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A low-cost medium for sweet potato micropropagation

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Abstract Tissue culture has not been optimised in sweet potato (*Ipomoea batatas* (L) Lam) propagation due to high costs. With an aim of producing affordable disease-free seedlings, a low cost medium was developed and used to regenerate two sweet potato varieties (KEMB 36 and Tainurey). The conventional sources of MS nutrients were substituted with locally available fertilisers. Thirty grammes per litre table sugar was used as a source of carbon. MS medium supplemented with $30g^{-1}$ table sugar and $3g^{-1}$ of gelrite was used as the control. The number of leaves, nodes, roots and plant height for the two cultivars were determined and compared. The low cost medium was significantly ($P<0.05$) cheaper compared to the MS medium costing 94.4% less per litre. KEMB 36 had a regeneration index of 7 nodes per plantlet on the low cost medium, while Tainurey had 3 nodes per plantlet.

Key words: *Ipomoea batatas* (L.), regeneration index, tissue culture

Introduction

Sweet potato (*Ipomoea batatas* (L.) productivity in the sub-Saharan Africa is limited due to abiotic and biotic constraints such as pests and diseases (Kapinga *et al.*, 2007). The crop is usually attacked by a wide range of pathogenic organisms with varying damage levels (Segundo, 2011). Sweet potato chlorotic stunt virus (SPCSV) and sweet potato feathery mottle virus (SPFMV) can cause severe losses of up to 90% (Karyeija *et al.*, 1998). Inefficient multiplication and distribution systems for disease-free planting materials has exacerbated the situation (ASARECA, 2008). This situation can only be reversed by adopting technologies that give farmers access to superior quality planting materials. The provision of improved planting materials for clonal crops has a proven track record in poverty alleviation through raising crop productivity, both through improved seed quality and through the dissemination of improved varieties with their associated pro-poor traits (Barker *et al.*, 2011). Production of pathogen-free materials is the first step of controlling viral diseases in vegetatively propagated crops including sweet potato (Mervat, 2007).

Tissue culture (TC) technology has been applied in plant propagation for many years now due to the opportunity it offers in producing healthy planting materials. TC techniques have opened a new frontier in agricultural science by addressing food security and agricultural production issues (Oggema *et al.*, 2007). The technology is efficient in establishing dependable seed systems for vegetatively propagated crops. Despite the enormous opportunities offered by TC technology, its uptake in developing countries especially the Sub-Saharan Africa region has been rather slow. This is mainly due to the high costs of operation. Tissue culture often requires sophisticated equipment and chemicals that are very expensive. To recover their investments, commercial TC operators are left with no option but to sell their seedlings at exorbitant costs. Smallholder farmers who are the majority in the region cannot afford the seedlings due to the high costs. They then resort to the traditional use of vines which contributes to disease spread and hence a decline in yields.

Strategies to reduce the cost of plantlet production are, therefore, necessary so that farmers can benefit from the technology. The situation calls for alternative low cost resources to reduce the cost of production and subsequently the cost of plant propagules. The objective of this study was to evaluate the possibility of using locally available fertilisers as alternative nutrient sources for sweet potato tissue culture.

Materials and Methods

Plant materials. Farmer-preferred sweet potato varieties were used. Vines of two sweet potato varieties, KEMB 36 and Tainurey were obtained from the Kenya Agricultural Research Institute station at Embu. They were planted in plastic pots and put in a net house to establish mother stock plants.

Media preparation. Local fertilisers were obtained from agrovet shops and used as alternative sources for the conventional Murashige and Skoog (MS) tissue culture salts. Individual substitutions were done for the sources of four macronutrients (potassium nitrate, ammonium nitrate, magnesium sulphate and potassium dihydrogen phosphate); while Stanes Iodised Microfood® was used as the alternative source of micronutrients (Table 1). A total of 100 ml^{-1} of the macronutrient stock solution, containing conventional MS calcium chloride, monopotassium phosphate, potassium nitrate and ammonium nitrate used in quarries, 10 ml^{-1} of magnesium sulphate stock solution and 2 g^{-1} of Stanes Iodised

Microfood® supplemented with 30 g^{-1} of table sugar and 3 g^{-1} of gelrite were used to prepare one litre of the low cost medium. The conventional MS medium for sweet potato tissue culture developed by the International Potato Center (Rolando *et al.*, 1992) supplemented with 30 g^{-1} of table sugar and 3 g^{-1} of gelrite was used as the control. Both media were dispensed into culture bottles and autoclaved at a temperature of 121°C and 15 pounds of pressure per square inch for 15 minutes in a pressure cooker.

Preparation, initiation and multiplication of explants. Healthy vines were obtained from the disease-free mother stock plants established in the net house. Leaves were excised and the vines cut into nodal cuttings each having a bud. The nodal cuttings were then sterilised in 40% v/v Jik® (commercial bleach), which contained 1.5% sodium hypochlorite and a drop of Tween 20®. This step took 20 minutes, after which the explants were immersed in 70% v/v ethanol for 6 minutes. The nodal explants were then washed four times with sterile distilled water and inoculated on the nutrient media. The culture bottles with explants were transferred into the growth room and incubated at a temperature of 28±2°C under an illumination of 2000 lux white light and a photoperiod of 16 hours light and 8 hours darkness. There were 9 replicates for each variety, arranged in a completely randomised design. Examination for growth and contamination was carried out after every three days. Any bottle that was found contaminated was discarded. The patterns of node, leaf and root formation plus plant heights were monitored over a period of six weeks and numbers of nodes, leaves, roots and plant height recorded after every two weeks. Multiplication of plantlets was achieved through two subcultures done after every six weeks.

***Ex vitro* hardening of plantlets.** Six weeks after the second subculture plantlets were removed from the growth room and transplanted on acclimatisation medium containing rice husks and red soil in the ratio 1:2. The acclimatisation medium was dispensed in rectangular trays, which were then put in a hardening chamber made of transparent polythene sheet. Survival of the plants was monitored over three weeks, after which surviving plants were transferred onto soil.

Data analysis. The collected data were subjected to analysis of variance using STATA® statistical software Version 11. Separation of means was done using Tukey's t test at $p<0.05$.

Results

Cost comparison. The use of alternative nutrient sources significantly ($P<0.05$) reduced the cost of sweet potato tissue culture (Table 2). Reductions of 87.8%, 68.6% and 97.1% in the costs of macronutrients, micronutrients and carbon were attained, respectively.

Node formation. The two sweet potato varieties showed significant differences in node formation on the two media (Fig. 1.). The variety KEMB 36 produced more nodes on the low cost medium compared to the conventional medium. This variety had average means of 7.2 nodes and 4.3 nodes per plantlet on the LCM and CM, respectively. Tainurey produced significantly ($P<0.05$) more nodes on the conventional medium compared to the low cost medium. KEMB 36 had superior node formation on both media, producing significantly ($P<0.05$) higher number of nodes compared to Tainurey.

Leaf formation. Variety KEMB 36 produced significantly ($P<0.05$) more leaves on the low cost medium than on the conventional medium (Fig. 2). Tainurey produced more leaves on the conventional medium than on the low cost

Table 1. Composition of the low cost medium (LCM).

Component	Concentration per litre of the stock solution (g^{-1})	Weight per litre of the medium (g^{-1})	Amount of stock solution per litre of the medium (ml^{-1})
Macronutrients			
Calcium chloride (conventional)	9	0.9	100
Monopotassium phosphate (MKP)	3.5	0.35	
Potassium nitrate fertiliser	40	4	
Ammonium nitrate (quarry explosive)	35	3.5	
Magnesium sulphate stock			
Epsom salt	37	0.37	10
Micronutrients			
Stanes iodised microfood®	-	0.2 [a]	-
Carbon source			
Table sugar	-	30 [a]	-
Gelling agent			
Gelrite	-	3 [a]	-

[a] were added during media preparation

Table 2. Cost analysis between the low cost medium and the conventional MS medium.

Conventional MS nutrient	Low cost substitute	Cost in one litre of the medium (KShs.)		Cost reduction (%)
Macronutrients		Conventional	Low cost	
$CaCl_2$	-	3.3	3.3	0
KH_2PO_4	Monopotassium phosphate (MKP)	1.2	0.084	93
KNO_3	Potassium fertiliser	14.4	0.72	95
$MgSO_4$	Epsom salt	1	0.07	93
NH_4NO_3	Ammonium quarry salt	21	0.812	96.1
Sub-total		40.9	4.986	87.8
Micronutrients				
$CoCl_2.6H_2O$		0.011		
$CuSO_4.5H_2O$		0.009		
Na_2EDTA		0.154		
$FeSO_4.7H_2O$	Stanes Iodised microfood	0.078		
H_3BO_3		0.17	0.24	
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	0.24	68.6
Carbon source				
Sucrose	Table sugar	105	3	97.1
Total		146.664	8.226	94.4

1 USD = Kshs. 83.

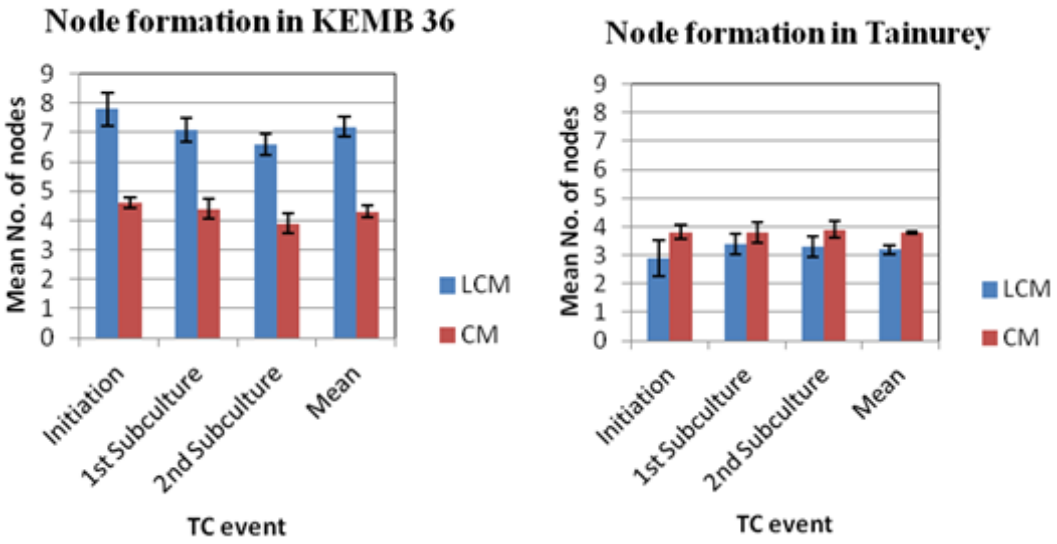

Figure 1. Node formation for sweet potato varieties, KEMB 36 and Tainurey, on low cost and conventional media.

medium which had an average mean of 3.5 leaves per plantlet. There were notable intervarietal differences in leaf formation with KEMB 36 producing significantly higher number of leaves compared to Tainurey.

Root development. Variety KEMB 36 had significantly ($P<0.05$) more roots on the conventional medium compared to the low cost medium while Tainurey had more roots on the low cost medium compared to the conventional medium (Fig. 3). Tainurey had more roots on the low cost medium compared to KEMB 36. It was *vice versa* on the conventional medium with the variety KEMB 36 producing significantly ($P<0.05$) higher number of roots compared to Tainurey.

Plant height. The two sweet potato varieties produced significantly taller plantlets on the low cost medium compared to the conventional medium (Fig. 4). Variety KEMB 36 produced plantlets that were taller on both media compared to Tainurey.

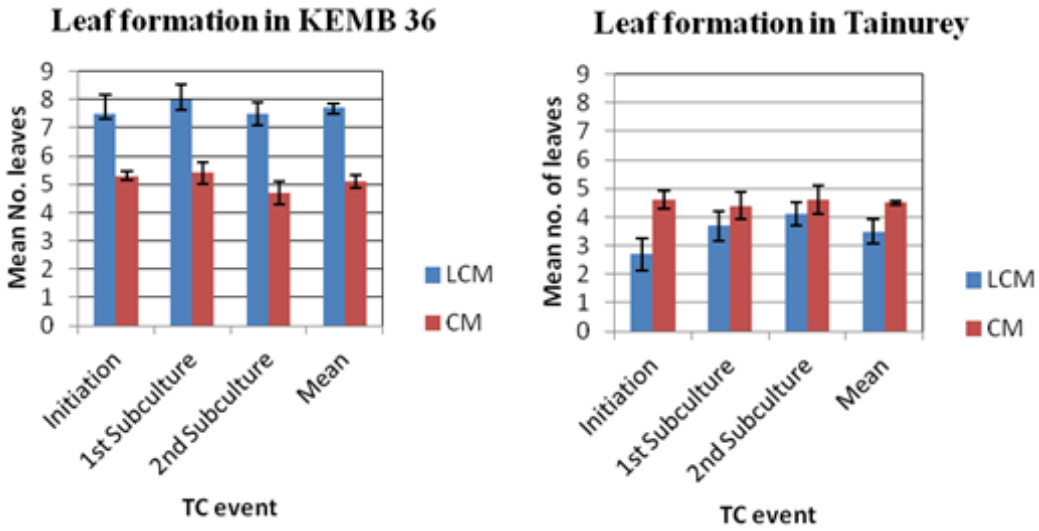

Figure 2. Leaf formation for the two sweet potato varieties, KEMB 36 and Tainurey, cultured on the low cost and conventional media.

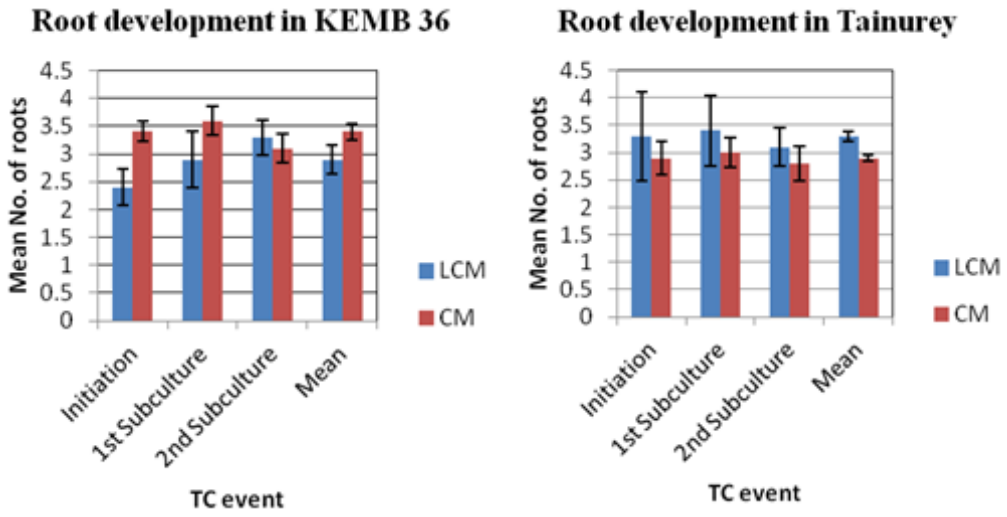

Figure 3. Root formation patterns for two sweet potato varieties cultured on low cost and conventional MS media.

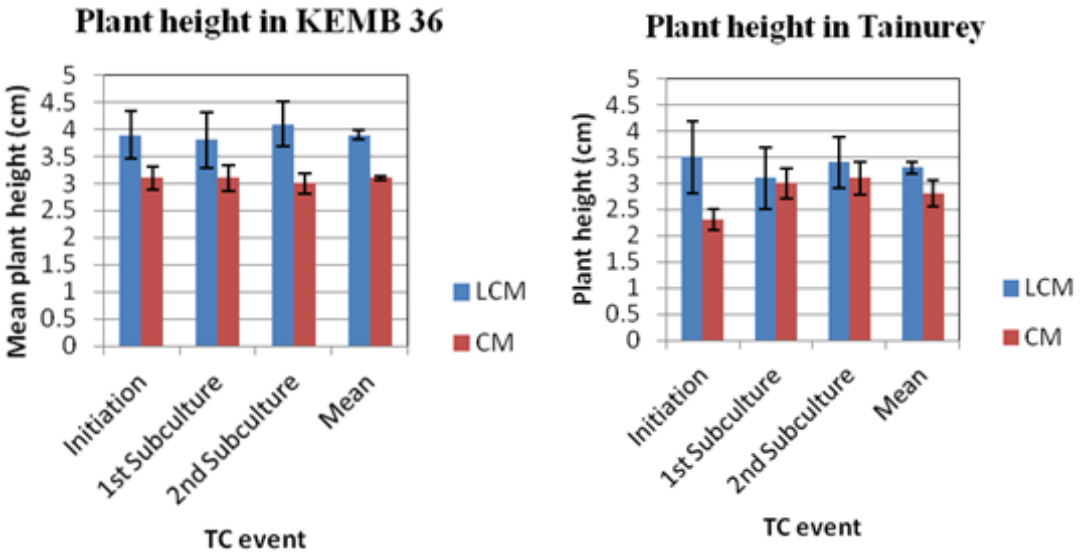

Figure 4. Mean plant height for two sweet potato varieties cultured on low cost and conventional MS media.

Figure 5. *Ex vitro* hardening of sweet potato on a medium consisting of red soil and rice husks in the ratio 2:1.

Figure 6. Sweet potato plantlets transplanted on soil.

Plant survival *ex vitro*. The sweet potato plants had a good survival rate during acclimatization with 80% of them surviving (Figs. 5 and 6).

Discussion

Tissue culture will continue to play a significant role in propagation of vegetative crops including sweet potato. However, unless interventions are made to lower the cost of production, the technology will remain a mirage to many smallholder farmers in the developing world. Scientists in a number of laboratories worldwide have been addressing this issue for quite some time with some achievements in lowering the cost of tissue culturing crops such as banana. Low cost tissue culture technology is a great tool in combating food insecurity since it contributes significantly to the improvement of agricultural productivity by availing affordable disease-free planting materials (Odame *et al.*, 2002).

Plant tissue culture has three components which can form points of intervention when addressing the issue of cost namely; nutrient media, equipment and the structures (Gitonga *et al.*, 2010). All these have been addressed in one way or another albeit for other crops. For instance, table sugar has been utilized in many laboratories as an alternative source of carbon (Kaur *et al.*, 2005; Demo *et al.*, 2008). Alternative low cost tissue culture materials and equipment have also been used in cassava micropropagation (Escobar *et al.*, 2001). Molasses has been used as a source of carbohydrates and vitamins for cassava tissue culture (Santana *et al.*, 2009). Results showed that plants regenerated on media containing molasses were stronger, greener and vigorous. The conventional gelling agents such as agar have been successfully replaced with support matrices (Goel *et al.*, 2007). The use of locally available fertilisers as alternative nutrient sources for the conventional MS salts was successful with significant ($P<0.05$) cost reductions achieved. The use of fertilisers as alternative tissue culture nutrient sources has been done but on other crops. Different kinds of fertilisers at different concentrations have been used in cassava tissue culture (Escobar *et al.*, 2005). In this study, the highest cost reduction was achieved in the substitution of the carbon source followed by macronutrients and micronutrients respectively. Further, cost reduction was achieved by not incorporating any growth regulator in the medium. Sweet potato has been reported as an easy to propagate crop capable of forming shoots and roots without inclusion of growth hormones in the media (Rolando *et al.*, 1992).

The rate of node formation increased consistently over the six weeks of each culture reaching a number that prompted subculture or acclimatisation for the case of the final subculture. Nodes are points of shoot development hence crucial during multiplication since one node represents one new plantlet (Mutegi, 2009). Tissue culture of sweet potato is mainly through direct organogenesis, through the use of nodal cuttings thus the higher the number of nodes the higher the number of plantlets. Variety KEMB 36 had better culture efficiency compared to Tainurey, having realised a regeneration index of 7 nodes per plantlet on the low cost medium and 4 nodes per plantlet on the conventional medium. This means that the variety is the most suitable for adoption into farm systems since high number of planting materials can be realised. KEMB 36 had a smaller inter-nodal length compared to Tainurey and this may be one of the qualities that made it produce more nodes compared to Tainurey. The small inter-nodal length was, however, a challenge when excising plantlets into nodal cuttings for introduction into culture medium during multiplication.

The *in vitro* plantlets cultured on the low cost medium had well developed leaves with the same morphology as the mother stock plants. Variety KEMB 36 had superior performance in terms of leaf formation on both the low cost and the conventional media compared to Tainurey.

Changes in leaf numbers throughout the culture periods were associated with changes in the number of nodes and plant height. Leaves contain chlorophyll which is essential in converting light energy to chemical energy, hence, a good leaf system is crucial for successful acclimatisation of plantlets. Plantlets with well structured leaves are photosynthetically efficient hence adapt quickly to the natural conditions.

Root development is critical for *ex vitro* survival of plantlets. *In vitro* root production enhances acclimatisation success because functioning roots create favorable plant water balance (Seelye *et al.*, 2003). They are believed to compensate for water loss caused by malfunctioning stomata. The physiological status of roots is critical for plant survival during the first few days of acclimatisation (Jorge, 2002). Well developed roots with strong connections on the plantlets are desirable for hardening. The two sweet potato varieties exhibited varying root formation patterns with Tainurey producing more roots on the low cost medium than on the conventional medium while KEMB 36 had significantly higher number of roots on the conventional medium compared to the low cost medium. This is an indication of genotype-dependent response to tissue culture by the two varieties. This was reinforced by the fact that KEMB 36 produced more roots on the conventional medium compared to Tainurey while on the low cost medium the latter had more roots.

The two varieties had taller plants in the low cost medium compared to the conventional medium which shows that it is possible to develop cost efficient nutrient medium that is better than MS media. Genotype-dependent growth was also evident in regard to this parameter with KEMB 36 producing significantly ($P<0.05$) taller plants compared to Tainurey on both media. Plant height is an important parameter when it comes to multiplication. Tall plants with intermediate internodal spacing produce many nodal cuttings and are easy to excise.

Genotype-dependent response to tissue culture was evident both on the low cost medium and the conventional medium. This differential response of sweet potato varieties to tissue culture has been reported elsewhere on different sweet potato varieties (Dessai *et al.*, 1995). He noticed significant differences in the regeneration frequencies of 27 sweet potato genotypes from a wide geographical distribution. These differences can be attributed to the differences in the genetic make-up of the different varieties. The differential response to tissue culture among different varieties means that *in vitro* regeneration procedures must be developed for each desirable genotype. A good tissue culture medium should be able to support the regeneration of a wide array of varieties of the target crop. However, given the fact that sweet potato is hexaploid and highly heterozygous small differences in response to tissue culture can be expected.

Acclimatisation of the plantlets was successful making this protocol a success. The benefit of any tissue culture procedure can only be realised after successful transfer of plantlets from *in vitro* culture conditions to *ex vitro* natural conditions. There is a lot of differences between the two environments including quantity and quality of light, relative humidity, nutrients, gaseous composition and medium substrate (Seelye *et al.*, 2003). The capability of plantlets to withstand *ex vitro* stress determines the success of any tissue culture protocol (Ahloowalia, 2002). The high survival rate of the sweet potato plantlets is attributed to the development of good root and leaf systems. Plantlets with well developed roots and leaves have been reported to adapt easily to natural conditions outside the growth room(Nowak & Pruski, 2002).

Conclusion

The successful use of alternative nutrient sources for sweet potato tissue culture is an indication that it is possible to produce disease-free seedlings at a lower cost without compromising the quality. This will reduce the cost of seedlings which will significantly increase productivity of the crop. Adoption of this protocol will greatly enhance establishment of affordable and reliable seed systems for sweet potato.

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