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# Low Cost Macronutrients in the Micropropagation of Selected Sweet Potato [*Ipomoea Batatas (L.) Lam*] Varieties

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**Abstract**

Sweet potato is a dicotyledonous plant in the family convolvulaceae family. Although i*n vitro* regeneration protocols have been developed for producing disease and pest free planting materials that can be produced throughout the year, micropropagation cost are high. The objective of this study was to assess *in vitr*o regeneration response of KSP 36 and KEMB 36 varieties using low cost macronutrients source to reduce the cost of micro-propagule production. Three conventional macronutrients; ammonium nitrate, potassium nitrate and magnesium sulphate were substituted each at a time with ammonium fertilizer, potassium fertilizer and Epsom salt respectively. Nodal cuttings were used as source of explants. Results obtained from the present investigation indicated that explants cultured in low cost macronutrient Epsom substitute media, performed better in regeneration in term of leaves and nodes formed compared to the conventional media, while in other substitute significant differences ($p > 0.05$) were not detected in varieties tested. Use of locally available macronutrients significantly ($p < 0.05$) reduced the cost of micropropagation of sweet potato. KEMB 36 performed better compared to KSP 36 in regeneration response. There is therefore a potential for using locally available low cost macronutrients source as alternative to the conventional costly laboratory macronutrients.

**Keywords**: low cost macronutrients; variety; sweet potato; *in vitro* regeneration; genotype-dependent

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## 1. Introduction

Sweet potato is considered to be versatile and the most under exploited of the developing world's major crops (Alam *et al.,* 2013) as evidenced by its breeding initiatives that are at relatively at early stages compared to other crop (Rees *et al.,* 2003). It's one of the world's highest yielding crops in term of production per unit areas, exceeding that of major cereals such as rice (Woolfe, 1992), with an average yield potential of 30 tons per hectare lower agricultural input. However yield in Africa are less than 5 tons per hectare in comparison with the world's average of 15 tons per hectare (Luo *et al*., 2006). Sweet potato ranks seventh among the most important food crop on flesh weight basis in the world (Alam *et al.,* 2013) and fifth in over 50 developing countries after rice, wheat, maize and cassava (CIP, 1999).

Sweet potato is an important food security crop for domestic consumption and as a household source of income in Eastern Africa countries. It is the third most significant tuber crop globally and staple food crop in most families in East Africa (Kyamanywa *et al*., 2011). The orange fleshed sweet potato genotypes have high concentration of beta-carotene a precursor for vitamin A (Burri, 2011). This means sweet potato has the potential of preventing vitamin A deficiencies such as blindness in children ( Williams *et al*., 2013).

Sweet potato is mainly propagated vegetatively through the use of vines; hence it is easier to pass diseases to the next generation if the vines contain disease causing pathogen (Coolong, *et al*., 2012). Viral diseases and pests are the major constrain hindering sweet potato production in Africa with a yield reduction of up to 98% and extinction of elite cultivars (Gasura, *et al*., 2008). Sweet potato viruses are mainly spread through healthy looking vines, which farmers collect from the previous crop for the next cropping cycle. The control of these viruses and pests is impossible once the plant is infected and this has made use of resistant cultivars an attractive option (Adane, 2010).

Biotechnological interventions such as tissue culture and genetic engineering offer great potential for improving sweet potato (Liu *et al*., 1998). Tissue culture refers to a set of techniques that permit the regeneration of cells, tissues and organs of plants, from parts of plant organs or tissues, using nutrient solutions in aseptic and controlled environment (Lima *et al.,* 2012).

Differences in tissue culture and transformation responses of sweet potato across the cultivars are due to the genotypic effect (Alam *et al*., 2013).

According to smith (2004), use of tissue-cultured sweet potato by smallholder farmers in Zimbabwe improved household food security. Some of the farmers obtained yield of up to 25 tons per hectare against a national average of 6 tons per hectare. This increase in yield was attributed to the disease free status of the planting materials.

Micropropagation or tissue culture method of propagation is one of the technologies with the potential of producing bulk of healthy planting materials without season limitation throughout the year. Recent studies done have shown that a single shoot tip has the potential of producing more than 60000 transferable plantlet per year (Amoo *et al.,* 2011).This will provide adequate planting materials, which are disease free. However, the cost of tissue-culture based plant propagation is high thus limiting the applicability of the technology in the developing countries, therefore there is need to develop low cost micropropagation protocol to lower the cost of micro-propagule to enable accessibility of tissue cultured planting material to farmers (Santana *et al.,* 2009).

In many developing countries, the establishment cost of facilities and unit production cost of micropropagated plants is high and often the return on investment is not proportional to the economic potential advantage of the technology (Savangikar, 2004). This necessitates the need to source for alternative low cost facilities, equipment and chemicals. Various studies have addressed the problem by decreasing the unit cost of production like low technology tissue culture materials for initiation and multiplication of banana (Gitonga *et al.,* 2010). A simple and low cost strategy for micropropagation of cassava (*Manihot esculenta crantz*) and role of low cost options in tissue culture has been reported (Savangikar, 2004; Santana *et al*., 2009). Santana (2009) reported that fully substituted media with commercially available nutrients (Hydro Agri's fertilizer) for the micropropagation of cassava reduced cost by 93.1 % and showed a good growth performance compared to the traditional media.

Low cost technology tissue culture can be achieved by improving process efficiency, better utilization of resources and use of available low cost resources. The objective of this work was to evaluate the potential for *in vitro* regeneration of selected sweet potato varieties using low cost macronutrients to reduce the cost of macro-propagule production thus reducing the unit cost of the vines. This could result to accessibility of adequate clean planting materials to farmers and this in turn could lead to poverty alleviation through increased nutrition and income among smallholder farmers who are involved in sweet potato production in sub-Saharan Africa.

## 2. Materials and Methods

### 2.1 Collection of Plant Materials

This study was carried out at tissue culture laboratory Kenyatta University. The sweet potato varieties used in this study were KSP 36 and KEMB 36 obtained from Kenyatta University, Biotechnology transformation laboratory. The cuttings of the vines were planted in pots and maintained in the greenhouse in the Department of Plant Sciences, Kenyatta University for bulking to obtain sufficient germplasm.

### 2.2 Culture Media Preparation

The procedure for the preparation of the media for sweet potato tissue culture by Sefasi and Nankinga (2010) which is a Modified Murashige and Skoog (MMS) media was used in this study (Table 1). Conventional macronutrients ammonium nitrate, potassium nitrate and magnesium sulphate were substituted each at a time with the low cost macronutrients as shown in Table 1 while the other nutrients remained the same. The conventional macronutrients were used as control in this study. The pH of all the media used was adjusted to the 5.8 before adding the gelling agent (gelrite). The media were sterilized at a pressure of 1.05 kg $cm^{-2}$ and a temperature of 121 $^{O}C$ for 15 minutes.

Table 1: Conventional macronutrient and corresponding low cost macronutrient substituted

| Conventional macronutrient | Low cost macronutrient |
|---|---|
| $NH_4NO_3$ | Ammonium fertilizer |
| $KNO_3$ | Potassium fertilizer |
| $MgSO_4$ | Epsom salt |

## 2.3 Preparation and Sterilization of Sweet Potato Explants

Vines from vigorously growing 5-week old plants were placed in sampling bags and taken to the tissue culture laboratory. Leaves were removed and the stem having 5-8 nodes were washed thoroughly in running tap water to remove dirt, before cutting them into one node segment. The nodal segment of 2-3 cm were surface sterilized using 70 % ethanol for 2 minutes, 5 % hydrogen peroxide for 2 minutes followed by 1.5 % commercial jik with 2 drops of Tween 20. They were rinsed six times with sterile distilled water.

## 2.4 Shoot and Root Induction

The ends of the nodal cutting were trimmed and the nodal segments were cultured into a solidified medium supplemented with 3 % table sugar and 1 mg $l^{-1}$ BAP for shoot induction. Five replicates of the explants per variety were cultured. The cultures were arranged in a completely randomized block design. The cultures were incubated in growth chamber at a temperature of 27±1 $^{O}$C, with a light intensity of 1000 lux provided by cool white fluorescent lamp with a photoperiod of 16/8h (day/night). The number of leaves and nodes formed were recorded after five weeks of culture. The initiation of roots was carried out in a Modified MS media containing half strength nutrients.

## 2.5 Acclimatization

The *in vitro* regenerated plantlets with well-developed root and leaf systems were washed with tap water to remove the media to avoid fungus growth. The plantlets were transplanted onto pots containing a mixture of rice husks and red soil. The pots were covered with transparent polythene sheets and the plants were watered for two weeks for acclimatization. The plants were the transplanted into polythene bags containing a mixture of red soil and manure in a ratio 2:1

2.6 Data Analysis

The data were analyzed using Analysis of variance (ANOVA) with MINITAB computer software version 23.22. Data analyzed included number of leaves and nodes of the varieties between the conventional and low cost micronutrients and the cost efficiency between the low cost macronutrient source and conventional macronutrient source.

## 3. Results

3.1 Cost Efficiency

Cost savings of 98.96% was recorded when ammonium fertilizer was used as a source of macronutrient. Similarly, cost saving of 97.30% and 97.43% was recorded when Epsom salt and potassium fertilizer were used as alternative source of micronutrient respectively. Overall substitution of conventional macronutrients with low cost locally available macronutrients reduced the overall cost of micropropagation of sweet potato by 97.9 % (Table 2).

Table 2: Cost efficiency of conventional macronutrients compared with low cost macronutrients

| Conventional macronutrient (CM) | Price per litre of the culture media (CM) | Low cost Macronutrient (LM) | Price perlitre of the culture media (LM) | % cost saving |
|---|---|---|---|---|
| $NH_4NO_3$ | Ksh 84 | Ammonium fertilizer | Ksh 0.875 | 98.96 |
| $MgSO_4$ | Ksh 2.59 | Epsom salt | Ksh 0.07 | 97.30 |
| $KNO_3$ | Ksh 70 | Potassium fertilizer | Ksh 1.8 | 97.43 |
| Total | 156.59 | | 2.75 | 97.9 |

CM, Conventional macronutrient; LM, Low cost macronutrient

### 3.2 Regeneration Response of Sweet Potato Varieties

KEM 36 and KSP 36 sweet potato varieties regenerated from nodal cuttings in both conventional and low cost macronutrients (Fig. 1A-D). The two varieties responded differently in the three-experimental set in term of leaves and nodes formed between the low cost and conventional macronutrient sources. There were significantly higher number of leaves and nodes formed in both KSP 36 and KEMB 36 in media containing low cost $MgSO_4$ (Epsom salt) compared to media with conventional $MgSO_4$ (Table 3). Significant differences were not detected ($p > 0.05$) on the number of leaves and nodes formed in both KSP 36 and KEMB 36 in media containing low cost $NH_4NO_3$ (ammonium fertilizer) compared with media containing conventional $NH_4NO_3$ (Table 4). In media containing low cost source $KNO_3$ (potassium fertilizer) there was no significant difference ($p > 0.05$) in number of leaves and nodes formed compared to media containing conventional $KNO_3$ for KSP 36 but the difference was significant ($p < 0.05$) in the case of KEMB 36 in which in media containing conventional $KNO_3$ more leaves and nodes were formed compared to low cost (Table 5). KEMB 36 had significantly ($p < 0.05$) high number of nodes and leaves overall compared with KSP 36 a medium containing $MgSO_4$ and $NH_4NO_3$ (Table 3 and Table 4).

Roots were formed in the same media used for regeneration when the cultures were left for one month and had no sub-roots in all the three varieties tested. However root induction in Modified MS media containing half strength nutrients formed sub-roots which grew vigorously (Fig. 1E). *In vitro* regenerated sweet potato plantlets were successfully transferred into the soil in the greenhouse (Fig. 1F).

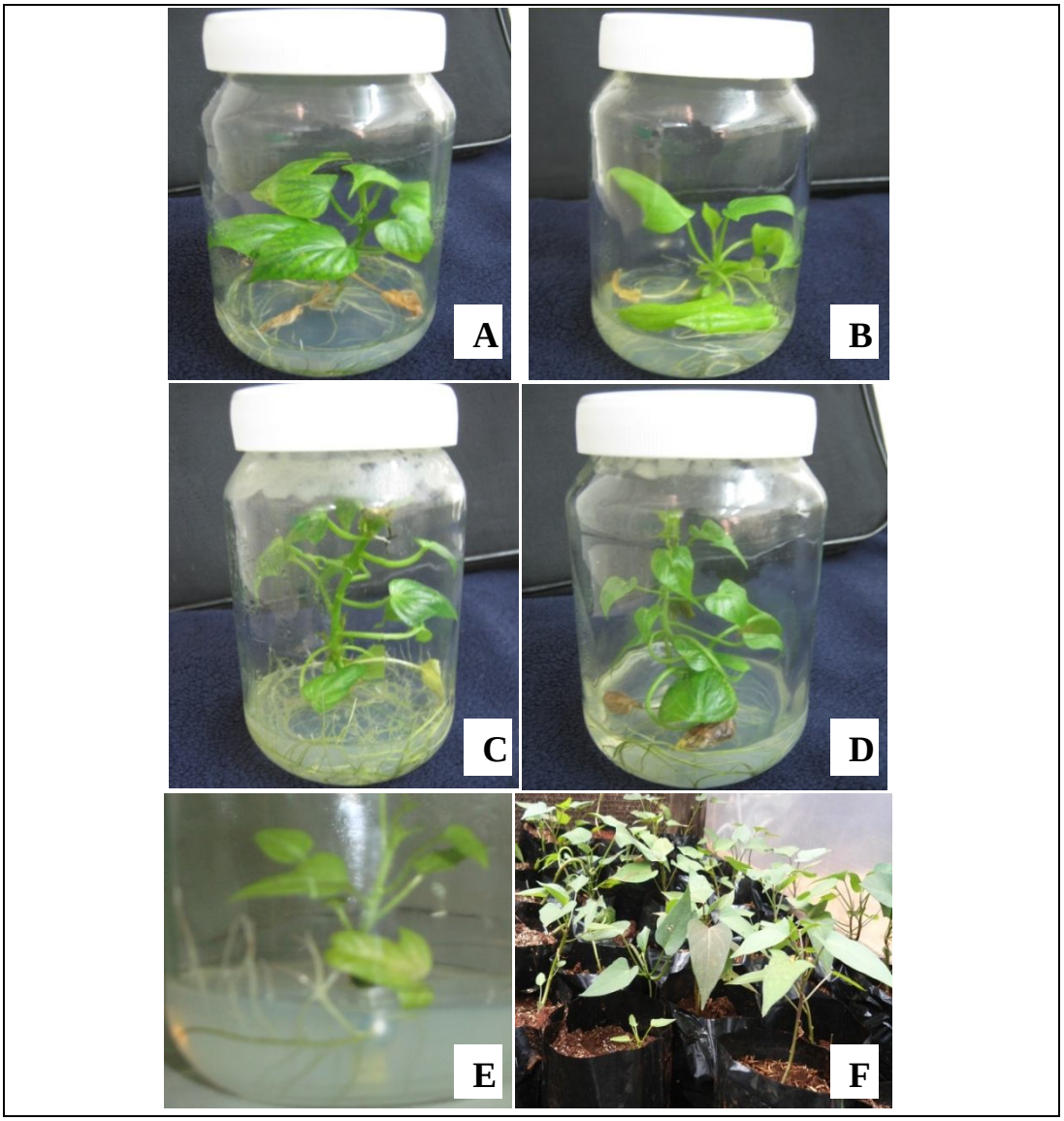


Figure 1: Regeneration response of different sweet potato on different media. A, KSP 36 plants on the medium containing conventional macronutrient (ammonium fertilizer); B, KSP 36 plants on the medium containing low cost conventional macronutrient; C, KEMB 36 plants on the medium containing conventional macronutrient; D, KEMB 36 plants on the medium containing low cost conventional macronutrient (ammonium fertilizer); .E, Root induction in half strength modified MS media. F, *In vitro* regenerated sweet potato plantlets in the greenhouse.

Table 3: Effect of the MMS media containing low cost $MgSO_4$ (Epsom salt) and conventional $MgSO_4$ macronutrient on the number of leaves and nodes formed on the 5th week after culture of nodal explants

| Parameter | Variety | Type of macronutrient | | Overall mean |
|---|---|---|---|---|
| | | Epsom salt | Conventional $MgSO_4$ salt | |
| Number of leaves | KSP 36 | 5.50 ± 0.93 | 3.95 ± 0.60 | 4.73 |
| | KEMB 36 | 7.55 ± 1.04 | 6.85± 0.93 | 7.20 |
| Number of nodes | KSP 36 | 6.40 ± 0.96 | 4.95± 0.68 | 5.68 |
| | KEMB 36 | 8.50 ± 1.030 | 7.90 ± 0.95 | 8.20 |

Table 4: Mean number of leaves and nodes formed on MMS media containing low cost $NH_4NO_3$ (Ammonium fertilizer) and conventional $NH_4NO_3$ macronutrient on the 5th week after culture of nodal explants

| Parameter | Variety | Type of macronutrient | | Overall mean |
|---|---|---|---|---|
| | | Ammonium fertilizer | Conventional $NH_4NO_3$ salt | |
| Number of leaves | KSP 36 | 3.63 ± 0.26 | 3.80 ± 0.49 | 3.72 |
| | KEMB 36 | 4.70 ± 0.44 | 4.30 ± 0.51 | 4.50 |
| Number of nodes | KSP 36 | 4.60 ± 0.39 | 4.90 ± 0.53 | 4.75 |
| | KEMB 36 | 5.30 ± 0.42 | 5.35 ± 0.49 | 5.33 |

Table 5: Mean number of leaves and nodes formed on MMS media containing low cost $KNO_3$ (Potassium fertilizer) and conventional $KNO_3$ macronutrient on the 5th week after culture of nodal explants

| Parameter | Variety | Type of macronutrient | | Overall mean |
|---|---|---|---|---|
| | | Potassium fertilizer | Conventional $KNO_3$ salt | |
| Number of leaves | KSP 36 | 4.15 ± 0.37 | 4.55 ± 0.63 | 4.35 |
| | KEMB 36 | 3.15 ± 0.29 | 4.20 ± 0.35 | 3.68 |
| Number of nodes | KSP 36 | 5.60 ± 0.43 | 5.50 ± 0.62 | 5.55 |
| | KEMB 36 | 4.40 ± 0.31 | 5.05 ± 0.30 | 4.73 |

## 4. Discussion

Sweet potato production is greatly constrained by diseases (mainly viruses and pest diseases) and lack of enough planting materials. The introduction of tissue culture technology leads to availability of sufficient clean planting material throughout the year without season limitation thus partially solving sweet potato production constraints. However, the cost of the plantlets has hindered accessibility of healthy planting material to farmers. The high cost is due to expenses incurred during *in vitro* micropropagation. In an effort to avail sufficient clean planting material for sweet potato to the resource poor smallholder farmers, this study developed a low cost medium which can be used to propagate sweet potato from nodal cuttings.

In this study the cost of medium reduced when low cost macronutrients source were used (Table 2). This is in agreement with the findings by Gitonga *et al.* (2010) in which the cost was reduced by. 96.2 %, 93.1 % and 95.0 % respectively when low cost macronutrients (ammonium fertilizer, Epsom salt and potassium fertilizer) were used in the in the initiation and multiplication of banana plants. According to Ogero *et al.* (2012a), the use of ammonium quarry salt, Epsom salt and potassium fertilizer in the regeneration of cassava reduced the cost of macronutrients by 96.2 %, 93.0 % and 94.9 % respectively.

In this study, KEMB 36 had the best regeneration response due to the higher number of leaves and nodes formed compared to KSP 36 (Table 2 and 3). This shows that regeneration was genotype dependent.

This is in agreement with result reported by Gasura *et al* (2008) which showed that regeneration response was genotype dependent. Sweet potato germplasm has high genetic variability; therefore differences in micropropagation responses across the varieties can be attributed to genotypic effect (Alam *et al.,* 2013). According to Ogero *et al*. (2012b), the response of KEMB 36 sweet potato variety was higher in a low cost medium compared to Tainurey. The sweet potato species varies widely in their response to micropropagation due to their genetic constitution. In addition, influence of tissue culture-based plant regeneration has been attributed to differential composition of phenolic compounds and anthocyanin in various sweet potato cultivars (Far and Taie, 2009). In all the media containing low cost macronutrient KSP 36 and KEMB 36 sweet potato varieties performed better compared to the media with conventional macronutrients (Tables 3 - 5). This is consistent with Ogero *et al* (2012c) who successfully substituted conventional Murashige and Skoog salts with Easygro vegetative fertilizer containing both micro and macronutrients in the micropropagation of sweet potato.

In conclusion, the study shows that the substitution of conventional macronutrients with locally available sources reduced the cost of the micropropagation sweet potato varieties. Thus, it is necessary to carry out further studies of other sources of nutrient that can cut down the cost without compromising the quality of plantlets. This will consequently lower the cost of the micro-propagules thus enabling accessibility of clean planting materials to the smallholder farmers who grow the sweet potato.

The regeneration response of the two sweet potato varieties used in this study were found to be genotype dependent; hence it is necessary to study regeneration response of other varieties to ascertain the applicability of this protocol.

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