerated on the conventional medium after five weeks of culture.

leaves per plantlet.

#### ***Effect of the type of the media on the number of roots formed during initiation***

Rooting occurred without incorporation of any growth hormone in the media. KME 1 variety had an average of 3.30 and 3.60 roots per plantlet on the low cost and

conventional media, respectively (Table 4). Muchericheri produced a mean of 3.20 roots per plantlet on the low cost medium and 4.80 roots per plantlet on the modified conventional medium. The number of roots produced was significantly higher on the CM compared to the LCM for both varieties. Significant ( $p > 0.05$ ) differences were not detected on the number of roots produced between the two varieties on the low cost medium. Muchericheri variety produced a significantly higher number of roots on

**Table 2.** Mean number of nodes for cassava varieties KME 1 and Muchericheri during initiation.

Medium	Variety <sup>†</sup>	
	KME 1	Muchericheri
LCM	3.70±0.29 <sup>ax</sup>	3.50±0.23 <sup>ax</sup>
CM	4.60±0.53 <sup>bx</sup>	6.20±0.48 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the nodes. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

**Table 3.** Mean number of leaves for cassava varieties KME 1 and Muchericheri during initiation.

Medium	Variety <sup>†</sup>	
	KME 1	Muchericheri
LCM	3.40±0.20 <sup>ay</sup>	2.70±0.21 <sup>ax</sup>
CM	5.10±0.55 <sup>bx</sup>	7.30±0.33 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the leaves. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

the modified conventional medium compared to KME 1.

#### ***Effect of the type of the media on plant height during initiation***

The two cassava varieties had significantly ( $p < 0.05$ ) taller plantlets on the modified conventional medium compared to the low cost medium. Muchericheri had significantly taller plants on the modified conventional medium with an average of 4.80 cm compared to KME 1 which had an average of 3.20 cm (Table 5). The two varieties did not show any significant difference in plant height on the low cost medium, with KME 1 and Muchericheri producing plantlets that were averagely 2.70 and 2.90 cm, respectively.

#### **Multiplication**

##### ***Effect of the type of the media on the number of nodes formed***

KME 1 variety produced significantly ( $p < 0.05$ ) higher number of nodes on the modified conventional medium during the first subculture with a mean of 4.60 nodes per plantlet compared to the low cost medium where it produced an average of 4 nodes per plantlet (Table 6). However, no significant differences were noticed in the number of nodes produced by this variety on the two media during the second subculture. Muchericheri produced significantly ( $p < 0.05$ ) higher number of nodes

**Table 4.** Mean number of roots for cassava varieties KME 1 and Muchericheri during initiation.

Medium	Variety <sup>†</sup>	
	KME 1	Muchericheri
LCM	3.30±0.36 <sup>ax</sup>	3.20±0.31 <sup>ax</sup>
CM	3.60±0.53 <sup>ax</sup>	4.80±0.17 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the roots. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

**Table 5.** Mean plant height for cassava varieties KME 1 and Muchericheri during initiation.

Medium	Variety <sup>†</sup>	
	KME 1	Muchericheri
LCM	2.70±0.25 <sup>ax</sup>	2.90±0.33 <sup>ax</sup>
CM	3.20±0.47 <sup>bx</sup>	4.80±0.43 <sup>by</sup>

<sup>†</sup>Mean ± standard error of plant height. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

on the modified conventional medium compared to the low cost medium during both subcultures (Table 6). KME 1 produced significantly higher number of nodes on the low cost medium compared to Muchericheri during the first subculture.

The two varieties did not show any significant difference in the number of nodes formed during the second subculture on the low cost medium. Muchericheri had significantly higher number of nodes on the modified conventional medium compared to KME 1 during both subcultures.

##### ***Effect of the type of the media on the number of leaves formed***

The two cassava varieties did not show any significant ( $p > 0.05$ ) difference in the number of leaves formed on the low cost medium during both subcultures (Table 7). However, on the modified conventional medium, Muchericheri produced significantly higher number of nodes compared to KME 1. KME 1 variety produced significantly ( $p < 0.05$ ) higher number of leaves per plantlet on the modified conventional medium compared to the low cost medium during the first subculture. However, this variety did not show any significant ( $p > 0.05$ ) difference in the number of leaves produced by the plantlets regenerated on the two media during the second subculture. Muchericheri variety produced significantly ( $p < 0.05$ ) higher number of leaves per plantlet on the modified conventional medium compared to the low cost medium during both subcultures.

**Table 6.** Mean number of nodes for cassava varieties KME 1 and Muchericheri during multiplication.

Medium	Variety <sup>†</sup>					
	KME 1			Muchericheri		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
LCM	4.00±0.33 <sup>ax</sup>	4.30±0.29 <sup>ax</sup>	4.15±0.15 <sup>ax</sup>	3.70±0.29 <sup>ay</sup>	4.10±0.30 <sup>ax</sup>	3.90±0.20 <sup>ax</sup>
CM	4.60±0.42 <sup>bx</sup>	4.30±0.37 <sup>ax</sup>	4.45±0.15 <sup>bx</sup>	5.60±0.42 <sup>by</sup>	4.9±0.55 <sup>by</sup>	5.25±0.35 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the nodes. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

**Table 7.** Mean number of leaves for cassava varieties KME 1 and Muchericheri during multiplication.

Medium	Variety <sup>†</sup>					
	KME 1			Muchericheri		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
LCM	4.30±0.45 <sup>ax</sup>	5.00±0.38 <sup>ax</sup>	4.65±0.35 <sup>ax</sup>	4.00±0.31 <sup>ax</sup>	5.00±0.27 <sup>ax</sup>	4.50±0.50 <sup>ax</sup>
CM	5.30±0.45 <sup>bx</sup>	4.90±0.30 <sup>ax</sup>	5.10±0.20 <sup>bx</sup>	6.40±0.50 <sup>by</sup>	5.80±0.37 <sup>by</sup>	6.10±0.30 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the leaves. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

**Table 8.** Mean number of roots for cassava varieties KME 1 and Muchericheri during multiplication.

Medium	Variety <sup>†</sup>					
	KME 1			Muchericheri		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
LCM	2.80±0.25 <sup>ax</sup>	2.60±0.30 <sup>ax</sup>	2.70±0.10 <sup>ax</sup>	2.90±0.26 <sup>ax</sup>	3.30±0.25 <sup>ay</sup>	3.10±0.20 <sup>ay</sup>
CM	3.40±0.32 <sup>bx</sup>	2.80±0.25 <sup>ax</sup>	3.10±0.30 <sup>bx</sup>	4.50±0.19 <sup>by</sup>	3.80±0.37 <sup>by</sup>	4.15±0.35 <sup>by</sup>

<sup>†</sup>Mean ± standard error of the roots. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

### **Effect of the type of the media on the number of roots**

KME 1 variety produced significantly ( $p < 0.05$ ) higher number of roots on the modified conventional medium compared to the low cost medium during the first subculture (Table 8). However, there were no significant differences in the number of roots formed by this variety on the two media during the second subculture. Muchericheri produced significantly more roots on the modified conventional medium compared to the low cost medium during both subcultures. The two varieties did not have any significant difference in the number of roots produced on the low cost medium during the first subculture but in the second subculture, Muchericheri produced more roots compared to KME 1. Muchericheri variety had a significantly ( $p < 0.05$ ) higher number of roots on the modified conventional medium compared to KME 1 during both subcultures.

### **Effect of the type of the media on plant height**

Plantlets of the two cassava varieties produced on the

modified conventional medium were significantly ( $p < 0.05$ ) taller compared to those produced on the low cost medium during both subcultures (Table 9). There were no significant differences ( $p > 0.05$ ) in the heights of plantlets of the two varieties cultured on the low cost medium during both subcultures (Table 9).

### **Plant survival ex vitro**

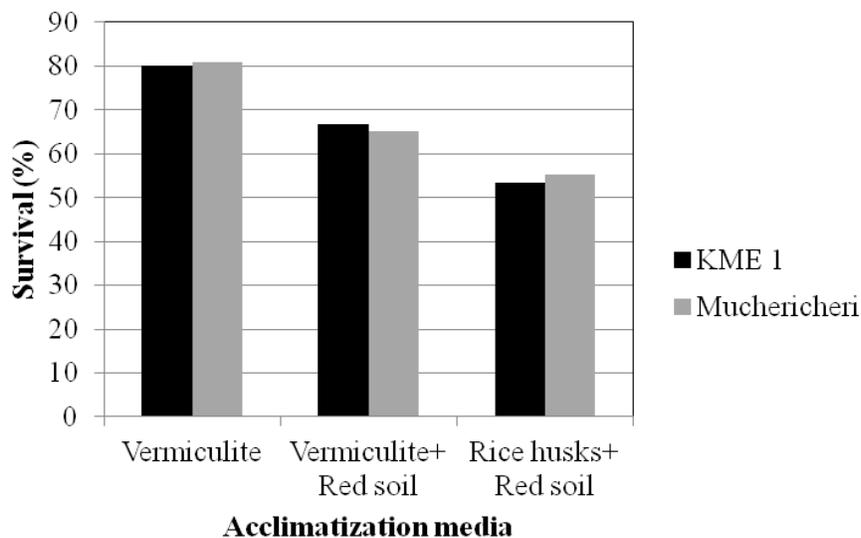
Plantlets of the two cassava varieties acclimatized on vermiculite recorded the highest survival rate of 80 and 81% for KME 1 and Muchericheri, respectively (Figure 3). This was followed by those which were acclimatized on a mixture of vermiculite and red soil, KME 1 (66.7%) and Muchericheri (67%).

The media comprising of rice husks and red soil recorded the lowest survival rate with 53.3 and 55.2% of plantlets surviving for KME 1 and Muchericheri, respectively. The plantlets adapted well in *ex vitro* conditions after transfer from the acclimatization media onto the soil (Figures 4 and 5).

**Table 9.** Mean plant height for cassava varieties KME 1 and Muchericheri during multiplication.

Medium	Variety <sup>†</sup>					
	KME 1			Muchericheri		
	1st subculture	2nd subculture	Mean	1st subculture	2nd subculture	Mean
LCM	2.90±0.20 <sup>ax</sup>	3.30±0.21 <sup>ax</sup>	3.10±0.20 <sup>ax</sup>	3.00±0.26 <sup>ax</sup>	3.30±0.17 <sup>ax</sup>	3.15±0.15 <sup>ax</sup>
CM	3.70±0.38 <sup>bx</sup>	3.80±0.26 <sup>bx</sup>	3.75±0.05 <sup>bx</sup>	4.90±0.35 <sup>by</sup>	4.80±0.31 <sup>by</sup>	4.85±0.05 <sup>by</sup>

<sup>†</sup>Mean ± standard error of plant height. Means having the same letters are not significantly different using Tukey's HSD at 5% level. <sup>a</sup> and <sup>b</sup>, Comparisons within columns; <sup>x</sup> and <sup>y</sup>, comparisons within rows.

**Figure 3.** Survival rate of plantlets of cassava varieties KME 1 and Muchericheri acclimatized on different media.**Figure 4.** Regenerated plantlets of Muchericheri cassava variety two weeks after transfer onto the soil.



**Figure 5.** Regenerated plantlets KME 1 of cassava variety two weeks after transfer onto the soil.

## DISCUSSION

The success of tissue culture in improving crop production largely depends on the affordability of seedlings produced. The cost of plantlets is however determined by the expenses incurred during production. Tissue culture technology is usually costly, which makes the seedlings out of reach for resource poor farmers. In an effort to avail healthy planting materials for cassava, this study developed a low cost medium which can be used to propagate cassava from nodal cuttings. The use of locally available salts as sources of the Murashige and Skoog nutrients led to a significant reduction in the cost of production. This was based on the fact that tissue culture is not a very rigid technique; hence can be modified. The media constituents may be substituted with locally available nutrients, hormones or chemicals. Significant cost reduction was achieved when locally available fertilizers were used as alternative tissue culture nutrient sources. Low cost protocols for tissue culture of cassava and other plants have been reported elsewhere. For example, farmers in Colombia with the help of CIAT scientists improvised a medium for cassava tissue culture that was 5 times cheaper than the conventional medium (Escobar et al., 2001). Cassava flour has also been reported as a low cost alternative for the gelling agent (Maliro and Lameck, 2004). The low cost medium

developed in this research was efficient in the regeneration of cassava plantlets. The plantlets had good multiplication rate of 3.50 to 6.20 nodes per plantlet, making this medium suitable for cassava multiplication. A multiplication rate of 3 to 7 nodes per cycle has been reported previously for IDEA87 and CM6740-7 cassava varieties (Santana et al., 2009).

Leaf production was good with plantlets of both varieties producing a good number of leaves which is essential during acclimatization. Leaves are the main site for photosynthesis; hence of great importance during hardening of plants *ex vitro* (Bell and Bryan, 1993). They are vital in the conversion of light energy into chemical energy. An ideal tissue culture medium should therefore be able to support development of well-structured leaves to ensure *ex vitro* plant survival.

In this study rooting of the two cassava varieties was achieved in a medium devoid of growth hormones. Use of the media without rooting hormone is a way of reducing the cost of cassava plantlet production. Cassava has been reported as an easy to root crop and does not necessarily require a growth hormone (Zimmerman et al., 2007; Yona et al., 2010). Roots are important in the absorption of water and extraction of nutrients from soil. Acclimatization of plantlets requires well-structured functional root system. *In vitro* root development usually enhances transplanting success because functioning

**Table 10.** Substitution of conventional MS nutrients with low cost alternatives.

Modified conventional MS nutrient (CM)	Low cost substitute (LCM)	Amount in stock solution (g/l)	Amount in culture medium (g/l)
<b>Macronutrients</b>			
CaCl <sub>2</sub>	-	11.00	0.44
KH <sub>2</sub> PO <sub>4</sub>	Monopotassium phosphate (MKP)	4.25	0.17
KNO <sub>3</sub>	Potassium fertilizer	47.5	1.90
MgSO <sub>4</sub>	Epsom salt	9.25	0.37
NH <sub>4</sub> NO <sub>3</sub>	Ammonium quarry salt	41.25	1.65
<b>Micronutrients</b>			
CoCl <sub>2</sub> .6H <sub>2</sub> O			
CuSO <sub>4</sub> .5H <sub>2</sub> O			
Na <sub>2</sub> EDTA			
FeSO <sub>4</sub> .7H <sub>2</sub> O			
H <sub>3</sub> BO <sub>3</sub>	Stanes iodized microfood <sup>®</sup>	N/A	0.20 <sup>β</sup>
KI			
MnSO <sub>4</sub> .4H <sub>2</sub> O			
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O			
ZnSO <sub>4</sub> .7H <sub>2</sub> O			
<b>Carbon source</b>			
Sucrose	Table sugar	N/A	30 <sup>β</sup>
Gelling agent			
Gelrite	-	N/A	3 <sup>β</sup>

<sup>β</sup>Were added during media preparation.

roots can create a favorable plant water balance (Diaz-Perez et al., 1995). Multiplication of *in vitro* cassava plantlets requires good plant height to enable easy splicing into nodal cuttings. The two cassava varieties used here did not show any significant difference in plant height on the low cost medium used here, hence this medium can be adopted in micro propagation of a wide array of cassava varieties. Plantlets were successfully acclimatized to natural conditions. This shows that the protocol developed in this study is appropriate in cassava regeneration. However, the differential survival rates on the different acclimatization media should be investigated further. Adaptation of *in vitro* seedlings to natural conditions is crucial for any protocol to be successful. This is because there are great differences between the artificial culture conditions in the growth room, the green house conditions and the natural conditions in terms of quantity and quality of light; relative humidity; nutrients and medium substrate. Cassava is a delicate plant to harden, and huge losses occur during transfer from *in vitro* laboratory to *ex vitro* field conditions (Jorge, 2002) which requires care and media optimization.

## Conclusion

Great milestones have been made in plant tissue culture

since its inception with micropropagation established as a commercially viable form of vegetative propagation (Gamborg, 2002). Strategies to reduce the cost of production and make plantlets affordable are among these achievements. This research has shown that there is a potential of cutting down the costs incurred during tissue culture by using fertilizers as alternative nutrients sources. The procedure used here, if adopted for commercial purposes, can greatly reduce the cost of producing cassava seedlings, thus boosting production of the crop.

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