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Research Paper

Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (*Ipomoea batatas* (L) Lam)

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Abstract

Sweet potato production in Eastern Africa has been declining due to lack of healthy planting materials. Developing countries have not maximised *in vitro* sweet potato regeneration due to the high costs incurred. The objective of this research was to reduce the cost of sweet potato tissue culture nutrients by using affordable alternative nutrient sources. The conventional sources of Murashige and Skoog (MS) salts were substituted with Easygro® vegetative fertilizer containing both macro and micronutrients. Two grams of the fertilizer were supplemented with 30 g/L of table sugar and 9 g/L of agar. Conventional MS medium supplemented with 30 g/L of sucrose and 3 g/L of gelrite was used as the control. Two farmer-preferred sweet potato varieties, Kemb-36 and Tainurey were initiated on the two media. The mean number of nodes, leaves, roots and plant height were determined and comparisons made between the two media. There was 96.9% reduction in the cost of the nutrients used in media preparation. Significant differences were detected on the number of nodes produced by Kemb-36 on the two media with plantlets cultured on the low cost medium producing four nodes per plantlet while those cultured on the conventional MS medium had an average of five nodes per plantlet. Significant differences were not detected on the number of nodes produced by Tainurey on the two media. The developed low cost medium can be used to boost the production of affordable disease-free sweet potato seedlings.

Keywords: *Low Cost Medium, Sweet Potato, In Vitro Plant Regeneration*

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is the second most important root crop after cassava in Africa. Most of it is cultivated in the East African countries around Lake Victoria. The crop is grown in several agro-ecological zones and usually plays significant roles in the farming and food systems. The crop has a short growing period, stores well in the soil and performs well in marginal lands hence referred to as a food security crop (Kapinga et al, 2007). The yellow and orange-fleshed sweet potato varieties serve as good sources of vitamin A that is frequently lacking in diets of most African farming communities. Apart from these uses, sweet potato can also be used to produce renewable plant products such as ethanol (Woolfe, 1992).

With climate change threatening maize production in Kenya, sweet potato offers an important alternative source of carbohydrates. However, sweet potato production has been on the decline in the country. This is due to its susceptibility to viral diseases such as Sweet Potato Feathery Mottle Virus (SPMV) which cause enormous yield losses (Karyeija et al, 2000; Odame et al, 2002). The frequent introduction and exchange of sweet potato cultivars and clonal propagation exposes the crop to viral infections (Xiansong, 2010). Traditional planting methods using cuttings are time consuming and labor intensive. If infected, the parent plant can transmit the disease to the next generation when cuttings are used as propagating

material (Nandwani and Tudela, 2010).

Production of pathogen-free materials and disease indexing are the first steps of a proper control strategy of viral diseases in vegetatively propagated crops, such as sweet potato (Lepoivre, 1998). Tissue culture has many advantages such as production of disease-free planting materials in large numbers hence permits rapid dissemination of healthy and improved plants within and among countries, as the materials are readily certified as disease-free (FAO, 2003) and grows uniformly hence they are highly marketable (Vuylsteke and Talengera, 1998). Optimization of sweet potato improvement depends on robust transformation and regeneration procedures (Santa-Maria et al, 2009).

High production cost has been an impediment to tissue culture adoption especially in the sub-Saharan Africa. This has limited the technology to a few institutions and rich farmers while locking out the resource-challenged subsistence farmers. One factor contributing to the high cost of production is the cost of the culture nutrient medium which requires chemicals that are often very expensive (Savangikar, 2002). In order to increase application of tissue culture technology in sweet potato farming, innovative approaches are needed to lower the cost of micropropagule production. The objective of this study was to generate an efficient and affordable protocol for the micropropagation of sweet potatoes.

2. Materials and Methods

2.1. Cost Analysis

The cost of each compound used was calculated as follows based on the quantities used per litre of the medium:

$$\frac{\text{(Amount Used in Culture Medium \{g\}} \times \text{Price of Amount Bought \{KShs)}}{\text{Amount Bought \{g\}}}$$

Differences in cost between the conventional and alternative nutrient sources were then determined and their percentages evaluated.

2.2. Plant Materials

Two Kenyan sweet potato varieties, Kemb-36 and Tainurey were used in this research. Cuttings of certified disease-free plants were obtained from the Kenya Agriculture Research Institute and planted in potted soil at Kenyatta University, Kenya. The research was carried out from February 2011 to January 2012.

2.3. Media Preparation

A cost-efficient media was developed and used alongside the conventional MS medium to regenerate sweet potato from nodal cutting explants. Easygro® vegetative fertilizer, a locally available foliar feed was used as the alternative source of MS nutrients. Two grams of the fertilizer which contains both macro and micronutrients were used to make one litre of medium. This was supplemented with 30 g/L of table sugar and 9 g/L of agar. Table sugar was used as the alternative low cost source of sucrose. The conventional MS nutrient salts supplemented with 3 g/L of gelrite and 30 g/L of sucrose was used as the control. Growth regulators were not added into both media.

2.4. Sterilization and Initiation of the Cultures

Healthy vines were collected from the net house and leaves were excised. The stem pieces were then cut into 2 cm long nodal cuttings, each having a bud. The nodal cuttings were washed in running tap water and then surface-sterilized with 40% (v/v) Jik®(commercial bleach) containing 1.5% sodium hypochlorite and a drop of Tween 20® for 20 minutes. The explants were immersed in 70% (v/v) ethanol for six minutes and then rinsed four times with sterile distilled water. The damaged parts were excised off using a sterile scalpel and the explants introduced into the nutrient media. The cultures were incubated at a temperature of 28±2° C and a photoperiod of 16 hours light and 8 hours darkness at a light intensity of 2000 lux. The numbers of leaves, nodes, roots and plant height were determined and recorded after six weeks. This experiment was repeated twice to test reproducibility of the results.

2.5. Multiplication Experiments

Multiplication was carried out twice to increase the number of plantlets. Plantlets that had 4-6 nodes were selected and spliced into nodal cuttings. The nodal cuttings were put in fresh medium of the same composition as the initiation medium and incubated at temperature of 28±2°C and a photoperiod of 16 hours light and 8 hours darkness at a light intensity of 2000 lux. Morphological changes were observed and the number of nodes, leaves, roots and plant height recorded after the sixth week of culture.

2.6. Acclimatization

Plantlets with well-developed root and leaf systems were washed with tap water to remove adhering media to avoid mould growth. They were then transplanted onto a mixture of red soil and rice husks in the ratio 2:1 and dispensed in rectangular trays. The trays containing plantlets were kept in an acclimatization chamber made with transparent polythene sheet for 21 days. The number of surviving plantlets was recorded and the plantlets transplanted onto the soil in polythene bags.

2.7. Experimental Design and Data Analysis

A completely randomized design with two treatments and nine replicates per variety was used during the initiation and multiplication experiments. Analysis of variance (ANOVA) using STATA® statistical program version 11 was carried to compare the number of nodes, leaves and plant height formed in the conventional MS medium and the alternative medium. Means were separated using Tukey's test at 5% level.

3. Results

3.1. Cost Analysis Per Litre of Medium

The use of Easygro vegetative fertilizer as the alternative source of MS nutrients reduced the cost of the nutrient medium by 96.2% while the use of table sugar led to 97.1% savings in regard to the source of carbon. A total cost reduction of 96.9% was realized as shown in Table 1.

3.2. Initiation

3.2.1. Effect of the Type of the Media on the Number of Nodes

The variety Tainurey produced significantly ($p<0.05$) higher number of nodes on the low cost medium compared to Kemb-36. Tainurey had an average of 3.3 nodes per plantlet at the end of the culture period while Kemb-36 had an average of three leaves per plantlet. On the conventional medium, Kemb-36 produced significantly ($p<0.05$) more nodes with an average of 4.6 leaves per plantlet compared to Tainurey which had an average of 3.8 nodes per plantlet. Both varieties produced significantly higher number of leaves on the conventional medium compared to the low cost medium.

3.2.2. Effect of the Type of the Media on the Number of Leaves

Table 1. Cost Comparison Between the Low Cost Medium and the Conventional Medium Sources

Conventional TC Nutrient	**Low Cost Substitute**	**Cost in one Litre of the Medium (KShs.)**		**Cost Reduction (%)**
		Conventional	**Low Cost**	
Macronutrient				
$CaCl_2$	Easygro Vegetative Fertilizer	3.3	1.6	
KH_2PO_4		1.2		
KNO_3		14.4		
$MgSO_4$		1		
NH_4NO_3		21		
Sub-TOTAL		**40.9**		
Micronutrients				
$CoCl_2.6H_2O$		0.011		
$CuSO_4.5H_2O$		0.009		
Na_2EDTA		0.154		
$FeSO_4.7H_2O$		0.078		
H_3BO_3		0.17		
KI		0.017		
$MnSO_4.4H_2O$		0.27		
$Na_2MoO_4.2H_2O$		0.017		
$ZnSO_4.7H_2O$		0.038		
Sub-TOTAL		**0.764**	1.6	
TOTAL		**41.664**	**1.6**	**96.2**
Carbon Source				
Sucrose	Table sugar	105	3	97.1
TOTAL		**146.664**	**4.6**	**96.9**

The two sweet potato varieties produced significantly ($p<0.05$) higher number of leaves on the conventional medium compared to the low cost medium. Tainurey had a better response on the low cost medium with an average of 4.3 leaves per plantlet compared to Kemb-36, which had an average of four leaves per plantlet after six weeks of culture. The variety Kemb-36 produced significantly higher number of nodes on the conventional medium compared to Tainurey.

3.2.3. Effect of the Type of the Media on the Formation of Roots

The two sweet potato varieties had no significant difference ($p>0.05$) in the number of roots produced on the low cost medium but on the conventional medium Kemb-36 produced significantly ($p<0.05$) higher number of roots compared to Tainurey. The Kemb-36 variety produced an average of 2.5 roots per plantlet on the low cost medium while Tainurey had an average of 2.7 roots per plantlet after six weeks of culture. Kemb-36 had an average of 3.4 roots per plantlet on the conventional medium while Tainurey had an average of 2.9 roots per plantlet.

3.2.4. Effect of the Type of the Media on the Height of In Vitro Plantlets

The two sweet potato varieties did not show any significant difference ($p>0.05$) in the height of *in vitro* plantlets on the low cost medium. However, on the conventional medium significant ($p<0.05$) differences were detected with Kemb-36 producing taller plantlets compared to Tainurey.

3.3. Multiplication

3.3.1. Effect of the Type of the Media on the Number of Nodes

Significantly ($p<0.05$) higher number of nodes were produced in the conventional medium compared to the low cost medium during the first subculture while no significant differences ($p>0.05$) were detected during the second subculture in the two varieties of sweet potatoes as shown in Table 2.

In overall, the two sweet potato varieties did not show any significant difference ($p>0.05$) in the number of nodes produced on the low cost medium but Kemb-36 had a significantly more nodes on the conventional medium compared to Tainurey.

3.3.2. Effect of the Type of the Media on the Number of Leaves

In both instances of subculture, the Kemb-36 variety had significant differences ($p<0.05$) in the number of leaves produced between the low cost and conventional media. The said variety produced more leaves on the conventional medium compared to the low cost medium with the overall mean of 5.05 and 4.15 respectively as shown in Table 3.

There were no significant differences ($p>0.05$) detected on the number of leaves produced in the two media during both subcultures in Tainurey variety. During the first subculture Tainurey produced significantly higher number of leaves on the low cost medium compared to Kemb-36 while Kemb-36 produced significantly more leaves on the conventional medium compared to Tainurey. Significant differences were not detected in leaf formation for the two varieties on both media during the second subculture.

3.3.3. Effect of the Type of the Media on the Formation of Roots

Kemb-36 variety produced significantly ($p<0.05$) higher number of roots on the conventional medium compared to the low cost medium during both subcultures as shown in Table 4.

Tainurey did not show any significant differences ($p<0.05$) in the number of roots formed in the two media during the first subculture but in the second subculture more roots were produced on the low cost medium compared to the conventional medium. The Tainurey variety produced significantly ($p<0.05$) higher number of roots compared to Kemb-36 on the low cost medium during both subcultures. Kemb-36 had better root production compared to Tainurey on the conventional medium.

3.3.4. Effect of the Type of the Media on the Height of In Vitro Plantlets

The two sweet potato varieties produced significantly ($p<0.05$) taller plantlets on the conventional medium compared to the low cost medium during both subcultures as shown in Table 5.

The Tainurey variety produced significantly taller plantlets on the low cost medium compared to Kemb-36 during the first subculture. Significant differences were not observed in the heights of plantlets of the two varieties on the low cost medium during the second subculture. There were no significant differences in the heights of plantlets of the two sweet potato varieties regenerated on the conventional medium in both subcultures.

3.4. Hardening of Plantlets

The *in vitro* regenerants of the two sweet potato varieties adapted well when they were transplanted onto a mixture of red soil and rice husks The survival rate was 66.7% and 67% for Kemb-36 and Tainurey respectively. The

Table 2. Mean Number of Nodes Produced by Two Sweet Potato Varieties Cultured on Low Cost and Conventional Tissue Culture Media

	Mean Number of Nodes*					
	Kemb-36			Tainurey		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM	3.50 ± 0.33^{ax}	4.00 ± 0.31^{ax}	$\mathbf{3.75\pm0.25^{ax}}$	3.5 ± 0.38^{ax}	3.7 ± 0.42^{ay}	$\mathbf{3.6\pm0.10^{ax}}$
CM	4.40 ± 0.32^{bx}	3.90 ± 0.34^{cx}	$\mathbf{4.15\pm0.25^{bx}}$	3.8 ± 0.37^{by}	3.9 ± 0.30^{ax}	$\mathbf{3.85\pm0.05^{ay}}$

**Values are expressed as mean± standard errors of the mean. Same letters represent values without significant differences (^{a} and ^{b} represent comparisons between media (within rows) while ^{x} and ^{y} represent comparisons between the varieties (within columns)*

Table 3. Mean Number of Leaves Produced by two Sweet Potato Varieties Cultured on Low Cost and Conventional Tissue Culture Media

	Mean Number of Leaves*					
	Kemb-36			Tainurey		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM	3.90 ± 0.30^{ax}	4.40 ± 0.30^{ax}	$\mathbf{4.15\pm0.25^{ax}}$	4.30 ± 0.37^{ay}	4.40 ± 0.37^{ax}	$\mathbf{4.35\pm0.05^{ax}}$
CM	5.40 ± 0.38^{bx}	4.70 ± 0.42^{bx}	$\mathbf{5.05\pm0.35^{bx}}$	4.40 ± 0.46^{ay}	4.60 ± 0.50^{ax}	$\mathbf{4.50\pm0.10^{ay}}$

**Values are expressed as mean± standard errors of the mean. Same letters represent values without significant differences (^{a} and ^{b} represent comparisons between media (within rows) while ^{x} and ^{y} represent comparisons between the varieties (within columns)*

Table 4. Mean Number of Roots Produced by two Sweet Potato Varieties Cultured on Low Cost and Conventional Tissue Culture Media

	Mean Number of Roots*					
	Kemb-36			Tainurey		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM	2.40 ± 0.26^{ax}	2.70 ± 0.29^{ax}	$\mathbf{2.55\pm0.15^{ax}}$	3.0 ± 0.27^{ay}	3.30 ± 0.42^{by}	$\mathbf{3.15\pm0.15^{aby}}$
CM	3.60 ± 0.26^{bx}	3.10 ± 0.26^{bx}	$\mathbf{3.35\pm0.25^{bx}}$	3.0 ± 0.27^{ay}	2.80 ± 0.31^{ax}	$\mathbf{2.9\pm0.10^{by}}$

**Values are expressed as mean± standard errors of the mean. Same letters represent values without significant differences (^{a} and ^{b} represent comparisons between media (within rows) while ^{x} and ^{y} represent comparisons between the varieties (within columns)*

Table 5. Mean Plant Heights for Plantlets of Two Sweet Potato Varieties Cultured on Low Cost and Conventional Tissue Culture Media

	Mean Plant Height*					
	Kemb-36			Tainurey		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM	1.50 ± 0.15^{ax}	2.70 ± 0.44^{ax}	$\mathbf{2.10\pm0.60^{ax}}$	2.20 ± 0.38^{ay}	2.60 ± 0.32^{ax}	$\mathbf{2.40\pm0.20^{ay}}$
CM	3.10 ± 0.23^{bx}	3.00 ± 0.19^{bx}	$\mathbf{3.05\pm0.05^{bx}}$	3.00 ± 0.29^{bx}	3.10 ± 0.31^{bx}	$\mathbf{3.05\pm0.05^{bx}}$

**Values are expressed as mean± standard errors of the mean. Same letters represent values without significant differences (^{a} and ^{b} represent comparisons between media (within rows) while ^{x} and ^{y} represent comparisons between the varieties (within columns)*

surviving plants were successfully transplanted onto soil in *ex vitro* conditions (Figures 1 and 2).

Figure 1. Plants of Sweet Potato Variety KEMB-36 Two Weeks after Transfer onto Soil

Figure 2. Plants of Sweet Potato Variety Tainurey Two Weeks after Transfer onto Soil

4. Discussion

In vitro regeneration of sweet potato through tissue culture can significantly boost production of the crop by availing healthy planting materials. However, this is usually constrained by high cost of plantlet production. Findings of this study, however, show that this hindrance can be overcome. The successful regeneration of the two sweet potato cultivars on the developed low cost medium indicates that locally available salts such as fertilizers can be used as affordable alternative sources of the commonly used Murashige and Skoog media salts. This can greatly reduce the cost of nutrient medium and hence the cost of plantlet production which will in turn lower the cost of the plantlets. Strategies to reduce the cost of tissue culture nutrient media have been reported on other plants. Combination of starch, semolina and potato powder or combination of starch and agar can be a low cost option for shoot induction in African violet (Sharifi et al, 2010). A low cost protocol for multiplication of healthy banana seedlings has also been reported (Gitonga et al, 2010). Despite these tremendous achievements in cost reduction in the *in vitro* seedling production for other crops nothing has been reported in regard to sweet potato varieties available in Kenya and hence this study was carried out to address this gap.

The use of Easygro® vegetative fertilizer as the alternative source of MS nutrients was on the basis that this foliar feed contains both the macro- and micro- elements required for plant growth. The medium developed supported plant growth hence can easily be adopted for regeneration of sweet potato. The superior node production pattern on the conventional medium compared to the low cost medium may be due to insufficient amount of essential elements. Adjustments of the amount of Easygro® vegetative fertilizer can help to overcome this. Differences were noted between the two varieties in regard to the number of nodes formed, which can be attributed to genetic differences that exist among the varieties. This is a challenge since an efficient tissue culture medium should be capable of supporting many varieties of a crop to avoid developing crop-specific media. According to Dessai et al (1995) genotype-dependent regeneration efficiency increases the cost of media design. The differences observed in leaf production between the two varieties can also be attributed to genotypic differences and the fact that sweet potato is highly heterozygous.

A good root system is essential for successful acclimatization of the plantlets and subsequent growth in the field since roots facilitate the absorption of nutrients from the soil (Xiansong, 2010). The physiological status of roots is critical for plant survival during the first few days of acclimatization (Jorge, 2002). The two sweet potato varieties produced roots without incorporating any auxin. Tainurey produced more roots in the low cost medium during initiation compared to Kemb-36 indicating that the media composition in low cost medium was more appropriate for root formation in Tainurey compared with to Kemb-36. This can be attributed to the differences in the genetic constitution between the two varieties.

The two varieties also had differences in plant height on the two media. This affects multiplication since plant height is an important parameter during multiplication. Tall plants with intermediate inter-nodal length produce many nodal cuttings and are easy to excise. The differences exhibited by the two varieties in root production and plant height on the low cost medium may be due to inherent genotypic differences, which have also

been reported in sweet potato (Dessai et al, 1995) and cassava (Santana et al, 2009). Dessai et al (1995) reported differential response in node production of 27 sweet potato varieties during *in vitro* propagation. Santana et al (2009) also reported some differences in the regeneration efficiency of cassava varieties IDEA87 and CM6740-7 on a low cost tissue culture medium developed in Colombia.

Successful acclimatization of the sweet potato plantlets was achieved in this study that can be attributed to the development of good root and leaf systems. Plantlets with well developed roots and leaves have been reported to adapt quickly to natural conditions outside the growth room (Nowak and Pruski, 2002).

5. Conclusion

In this study a low cost medium for sweet potato regeneration was developed. This can contribute to increased sweet potato production not only in sub-Saharan Africa but also worldwide. Adoption of this protocol can empower farmers to set up low cost tissue culture laboratories in their localities hence increase seedling production. Given the ability of sweet potato to tolerate adverse conditions such as drought, increase in its production can greatly reduce food insecurity.

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