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Response of two sweet potato varieties regenerated on low cost tissue culture medium

K.O. OGERO[1], N.M. GITONGA[3], M. MWANGI[1], O. OMBORI[2] & M. NGUGI[2]
[1]Department of Agricultural Science and Technology, Kenyatta University, P. O. Box 43844-00100, Nairobi, Kenya
[2]Department of Plant and Microbial Sciences, Kenyatta University, Kenya
[3]Department of Agriculture, Meru University College of Science and Technology, P. O. Box, 972 - 60200, Meru, Kenya
Corresponding author: Ogero.ko@gmail.com

Abstract Plant tissue culture continues to be of great interest within the realms of molecular biology, plant breeding and plant health. However, different plant cultivars have different culture efficiency to tissue culture. In this research, the response of two Kenyan sweet potato varieties cultured on a low cost tissue culture medium was evaluated. The low cost medium contained plant nutrients that were obtained from locally available materials such as fertilizers. Each conventional Murashige and Skoog (MS) macronutrient was individually substituted with a locally available fertilizer. The conventional source of micronutrients was substituted with Stanes® Iodized Microfood while sucrose was obtained from table sugar. Performance of the two cultivars was monitored over a period of six weeks. KEMB 36 had a better performance than Tainurey with an average of 8 nodes, 7 leaves, 3 roots and height of 4 centimeters per plantlet indicating genotype-dependent response.

Key words: Culture efficiency, low cost tissue culture medium, cultivar, genotype-dependent

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam) is one of the most important staple crops in the sub-Saharan Africa. The crop which belongs to the morning-glory family (Convolvulaceae) and which originated from Latin America is widely grown within the region and its ability to thrive well in marginal areas appeals to many farmers (Ayabei, 2010). The crop is the sixth important food crop worldwide after rice, wheat, Irish potatoes, maize and cassava. In Kenya, the crop is important for food security and is largely grown by smallholder farmers in rural areas. With the increasing emphasis on commercially oriented agriculture, sweet potato stands out as one of the crops that can earn farmers huge incomes. Any efforts geared towards improving its productivity will therefore have positive impacts on the small scale farmers.

Despite its potential in improving food security in the sub-Saharan Africa region, sweet potato production has been on the decline due to a number of constraints such as viral diseases and insect pests. The complexity of farming systems in rural Africa makes control of these constraints difficult. Sweet potato is commonly grown by farmers in complex, mixed cropping systems where they normally plant different varieties with different characteristics on the same plot (Kapinga *et al.*, 2007). Biotechnological interventions such as tissue culture (TC) and genetic engineering can offer alternative strategies to alleviate these constraints. Development of a reliable *in vitro* plant regeneration procedure for sweet potato is a pre-requisite for its improvement by genetic transformation. Tissue culture offers the opportunity of producing large numbers of disease-free sweet potato seedlings. Virus-indexing usually done before the tissue culture process is efficient in virus detection hence assuring one that the explants being used are viral-free. Tissue culture is however, an expensive venture which has slowed down its adoption in the developing countries. To ensure the trickling down of TC benefits to small holder farmers, low cost tissue culture interventions are needed. There is a need for low-cost plant tissue culture systems, applicable for micropropagation and *in vitro* conservation of plant genetic resources in order to increase adoption of TC in developing countries (Savangikar, 2002). Micropropagation costs include those for nutrient media chemicals. Low cost tissue culture protocols have been developed for other crops and have proven to be efficient. Cost reductions of up to 73% have been recorded for plant regeneration and *in vitro* conservation of Turmeric (Tyagi *et al.*, 2007). Tremendous work has also been achieved in reduction of cassava micropropagation costs (Santana *et al.*, 2009).

However, despite all the efforts that have been made in lowering the costs of tissue culture, genotype-dependent response to various TC media remains an impediment. Genotype-dependent morphogenetic response has been reported in pigeon pea (Naidu *et al.*, 1995). Genotype-dependent effects imply that tissue culture and transformation strategies must be re-designed for poorly performing genotypes and different protocols developed for different genotypes. This study sought to monitor the response of two sweet potato varieties, to a low cost tissue culture medium.

Materials and Methods

Plant materials. The plant materials used in this research were two sweet potato varieties, KEMB 36 and Tainurey.

The two varieties were developed by the Kenya Agricultural Research Institute and were chosen on the basis of farmer-preference.

Media preparation. A low cost medium consisting of locally available fertilisers, as alternative nutrient sources, was developed and used (Table 1). The conventional source of all the macronutrients apart from calcium chloride was substituted with locally available fertilizers. A single substitution was done for the case of the source of micronutrients. Stanes Iodized Microfood® that contains microelements required for plant growth was used as the alternative source for the micronutrients. Table sugar obtained from local shops was used as the alternative source of sucrose. The low cost medium consisted of 100ml/l of macronutrients' stock solution, 10ml/l of magnesium sulphate stock solution, 0.2g/l of Stanes Iodized Microfood®, 30g/l of table sugar and 3g/l of gelrite. The MS salts supplemented with 30g/l of table sugar and 3g/l of gelrite was used as the control. Both media were sterilized by autoclaving at a temperature of 121°C and 15 pounds of pressure per square inch for 15 minutes.

Preparation of explants. Nodal explants were obtained from healthy mother stock plants and washed with running tap water. They were then disinfected with 1.5 % sodium hypochlorite containing a drop of Tween 20® for 20 minutes before immersing them in 70% v/v ethanol for 6 minutes. The explants were then rinsed four times using sterile distilled water and kept in the laminar hood flow under sterile conditions.

Culture initiation. The damaged end points of the sterile explants were spliced off with a sterile scalpel into 2 cm long pieces. They were then inoculated on the culture media and the culture bottles labeled with the variety type and date of culture. The cultures were then transferred into the growth room where they were arranged in a completely randomized design with nine replications per variety and incubated at a temperature of 28ºC with a photoperiod of 16 hours light and 8 hours darkness. The cultures were regularly checked and the progress in leaf formation, node development, root production and plant height recorded at intervals of 14 days for six weeks.

Data analysis. Analysis of variance was done using STATA® statistical program to ascertain the differences between the two sweet potato varieties for the parameters measured. Separation of means was done using Tukey's test at 5% significance level.

Results

Node formation. The two sweet potato varieties exhibited significant ($P<0.05$) differences in node development from the first week of culture with KEMB 36 producing more nodes compared to Tainurey (Fig. 1). The two varieties showed an upward trend in node formation with the highest number of nodes being realized in the sixth week.

Leaf formation. The variety KEMB 36 produced significantly ($P<0.05$) higher number of leaves compared to Tainurey throughout the culture period (Fig. 2). KEMB 36 had a mean of 7.5 nodes per plantlet at the end of the culture period while Tainurey had a mean of 2.7 nodes per plantlet.

Root production. No significant ($P>0.05$) differences were recorded in root formation for the two varieties during the second week of culture with KEMB 36 producing an average of 1.4 roots per plantlet while Tainurey had 1.5 roots per plantlet (Fig. 3). However, there were significant differences in root production between the two varieties during the fourth and sixth weeks with KEMB 36 having more roots compared to Tainurey in the fourth week of culture while Tainurey had more roots compared to KEMB 36 at the end of the culture period.

Plant height. The two sweet potato varieties had significant ($P<0.05$) differences in plant elongation from the first week of culture with KEMB 36 having taller plants compared to Tainurey throughout the culture period (Fig. 4).

Table 1. Composition of the low cost medium used in sweet potato tissue culture.

Component	Amount in stock solution (g/l)	Amount in culture medium (g/l)	Amount of stock solution per litre of the medium (ml/l)
Macronutrients			
Calcium chloride (conventional)	9	0.9	100
Monopotassium phosphate (MKP)	3.5	0.35	
Potassium nitrate fertilizer	40	4	
Ammonium nitrate (quarry explosive)	35	3.5	
Magnesium sulphate			
Epsom salt	37	0.37	10
Micronutrients			
Stanes iodised microfood®	-	0.2α	-
Carbon source			
Table sugar	-	30α	-

αWere added during media preparation

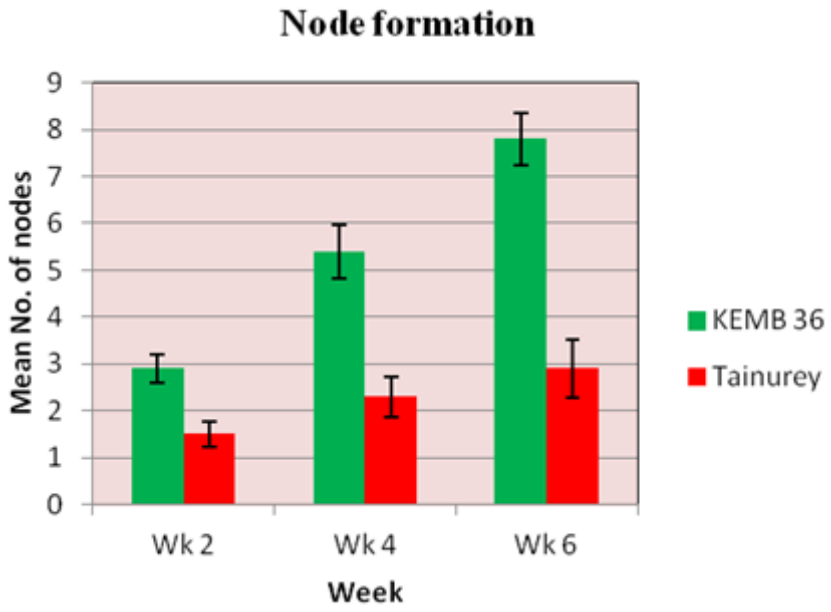

Figure 1. Sweet potato varieties, KEMB 36 and Tainurey, showing differences in node formation on the low cost medium.

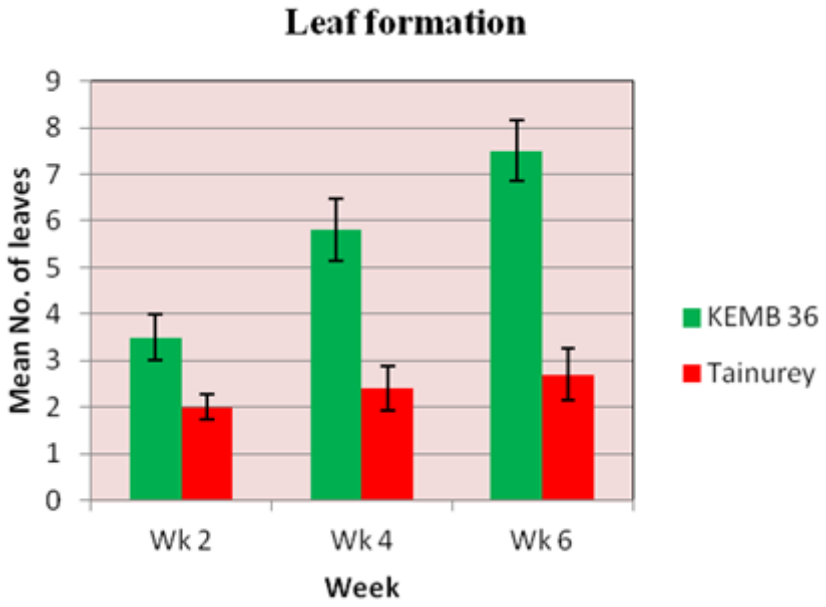

Figure 2. Leaf development for two sweet potato varieties, KEMB 36 and Tainurey, cultured on low cost medium.

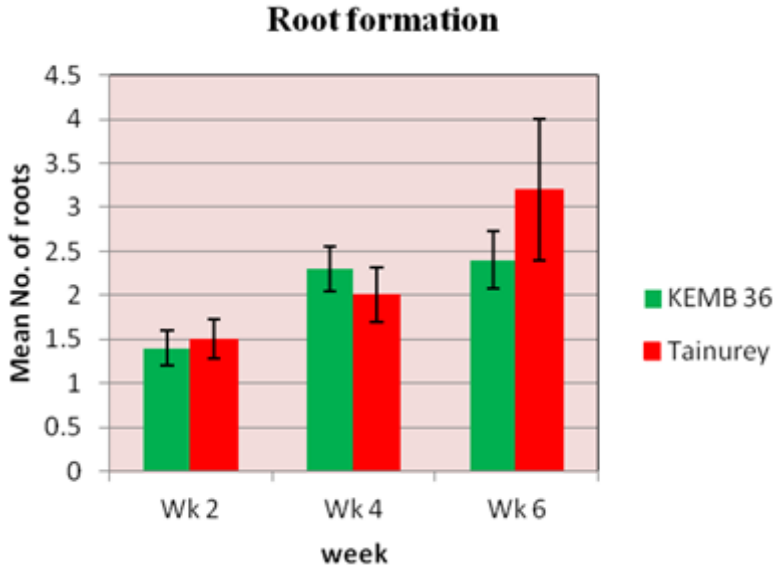

Figure 3. Intervarietal differences in root formation for two sweet potato varieties cultured on low cost medium.

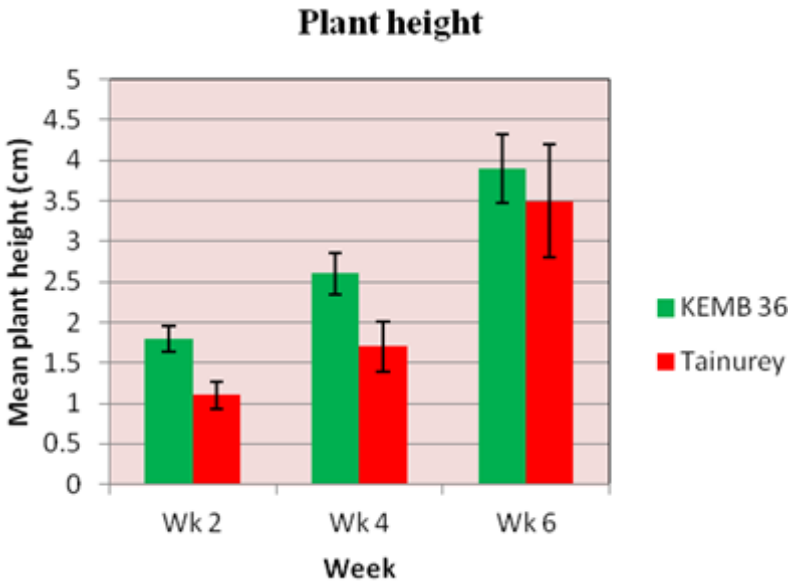

Figure 4. Differences in plant height for two sweet potato varieties cultured on low cost medium.

Discussion

With the increasing human population there is an urgent need to increase food productivity so as to meet the expected rise in demand. Attention has shifted to biotechnological techniques to increase food production. Tissue culture is one of these techniques and has really boosted propagation of vegetative crops and aided crop transformation through genetic engineering. Tissue culture involves asexual propagation to generate whole plants from small plant parts or cells (Chawla, 2000). The technique allows thousands of genetically identical plants to be derived from a single cell or tissue within a short time. Successful *in vitro* plant regeneration protocols are also paramount in successful genetic modification. Tissue culture has been applied for many years now in production of seedlings for many vegetatively propagated crops including sweet potato. However, farmers from many developing countries have not benefited fully from this technology, a factor attributed to the high cost of production. Efforts have been made to lower production costs but this has mainly concentrated on crops such as banana and cassava with little done on sweet potato. Sugarcane juice has been reported as an alternative source of carbon for banana and plantain tissue culture (Buah *et al.*, 2011). A lot of work has also been done in reducing the cost of tissue culture for cassava (Escobar *et al.*, 2001; Santana *et al.*, 2009). However, the differential response of various crop varieties to tissue culture makes the work of designing cost efficient media even more difficult. The media developed here significantly reduced the cost of sweet potato tissue culture but there were notable differences in the response of the two varieties used for all the parameters tested.

The variety KEMB 36 had a high regeneration index compared to Tainurey meaning it was the best suited for this medium because a high number of planting materials can be obtained. However, the variety had small internodal space making it difficult to excise during multiplication. Sweet potato varieties with small internodal spacing have been reported to be difficult to manipulate (González *et al.*, 1999). This variety also had taller plants which further augment its suitability for multiplication. Sweet potato has been reported as a recalcitrant crop to regenerate and often has genotype-dependent response to *in vitro* regeneration (González *et al.*, 2008). Results showed differences in culture efficiency of the sweet potato varieties Jewel and CEMSA 78354. This genotype-dependent response to regeneration methods has made sweet potato to lack an efficient and reliable system which further compromises transformation strategies. It has been reported that one of the challenges in developing transgenic sweet potatoes is that novel or modified *in vitro* regeneration procedures must be developed for each desirable genotype because of the significant variability in the response to hormone combinations (Santa-Maria *et al.*, 2009).

The differences in the responses of the varieties used here indicate that the low cost medium developed is more suitable in regenerating KEMB 36 while Tainurey may require some adjustments. Every cultivar vary widely in their response to tissue culture and plant regeneration because of the inherent genetic make up (Gosukonda *et al.*, 1995b). Genotypic characteristics therefore influence the success of *in vitro* regeneration and have been attributed to differential composition of phenolic compounds and anthocyanins in various sweet potato cultivars (Gosukonda *et al.*, 1995a; Islam *et al.*, 2002)

Conclusion

As efforts are made towards development of low cost media for sweet potato tissue culture consideration should also be put on intervarietal differences in response that exist in sweet potato. An efficient media should support regeneration of a wide array of cultivars.

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