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Cost effective medium for *in vitro* propagation of Tanzanian cassava landraces

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Cassava (*Manihot esculenta* Crantz) is a staple food for over 800 million people in the tropics. However, its production is constrained by an inadequate supply of clean planting materials. Tissue culture carried out in laboratories is one established method for mass production of clean planting materials. However, the cost of conventional tissue culture is high and the cassava industry would benefit from an alternative means of propagation. In the current study, a cost-effective protocol for micropropagation of the farmer-preferred cassava landraces 'Kibandameno' and 'Paja la mzee' in Tanzania was evaluated. Ammonium fertilizer, potassium fertilizer, epsom salt, monopotassium phosphate and calcinit were used as alternative source to conventional Murashige and Skoog (MS) macronutrients, while Stanes Iodized Microfood® was used as alternative to MS micronutrients. Nodal cuttings of the 2 cultivars were initiated in either conventional MS or cost-effective medium supplemented with 20 g/l table sugar and 3 g/L agar. Conventional MS was used as the control in this study. Four parameters namely plant height, number of leaves, number of nodes and number of roots were recorded from the two media and the differences were determined. For all 4 parameters, both cultivars performed better in the cost-effective medium as compared to conventional MS. More than 75% of plantlets acclimatized to greenhouse conditions from both types of media survived. The cost of production of cassava plantlets in both types of media was then calculated and compared. The use of the cost effective medium led to a cost reduction of 93% over conventional MS medium, which makes it a feasible and attractive alternative for growers.

Key words: *In vitro* culture, cassava, cost-effective medium, Tanzania, tissue culture, Murashige and Skoog (MS) medium.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a food crop for millions of people in East Africa and rural areas throughout the tropics and Asia. It is the second staple food after maize (Perez et al., 2011). It is a shrub plant with a starchy, tuberous root and easy to cultivate once established; it can be transformed into different products and can be stored for several years (Nassar et al., 2009). Moreover, it is a valuable source of calories, especially in countries where malnutrition is widespread (Ceballos et al., 2004). Because of these qualities, intensive efforts have been made to breed better cultivars. However, the most common method of propagation, the use of cuttings

from old plants, makes cassava multiplication tedious and slow (Santana et al., 2009), leading to insufficient planting materials which may restrict productivity.

Tissue culture is a technique with the potential to produce a massive number of healthy plantlets regardless of the growing season, thereby ensuring the availability of planting material throughout the year. Nevertheless, the adoption of tissue culture techniques is hindered by the high cost of production (Prakash et al., 2004). Establishing a tissue culture facility is highly expensive and hence limits the application of this method in many institutions.

To optimize the use of tissue culture, there is need to develop cost-effective technology in terms of equipment, chemicals, protocols and required skills so that a reduction in the unit cost of production can take place without compromising plant quality (Gitonga et al., 2010). The aim of this study was to evaluate a cost-effective micropagation protocol for Tanzanian cassava landraces by replacing the macronutrients and micronutrients used in Murashige and Skoog (MS, 1962) medium with locally available nutrients, particularly fertilizers, for the mass production of farmer-preferred cassava landraces.

MATERIALS AND METHODS

Study area and collection of plant materials

Two farmer-preferred cassava landraces, 'Kibandameno' and 'Paja la mzee' were obtained from coastal Tanzania (Tanga and Bagamoyo). These were preferred areas since most of the people grow cassava for different uses. The cuttings from these materials were planted in 4 L buckets containing loamy soil and allowed to sprout in a greenhouse at an average temperature of 28 to 30°C.

Preparation of culture media

Two types of culture media, MS and cost effective (CE) media were prepared. MS was supplemented with 20 g/L sucrose and 2 g/L phytagel (Table 1). CE medium was prepared using locally available materials where the MS macronutrients and micronutrients listed in Table 2 were substituted with local fertilizers from Yale industry Ltd; all other nutrients remained the same. The pH of the 2 types of media were adjusted, they were dispensed into culture bottles, and sterilized at 121°C and 15 psi for 15 min.

Preparation and sterilization of explants

Nodal cuttings of each cultivar were collected and put into bottles with detergent (Tarmol®) and 3 drops of Tween-20 and washed vigorously with tap water to remove soil particles. The explants were then taken to a safety clean bench and surface sterilized in70% ethanol for 5 min. They were then rinsed three times in sterile distilled water, followed by 100% JIK® with 2 drops of Tween-20 for 2 min and rinsed in sterile distilled water until no foam remained.

Initiation and multiplication

Using a sharp scalpel blade, the damaged parts of each explant were removed and cultured onto the 2 types of media. The cultures were moved to the growth room and incubated at 28 ± 2°C with a photoperiod of 16 h light and 8 h dark for 3 weeks and then subcultured onto fresh media for shoot multiplication.

Acclimatization

Plantlets were washed with sterile water to remove media in order to avoid fungal growth. The plantlets were then transferred into small pots containing sand, coconut husk and starter fertilizer. The pots were covered with polythene transparent sheets to ensure optimum humidity to protect the plantlets from withering. After 2 weeks, the plantlets were transferred into larger pots with soil and finisher fertilizer. Survival rates were recorded after 4 weeks.

Cost analysis

The price of alternative sources of nutrients were obtained and analysis of each nutrients used in the media was calculated for both conventional and alternative medium (Table 3). The cost efficiency differences between the two media were determined depending on the amount used in the media using the following formula:

$$\frac{\text{Amount of nutrient used in medium (g/l) x price of the amount of nutrient bought (Tsh)}}{\text{Amount of nutrient bought (g)}}$$

Research design and data analysis

There were four replicates for each variety in the 2 types of media and the plants were arranged in a completely randomized design within the growing area. *In vitro* plantlets were observed for any bacterial and fungal contamination and growth on a daily basis and results were recorded. Comparison between the two types of media was recorded based on four parameters, plant height, number of leaves, number of nodes and number of roots were recorded at 14 day intervals for 6 weeks. Data collected were subjected to analysis of variance using GenStat computer software 15th edition using a 5% level of significance.

RESULTS

The two cassava cultivars performed better in all 4 parameters on CE medium as compared to MS medium during the post-multiplication stage (Figures 1 and 2).

Cost efficiency

In total, 96 and 90% saving were achieved in the cost of

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Table 1. Composition of MS medium used in the micropropagation of cassava (Yona et al., 2010).

MS* macronutrient	Concentration in stock solution (g/l)	Concentration in 1 L of medium (g/l)	Amount of stock solution used per liter of medium (ml/l)
Ammonium nitrate	41.25	1.65	
Potassium nitrate	47.5	1.9	
Magnesium sulfate heptahydrate	9.25	0.37	40
Potassium phosphate monobasic	4.25	0.17	
Calcium chloride dihydrate	11	0.44	
MS micronutrient			
Boric acid	0.62	0.0031	
Manganese sulfate monohydrate	1.69	0.0085	5
Zinc sulfate heptahydrate	0.86	0.0043	
Potassium iodide	83	0.083	1
Sodium molybdate dehydrate	2.5	0.0025	1
Copper sulfate pentahydrate	2.5	0.0025	1
Cobalt chloride hexahydrate	2.5	0.0025	1
Vitamin			
Myo inositol	10	0.01	
Glycine	4	0.0002	
Nicotinic acid	0.1	0.0001	1
Pyridoxine HCl	0.1	0.0001	
Thiamine HCl	0.2	0.001	
Iron source			
Ferrous sulphate heptahydrate	5.56	0.0278	5
Disodium EDTA dihydrate	7.44	0.0372	
Carbon source			
Sugar	-	20	-
Gelling agent			
Phytagel	-	2	-
pH	5.7-5.8		

*MS = Murashige and Skoog.

Table 2. Composition of CE medium used in the micropropagation of cassava (with substitution of macronutrients and micronutrients).

Component	Weight per liter of stock solution (g/L)	Weight per liter of medium (g/L)	Amount of stock solution used/liter of medium (ml/L)
Macronutrient			
Ammonium nitrate	41.25	1.65	
Potassium fertilizer	47.5	1.9	
Epsom salt	9.25	0.37	40
Monopotassium phosphate	4.25	0.17	
Calcinit	11	0.44	
Micronutrient			
Microfood®		0.2*	
Carbon source			
Table sugar		20*	
Gelling agent			
Phytagel		2*	
pH	5.7-5.8		

*****Ingredients that were added during culture media preparation.

Table 3. Cost efficiency of MS medium compared to CE medium.

MS medium	CE medium	Cost per liter of the medium (Tshs)		Cost reduction (%)
		MS medium	CE medium	
Macronutrient				
NH_4NO_3	Ammonium fertilizer	5791	82.5	98.58
KNO_3	Potassium fertilizer	555.75	10.175	98.17
$MgSO_4$	Epsom salt	765.9	22	97.13
KH_2PO_4	Monopotassium phosphate	478.13	62.37	86.96
$CaCl_2$	Calcinit	842.6	4.08	99.51
Sub-total				96.06
Micronutrient				
H_3BO_3		39.6		
$MnSO_4.4H_2O$		1.434		
$ZnSO_4.7H_2O$		3.24		
KI	Stanes iodized microfood®	0.171	37.5	
$Na_2MoO_4.2H_2O$		50.22		
$CuSO_4.5H_2O$		200.772		
$CoCl_2.6H_2O$		74.304		
Ferrous sulphate		15.6		
Disodium EDTA		1.56		
Sub-total				90.27
Total				93.12

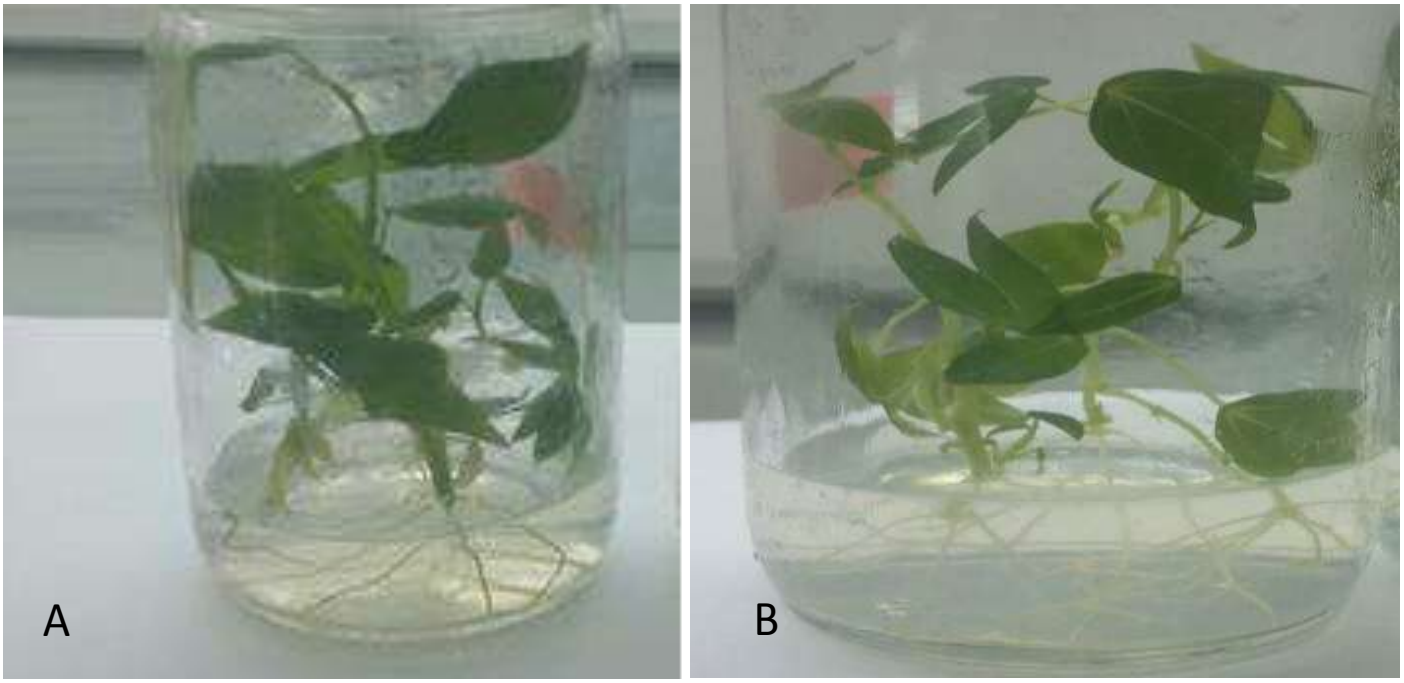

Figure 1. Six weeks old cassava plantlets of the varieties Paja la mzee (A) and Kibandameno (B) regenerated on cost-effective culture medium.

alternative macronutrients and micronutrients, respectively. This translated to a significant price reduction for plantlet production with an overall cost saving of 93% per liter of medium (Table 3).

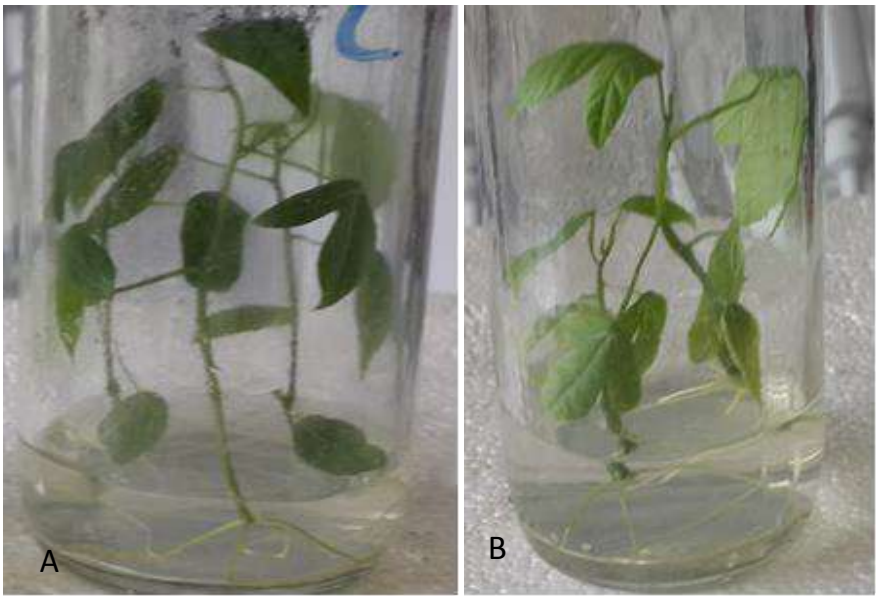

Figure 2. Six weeks old cassava plantlets of the varieties Paja la mzee (A) and Kibandameno (B) regenerated on conventional culture medium.

Table 4. Mean plant height produced by two cassava cultivars on MS medium and CE medium.

Medium	Kibandameno variety				Paja la mzee variety			
	2 Weeks	4 Weeks	6 Weeks	Grand Mean	2 Weeks	4 Weeks	6 Weeks	Grand Mean
MS	1.65 ±0.11^{bx}	2.19 ±0.13^{bz}	2.63 ±0.12^{bz}	2.15 ±0.12	1.23 ±0.09^{bz}	1.87 ±0.14^{bz}	2.37 ±0.14^{bz}	1.82 ±0.12
CE	1.53 ±0.12^{bz}	2.5 8±0.17^{ax}	3.26 ±0.20^{az}	2.46 ±0.16	1.60 ±0.08^{az}	2.74 ±0.17^{az}	3.35 ±0.19^{az}	2.56 ±0.15

*****Mean ± standard error of plant height. Means having same letters are not significantly different using Turkey's HSD at 5% level. ^{a} and ^{b} comparison within columns; ^{x} and ^{z} comparison within rows.

Regeneration response of cassava plants during the first 6 weeks after multiplication

Plant height

Cassava plants cultured on CE medium were significantly different from those on MS medium for both cultivars during the post-multiplication stage (Table 4). The height of 'Paja la mzee' plantlets was significantly taller ($p \leq 0.05$) than that of 'Kibandameno' on CE medium (means of 2.56 and 2.46 cm, respectively), whereas there was no significant difference ($p \geq 0.05$) between the heights of the two cassava cultivars on MS medium.

Leaves

There were significant differences ($p \leq 0.05$) in the number of leaves produced by the two cultivars on the two types of media at 4 weeks; 'Kibandameno' had a significantly ($p \leq 0.05$) higher mean number of leaves on CE medium (3.44) as compared to MS medium (3.06). However, no significant difference in leaf number of either variety was noted on either medium during the first 2 weeks (Table 5). On average, 'Kibandameno' produced the highest number of leaves on CE medium followed by MS medium.

Roots

'Kibandameno' produced a significantly ($p \leq 0.05$) higher number of roots on CE medium as compared to MS medium at every recording during subculture (Table 6). The averages of the mean number of roots for this variety for the six weeks on CE and MS medium were 3.31 and 2.55, respectively. 'Paja la mzee' also produced a significantly higher number of roots on CE medium than MS medium with an average of 4.0 and 1.58, respectively. 'Paja la mzee' produced a significantly ($p \leq 0.05$) higher number of roots on CE medium as compared to Kibandameno (Table 6).

Table 5. Mean number of leaves produced by two cassava cultivars on MS and CE media.

Medium	Kibandameno variety				Paja la mzee variety			
	2 Weeks	4 Weeks	6 Weeks	Grand mean	2 Weeks	4 Weeks	6 Weeks	Grand mean
MS	1.75 ±0.20^{bz}	2.58 ±0.25^{bz}	3.17 ±0.22^{bz}	2.50 ±0.22	1.42 ±0.24^{bz}	2.17 ±0.22^{bz}	3.08 ±0.27^{bz}	2.22 ±0.24
CE	1.58 ±0.20^{bz}	3.83 ±0.14^{ax}	4.92 ±0.28^{ax}	3.44 ±0.21	1.58 ±0.18^{bz}	3.42 ±0.24^{ax}	4.17 ±0.30^{az}	3.06 ±0.24

*****Mean ± standard error of leaves. Means having same letters are not significantly different using Turkey's HSD at 5% level. ^{a} and ^{b} comparison within columns; ^{x} and ^{z} comparison within rows.

Table 6. Mean number of roots produced by two cassava cultivars on MS medium and CE medium.

Medium	Kibandameno variety				Paja la mzee variety			
	2 Weeks	4 Weeks	6 Weeks	Grand mean	2 Weeks	4 Weeks	6 Weeks	Grand mean
MS	1.83 ±0.42^{bx}	2.50 ±0.40^{bz}	3.33 ±0.39^{bz}	2.55 ±0.40	0.42 ±0.43^{bz}	1.83 ±0.51^{bz}	2.50 ±0.47^{bz}	1.58 ±0.47
CE	2.33 ±0.52^{bz}	2.92 ±0.27^{bz}	4.67 ±0.43^{az}	3.31 ±0.41	1.58 ±0.42^{az}	4.25 ±0.46^{ax}	6.17 ±0.63^{ax}	4.00 ±0.50

*****Mean ± standard error of roots. Means having same letters are not significantly different using Turkey's HSD at 5% level. ^{a} and ^{b} comparison within columns; ^{x} and ^{z} comparison within rows.

Table 7. Mean number of nodes produced by two cassava cultivars on MS and CE media.

Medium	Kibandameno variety				Paja la mzee Variety			
	2 Weeks	4 Weeks	6 Weeks	Grand mean	2 Weeks	4 Weeks	6 Weeks	Grand mean
MS	1.08 ±0.11^{bz}	2.00 ±0.20^{bx}	2.50 ±0.19^{bz}	1.86 ±0.17	1.00 ±0.17^{ax}	1.58 ±0.18^{bz}	2.50 ±0.25^{bz}	1.69 ±0.20
CE	1.25 ±0.14^{bz}	3.50 ±0.29^{ax}	4.58 ±0.36^{ax}	3.11 ±0.26	0.75 ±0.16^{bz}	2.58 ±0.22^{az}	3.75 ±0.25^{az}	2.36 ±0.21

*****Mean ± standard error of nodes. Means having same letters are not significantly different using Turkey's HSD at 5% level. ^{a} and ^{b} comparison within columns; ^{x} and ^{z} comparison within rows.

Nodes

'Paja la mzee' had the highest number of nodes produced on CE medium followed by MS medium from weeks 2 to 6. On average, the mean number of nodes produced by 'Kibandameno' (biweekly) was 3.11 and 1.86 in CE and MS media, respectively (Table 7). 'Kibandameno' produced a significantly higher number ($p \leq 0.05$) of nodes in CE medium as compared to 'Paja la mzee' on all occasions. There was a significant difference ($p \leq 0.05$) in the number of nodes produced with the 2 cultivars in both media at 6 weeks; however, in the second week, no significant differences were observed between the 2 cultivars. For both cultivars, the highest number of nodes was recorded in CE medium.

Survival rate

Plantlets of the 2 cassava cultivars showed different survival rates when transplanted into small pots containing sterile soil/coconut husk (Figure 3). Plantlets from CE medium showed a higher survival rate than those from MS medium. On CE medium, survival rates were 85 and 79% for two varieties 'Kibandameno' and 'Paja la mzee', respectively (Figure 4). However, on MS medium, survival rates were 81 and 75% for Kibandameno and Paja la mzee, respectively (Figure 4).

DISCUSSION

Cassava productivity is mainly constrained by either lack of adequate planting materials or diseases (viral diseases and pests). Tissue culture technology can be used to produce healthy plantlets without seasonal limitations, hence solving one of the constraints of cassava production. However, tissue culture is generally costly, making such seedlings expensive to farmers; therefore, there is a low adoption of this technique in developing countries. In order to achieve sufficient clean plantlets, this study developed a CE medium that can be used to propagate cassava landraces from nodal cuttings.

Tissue culture technology can easily use locally available nutrients as sources of micropropagation medium nutrients, leading to a significant cost reduction in the production of cassava plantlets *in vitro.* In this study, the cost of media was reduced by over 90% when

Figure 3. Acclimatized cassava plants of the 2 varieties Paja la mzee (A) and Kibandameno (B).

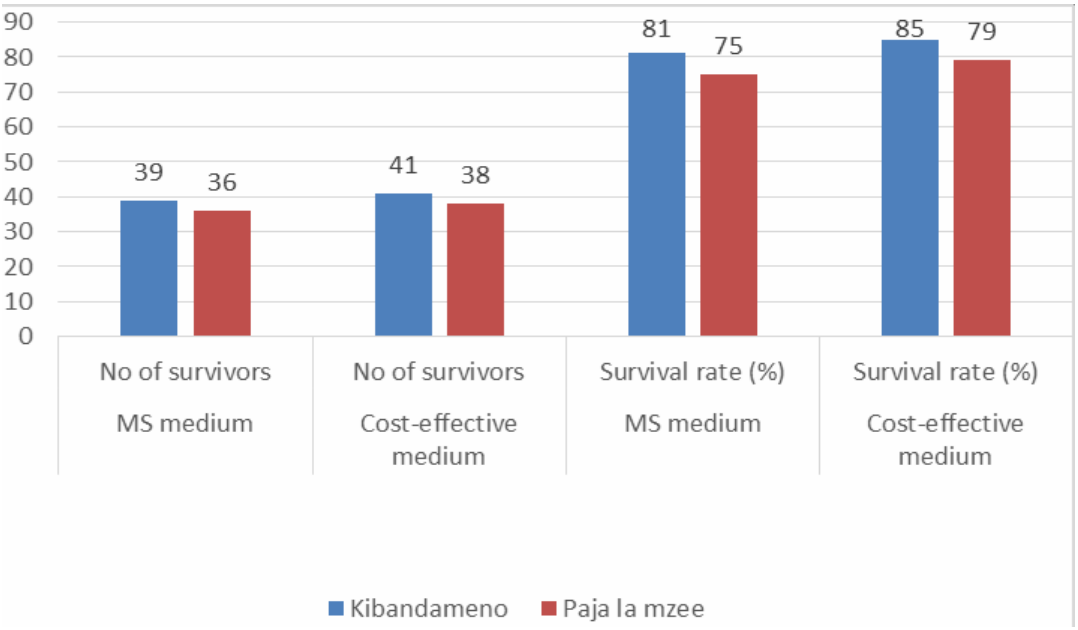

Figure 4. Survival rate (%) of cassava plantlets of the varieties Kibandameno and Paja la mzee, acclimatized from cuttings propagated in MS medium and cost-effective medium.

CE macronutrients and micronutrients were used. This agrees with the findings of Ogero et al. (2012b), where they reported a cost reduction of 95% when alternative sources of macronutrients and micronutrients were used in the multiplication of cassava plantlets in Kenya.

Gitonga et al. (2010) reduced the cost of producing tissue culture banana seedlings by 93.9% while using alternative nutrient sources. Several studies on cost-effective protocols for tissue culture have been reported in different areas. For instance, botanical starch (cassava, wheat and sweet potato) has been used as an alternative gelling agent (Madege et al., 2015; Mengesha et al., 2012). Mvuria and Ombori (2014) reported on the successful regeneration of sweet potato using low cost

macronutrients. Low cost micropropagation of pineapple is also described by Be and Debergh (2006). Ogero et al. (2012a) reported *in vitro* micropropagation of cassava through low cost tissue culture in Kenya.

In the current study, the two cassava landraces evaluated exhibited different responses to CE and MS media, perhaps due to genetic variation between the two cultivars. 'Paja la mzee' performed better on CE medium when compared with 'Kibandameno'; it produced a higher number of leaves, nodes and roots. The two cassavacultivars have a good mean plant height, which is necessary for rapid micropropagation as taller plants have higher number of nodes. This enables easy splicing of nodes during *in vitro* multiplication. Since the two tested cultivars performed well in the CE medium, it is suggested that it can be used effectively for other cultivars.

The number of leaves developed during the post-multiplication stage was higher in the CE medium than in the MS medium. Leaves are the major site of food production for the plant and a well-developed leaf system is important for plant survival during acclimatization (Bell and Bryan, 1993; Ogero et al., 2012a).

Using a media without plant growth regulators is one way of reducing costs. Roots are essential for plant growth and development as they facilitate the absorption of nutrients (Xiansong, 2010). The CE medium in the current study contained no plant growth regulators as Yona et al. (2010) had previously stated that cassava cuttings root easily without plant growth regulators. Furthermore, Dhanalakshml and Stephan (2014) also reported the rooting of banana cuttings on low cost tissue culture medium without any plant growth regulator. In the current study, roots grew well in the CE cost-effective medium. Regenerated plantlets were successfully acclimatized into coconut husks and then transplanted into potted soil in the greenhouse. Survival rates of 82 and 78% were observed in plants cultured in the CE and MS media, respectively.

Conclusion

This research has shown that replacement of conventional macronutrients and micronutrients with cheaper locally available nutrients reduces the cost of cassava plantlet production *in vitro*. The CE medium evaluated in this study can be used for the mass production of cassava plantlets and hence increase their availability to resource-challenged farmers. The adoption of such a program could increase cassava productivity in Tanzania and worldwide.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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