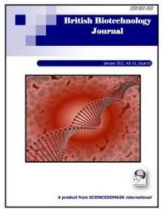

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Low Cost Micropropagation of Local Varieties of Taro (*Colocasia esculenta* spp.)

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Authors' contributions

All the authors collaborated in carrying out this work. Authors SR, OO and GN designed the study and supervised laboratory and greenhouse experiments. Authors AN and MN carried out the laboratory and greenhouse experiments and wrote the first draft. Authors FK and AP performed data analyses of the study and drafted the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to evaluate low cost protocol for the micropropagation of three varieties of taro (Dasheen, Eddoe and wild) from eastern Kenya.
Study Design: The plants were grown in polythene bags arranged in a completely randomized block design (CRBD) replicated nine times.
Place and Duration of Study: Department of plant sciences Kenyatta University in plant and tissue culture laboratory, between June 2010 and December 2011.
Methodology: The three media types tested were Omex foliar feed (LCM1), Stanes micronutrients (LCM2) and micro food (LCM3) as substitute for Murashige and Skoog (MS) media.

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Results: The results showed significant differences ($p<0.05$) in the shoot generation for Eddoe and wild varieties in LCM1 and LCM2 respectively compared to LCM3 and MS. Plants grown in MS media and LCM3 had the longest height compared to LCM1 and LCM2. Naphthalene Acetic Acid (NAA) and Citishooter did not show any significant differences on the number of roots. All the regenerated plants in this study were similar in morphology and vigour. Media cost was reduced by 94.7% (LCM1) and 96% for both LCM2 and LCM3.

Conclusion: This study indicates the potential of low cost media as a substitute for conventional micro propagation.

Keywords: Citishooter; conventional; micronutrients; micro propagation.

1. INTRODUCTION

Taro is an important staple food crop grown throughout many Pacific Island countries, Asia, the Caribbean and many parts of Africa, for its fleshy corms and nutritious leaves. In addition to contributing to sustained food security and export earnings [1]. The crop is one of the principal root crops that have shown great potential in generating income within the rural communities [2]. In Kenya, taro is a neglected crop grown primarily by farmers in marginal areas and its large-scale cultivation is constrained by the lack of high quality seed and the low productivity and profit. The plant is very susceptible to a wide range of pests, pathogens and diseases [3], such as *Pythium* rot, dasheen mosaic virus and nematode diseases [3]. In attempt to address these challenges, routine methods such as Tissue culture has been suggested however the cost of production is high for most of the countries in the sub-Saharan Africa including Kenya. The cost of the micro-propagules production has precluded the adoption of the technology for large scale micro-propagation [4]. Media cost including chemicals and energy account for 30–35% of the cost of micro-propagation of plants [5,6]. Many studies have reported that the production cost of tissue-cultured plants can be reduced by 50-90% using low cost media ingredients and containers [7]. The conventional method of taro cultivation is through vegetative propagation. The division of taro offshoots is not always suitable for this cultivation due to the weakness and susceptibility to pathological agents. However, there is limited availability of clean planting materials. The aim of this study was to provide a reliable, low cost and high quality taro planting materials.

2. MATERIALS AND METHODS

2.1 Study Site and Sample Collection

Plantable setts of Dasheen, Eddoe and Purple/Wild varieties of taro visibly free from diseases were collected from Meru central region, Eastern Kenya. Meru district receives an average annual rainfall ranging between 380 mm -2500 mm. The plants were transported to Kenyatta University Plant Sciences net house. The plants were grown a completely randomized block design (CRBD) in polythene bags containing soil enriched with diammonium phosphate (DAP) fertilizer consisting of four treatments with nine replications. The plants heights, number of shoots and roots were monitored.

2.2 *In vitro* Propagation of Taro

Plants were washed with tap water and outer leaves removed until inner cleaner section appears with 5cm of shoot and corm of 2 cm. Plants were then surface sterilized in 2.31% NaOCl (60% commercial Jik) containing a few drops of Tween 20 for 45 minutes under a laminar flow with frequent agitation. Outer leaves were separated from the dome in a circular fashion using a sterile surgical knife. The explants were then transferred to 90% of ethanol for 1.5 min after which it was further sterilized in 70% ethanol for 12.5 min. The final trimming was done until the meristem domes of about 1 cm^2 were obtained which were rinsed 4-5 times with autoclaved double distilled water.

2.3 Low Cost Media Formulation for Taro

Three low cost substitutes for MS salts were tested. Omex foliar feed 24-24-18 + trace elements (LCM1) from Murphy Chemicals (E.A) Limited–a complete substitute for MS salts since it contains both macronutrients and

micronutrients. The second treatment (LCM2) consisted of Stanes micronutrients from Osho Chemicals Limited while macronutrients came from low cost alternatives in the market that are used as fertilizers available in agrovet shops. The last treatment consisted of microfood ® horticulture from Osho Chemicals as source of micronutrients while macronutrients came from low cost alternatives in the market (LCM3) as shown in Table 1. Conductivity and pH of the fully substituted media was measured and adjusted to 5.7-5.8 using KOH and HCl then autoclaved at 15 psi and 121ºC.

2.4 Culture Conditions

For all treatments that is conventional, LCM1 LCM2 and LCM3 55 ml of medium was dispensed in 5.5x10 cm glass flasks (200 ml flask); one explant (approx. 1 cm long) was cultured on each flask. Sterile meristem explants were subsequently cultured on the MS basal medium supplemented with 8 mg/l Benzo Amino Purine (BAP) and 30g/l sucrose. The cultures were then taken to growth room which growth conditions were: 25±2ºC, 18 h (day)/6 h (night) photoperiod with light source provided by irradiation intensity of 40~44 µmol m^{-2} s^{-1}. Shooting was induced by transferring plants to new media after seven days and the treatments replicated four times. Multiple shoots was recorded after 4 weeks. Regenerated shoots were then transferred onto MS medium supplemented with 0.25 mg/l naphthalene acetic acid (NAA) and 30 g/l sucrose for induction of roots. Growth parameters such as number of shoots number and size, roots number and plant height, were recorded at 15 days interval for 2 months after raising the cultures.

2.5 Hardening, Acclimatization and Morphological Characterization

Regenerated plants were removed from individual glass jars. Roots were then rinsed with warm water to remove excess media and planted in small green pots containing vermiculite: Sand in the same ratio then transferred to greenhouse under 70% shade. They were covered with polythene paper to maintain humid conditions and reduce excess water loss for 2-3 weeks. They were then transplanted into medium sized black polythene pots containing soil with 5 g/kg DAP fertilizer and monitored for a month with daily watering every morning. Subsequently plantlets were transferred out of green house and planted in larger plastic pots containing loam soil with DAP fertilizer. The regenerants were studied morphologically with regard to general appearance, shoot number, length of shoot and number of roots formed in both conventional media in comparison to those of low cost media. Survival of plantlets was recorded after 3 weeks [Survival plantlet (%) = (Surviving plantlets/Total plantlets) x 100).

2.6 Data Analysis

Differences on the number of shoots, plant height and number of roots were subjected to analysis of variance (ANOVA). Means were separated by post hoc Tukeys at $p<0.05$. The cost efficiency (CE) was calculated by dividing the price of low cost media substitutes by conventional media per litre then subtracting from 100%.

3. RESULTS AND DISCUSSION

3.1 Cost Analysis between the Low Cost Medium and the Conventional (MS) Medium

The same media composition was used during initiation and multiplication for the four treatments (conventional media, LCM1, LCM2 and LCM3). Therefore cost reduction achieved at 94% for LCM1 (Table 2) and 96% LCM3 and LCM4 (Table 3 and Table 4 respectively).

3.2 Shoots Regeneration in Various Media

Significant differences ($p<0.05$) were observed in the number of shoots produced per plant among the treatments (Fig. 1). Dasheen had the highest number of shoots (5.92) on MS media followed by LCM3 (5.48), LCM2 (5.29) and LCM1 (4.7). The number of plant shoots produced in LCM1 was significantly lower number compared to LCM2, LCM3 and MS media.

No significant differences were observed on the growth of Dasheen, Eddoe and Wild/Purple plants on the MS media, LCM1, LCM2 and LCM3 (Fig. 2). Eddoe had the highest number of shoots (5.52) followed by Dasheen (5.5) and wild (5.05) on the media.

Table 1. Composition of each media tried

Media component		
Macronutrients	**Micronutrients**	**Media code**
Conventional	Conventional	Conventional
Omex	Omex	LCM1
Fertilizers	Stanes	LCM2
Fertilizers	Microfood	LCM3

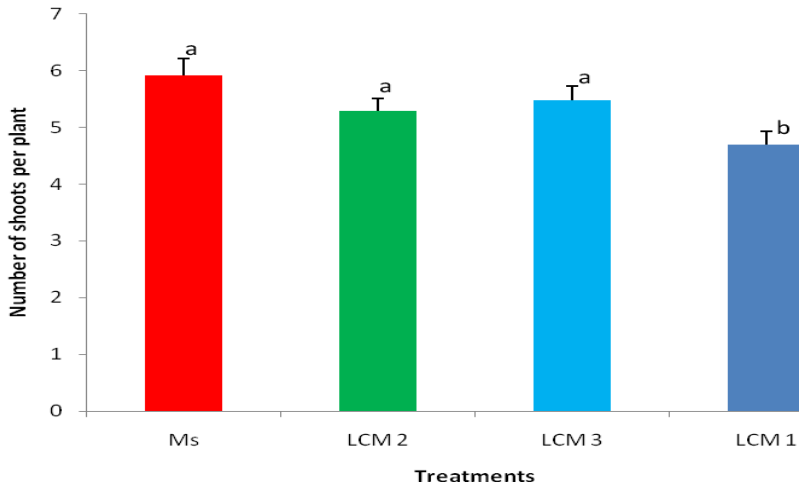

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Fig. 1. Shoot generation by use of different media

Table 2. Cost analysis of LCM1 compared to MS media

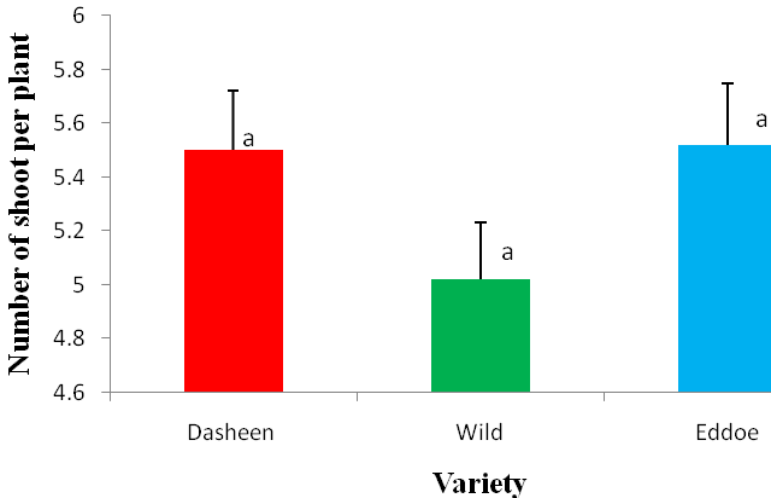
Conventional	Low cost	Cost of 1 litre of medium (Kenyan shillings)		Cost reduction
Macronutrients		**Conventional**	**Low cost**	
MgSO4		0.4810		
KNO_3		3.4200		
$CaCl_2$		0.7920	3.0000	
NH_4NO_3		4.9500		
KH_2PO_4		0.2890		
Sub-total		9.9320	3.0000	
Micronutrients				
$COCL_2.6H_2O$		0.0002		
$CuSO_4.5H_2O$		0.0002		
Na_2EDTA		0.0154		
$FESO_4.7\ H_2O$	Omex Foliar	0.0078		
H_3BO_3		0.0512		
KI		0.0035		
$MnSO4.4\ H_2O$		0.0605		
$Na_2MoO_4.2\ H_2O$		0.0039		
$ZnSO_4.7\ H_2O$		0.0008		
Sub-total		0.1436	3.0000	70.2200
Total		10.0756	3.0000	
Sucrose	Sugar	105.0000	3.0000	97.1000
Total		115.0756	6.0000	94.7860

Table 3. Cost analysis of LCM2 compared to MS media

Conventional	Low cost substitute	Cost of 1 litre of medium (Kenyan shillings)		Cost reduction %
Macronutrients		**Conventional**	**Low cost**	
MgSO4	Epsom salt	0.4810	0.0330	96.1670
KNO_3	Potassium Fert.	3.4200	0.1710	95.0000
$CaCl_2$	Calcinit	0.7920	0.7920	0
NH_4NO_3	Ammonia Fert.	4.9500	0.1897	95.3620
KH_2PO_4	MonoPotassium	0.2890	0.0204	92.9410
SUBTOTAL		9.9320	1.1951	95.8270
Micronutrients				
$COCL_2.6H_2O$		0.0003		
$CuSO_4.5H_2O$		0.0002		
Na_2EDTA	Stanes	0.0154		
$FESO_4.7\ H_2O$		0.0078		
H_3BO_3		0.0512	0.4049	
KI		0.0035		
MnSO4.4 H_2O		0.0605		
$Na_2MoO_4.2\ H_2O$		0.0039		
$ZnSO_4.7\ H_2O$		0.0008		
Sub-total		0.1436	0.4049	
Total		10.0756	1.6000	
Sucrose	Table sugar	105.0000	3.0000	97.1000
TOTAL		115.0756	4.6000	96.0000

Table 4. Cost analysis of LCM3 compared to MS media

Conventional	Low cost substitute	Cost per litre of medium (Kenyan shillings)		Cost reduction (%)
Macronutrients		**Conventional**	**Low cost**	
MgSO4	Epsom salt	0.4810	0.0330	96.1670
KNO_3	Potassium Fer.	3.4200	0.1710	95.0000
$CaCl_2$	Calcinit	0.7920	0.7920	0
NH_4NO_3	Ammonia Fer.	4.9500	0.1890	95.3620
KH_2PO_4	MonoPotassium Phos	0.2890	0.0200	92.9410
Sub-total		9.932	1.1951	95.8270
Micronutrients				
$COCL_2.6H_2O$		0.0002		
$CuSO_4.5H_2O$		0.0002		
Na_2EDTA		0.0154		
H_3BO_3	Micro-food	0.0512		
KI	horticulture	0.0034		
MnSO4.4 H_2O		0.0605		
$Na_2MoO_4.2\ H_2O$		0.0039		
$ZnSO_4.7\ H_2O$		0.0008	0.4049	
Sub-total		0.1436		
Total		10.0756	1.6000	
Sucrose	Table sugar	105.0000	3.0000	97.1000
Total		115.0756	4.6000	96.0000



The same letter (s) expressed show no difference at P<0.05 level

Fig. 2. Shoot regeneration across the varieties

3.3 Height of the Regenerated Plants

The height of all the taro varieties was significantly different ($p<0.05$) in all the treatments. The three taro varieties grown on MS media were taller compared to LCM1, LCM2 and LCM3 (Fig. 4). Plants grown on LCM1 had the shortest heights ranging from 2.56 cm (Eddoe) to and 2.94 cm (Dasheen) compared to MS media that ranged from 6 cm (Dasheen) to 5.61 cm (wild) Fig. 5.

3.4 The Number of Roots and Transplant Survival Rate

Significant differences ($p<0.05$) were observed on the number of roots generated using 1mg/l (Indole Acetic Acid) IAA and Citishooter. IAA on Eddoe variety had the highest number of roots (6.56) compared to wild variety (5.56 cm) on LCM3 media. There was no significant variation on the *in vitro* plantlets survival rate after three weeks Fig. 7. The survival rates were 99.35% (MS media), 99.3% (LCM3) and 99.1% (LCM1)

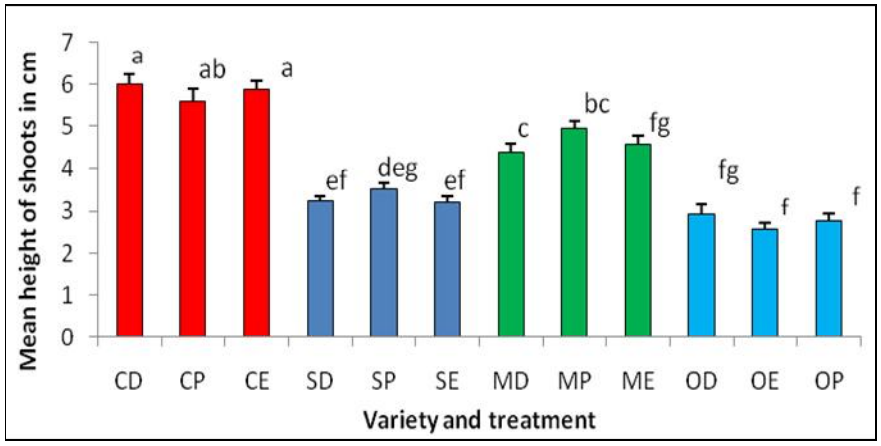
As observed in Fig. 6. The tested media supported growth of shoots with the same morphology and plant structure as those of conventional media.

The LCM1 substitute for Ms Media resulted in cost reduction by 94.786% (Table 2) while LCM2 and LCM3 each further reduced cost by 96.0% (Table 3 and Table 4 respectively). This supports previous studies by Gitonga et al. [4] who substituted macronutrients and micronutrients with the alternatives that reduced the cost by 94.2 and 97.8% respectively in banana micro-propagation. Ogero et al. [8] also studied how low cost nutrients can be substituted in cassava in which he was able to demonstrate that similar percentage of savings was achieved. This has further been shown by [9] low cost tissue culture of sweet potato. This reduction in the cost of media shows that the planting materials obtained can benefit resource poor farmers. The farmers will have access to planting materials at a cheaper price than those propagated using MS Media.

The substitute of plant culture media as a low-cost strategy to propagate planting material must guarantee high quality and well developed plants that compare well with conventional counterparts in both green house and field conditions. Multiple shoots were obtained after 40 days with transfer to new media after every 10 days (Fig. 3). The average number of shoots obtained for the control media were similar to those of Chien-Ying et al. [2] who recorded an average of 5.9 shoots per explants using 8 mg/l of BAP. In this study, the use of 10 mg/l of BAP produced an average of 6.44 shoots. The number of shoots obtained using low cost media was not significantly different except for LCM2 on wild variety and LCM1 for Eddoe variety. This is an indication that micropropation of taro using LCM3 can substitute MS media for production of all varieties of taro.

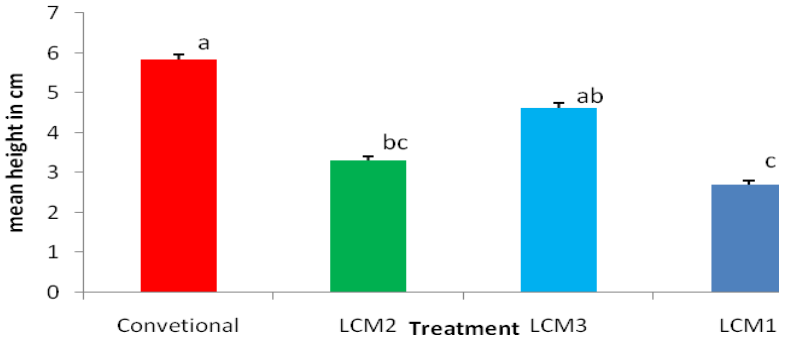
A: dasheen cultured on conventional media. B: eddoe cultured on LCM3 media. C: wild variety cultured on LCM2 media. D: dasheen variety cultured on LCM1 media. E: dasheen shoots rooted on media using citishooter as rooting hormone. F: rooted eddoe shoot on LCM3 media with citishooter as rooting hormone

Fig. 3. *In vitro* generated plants



The same letter(s) expressed show no difference at $P<0.05$ level; C: conventional, O: LCM1, S: LCM2, M: LCM3; D: dasheen, E: eddoe, P: wild varieties of taro; means within a column followed by the same letter(s) are not significantly different at $P<0.05$

Fig. 4. Height of plants in treatment per variety



Same letter(s) expressed show no difference at $P<0.05$ level

Fig. 5. Height of plants per treatment

(G) Wild taro on conventional media.

(H) Dasheen variety generated using LCM3 and citishooter

Fig. 6. *In vitro* generated wild and dasheen varieties plants ready for hardening off

(I) In vitro taro plants on soil generated by conventional media a month after hardening off

(J) In vitro taro plants generated on LCM3 a month after hardening off

Fig. 7. Hardened *in vitro* generated taro

The height of plants varied with conventional media having average height of 5.83 cm, LCM1 2.7 cm, LCM2 4.63 cm and LCM3 3.31cm. This shows that the best substitute for production of tall plants is LCM2 while LCM1 produced the shortest plants. This can be attributed to the low phosphate levels in the Omex media. The optimum amount of phosphate in MS is 1.65 g/l compared to 0.48 g/l in the media used in this study. Higher concentrations of fertilizer is very toxic to the tissues and causes chlorosis to plants at the beginning and necrosis after several days of exposure [10]. There was no significance in root formation between use of 1 mg/l NAA and the low cost alternative 1 ml/l of citishooter. The root formation appeared after two weeks even though Dasheen and Eddoe varieties caused delay in rooting for 5 days. Roots have an essential role and function in plant life and development, supplying water and nutrients to the plant from the environment [11]. Acclimatisation and high percentage survival of plantlets shows the plants ability to withstand transplanting stress [12]. The high percentage of acclimatisation observed in this study may be attributed to plantlets with functional root system during *ex vitro* acclimatization [13]. Also the plantlets produced through micro-propagation technique were of high quality compared to those of vegetative means and vigorous with well developed leaves. The plants were also did adjust to the field conditions. The capability of plantlets to withstand *ex vitro* stress determines the success of any tissue culture protocol [7].

4. CONCLUSION

In this study the low cost alternative showed potential in the production of taro plantlets and should therefore be considered for adoption. This will ensure that there is availability of cheaper planting material to the farmers. This will contribute to food security as well as saving on the available resources especially in sub-Saharan Africa.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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