LOW COST TISSUE CULTURE OF SELECTED CASSAVA (*Manihot esculenta* Crantz) AND SWEET POTATO (*Ipomoea batatas* (L) Lam.) VARIETIES

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DECLARATION

This thesis is my original work and has not been submitted for the award of a diploma or degree in any other University or College.

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DEDICATION

I dedicate this work to my parents Callen Kemunto and James Ogero for instilling in me a sense of discipline and hard work. Particular dedication goes to my sister Charity Kerubo for her encouragement and support during my studies. Lastly, to my late friend Bethuel Tanui whose life is a priceless inspiration to me.

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ACRONYMS AND ABBREVIATIONS

MS	Murashige and Skoog
ANOVA	Analysis of Variance
ASARECA	Association for Strengthening Agricultural Research in Eastern and Central Africa
TC	Tissue Culture
ELISA	Enzyme Linked Immunosorbent Assay
KARI	Kenya Agricultural Research Institute
KU	Kenyatta University
LCM 1	Low Cost Medium 1
LCM 2	Low Cost Medium 2
CM	Modified conventional MS Medium
OCIL	Osho Chemical Industries Limited

ABSTRACT

Drought has become a major contributor to food insecurity in sub-Sahara Africa. Drought-tolerant crops such as cassava and sweet potato can help alleviate this situation. However, lack of affordable healthy planting materials of farmer-preferred varieties is a major constraint to sweet potato and cassava production. Conventional tissue culture technology offers an important solution to this but it is very costly, making plantlets out of reach for resource poor farmers. Hence, it is necessary to have low cost options for micropropagation of planting materials. One way of doing this is to substitute the conventional source of Murashige and Skoog (MS) media salts with alternative sources that are available locally. This study sought to compare the cost of regenerating selected cassava and sweet potato varieties using alternative nutrient sources and the conventional MS nutrient sources. Another objective was to find out if there is any genotype-dependent response to regeneration using different media. Two varieties of each crop (KEMB 36 and Tainung for sweet potato and Muchericheri and KME 1 for cassava) were subjected to three treatments with different media composition and replicated nine times. In the first medium (LCM 1), Easygro® vegetative fertilizer (27:10:16 (N: P: K) + microelements) from Osho Chemical Industries Limited was used as an alternative source for MS basal salts. In the second medium (LCM 2), the conventional sources of four MS macronutrients were substituted individually with locally available fertilizers while Stanes Iodized Microfood® was used as the low cost source of micronutrients. Table sugar was used as an alternative source of carbon while the modified conventional MS medium (CM) was used as the control. Growth parameters including numbers of nodes, roots, leaves and plant height were recorded at 7 and 14 days intervals for cassava and sweet potato, respectively. This was carried out over a period of five weeks for cassava and six weeks for sweet potato. The use of LCM 1 for cassava micropropagation led to a reduction of 96.3% in the cost of the nutrient medium while LCM 2 led to savings of up to 95.5%. For sweet potato a cost reduction of 96.9% was realized with LCM 1 while LCM 2 led to cost reduction of 94.4%. LCM 1 produced better results compared to LCM 2 for cassava regeneration for all the four parameters assessed. The two cassava varieties had regeneration indices of 3-7 nodes per plantlet during initiation and 3.7-6.9 nodes per plantlet during multiplication on all the media. Leaf formation for the two cassava varieties after the sixth week of culture ranged between 2.7 and 7.3 leaves per plantlet on all the media during initiation and 4-7 leaves during multiplication. Muchericheri produced significantly ($p \leq 0.05$) higher number of roots on LCM 1. The two cassava varieties had no significant ($p \geq 0.05$) differences in root production on LCM 2. Sweet potato varieties produced better results on LCM 2 compared to LCM 1. KEMB 36 had a regeneration index of 7.8 nodes per plantlet on LCM 2 during initiation while Tainung had a mean of 3.8 nodes per plantlet after the sixth week of culture. The two sweet potato varieties recorded regeneration indices of 3.3-7.1 nodes per plantlet during multiplication. The variety KEMB 36 had better leaf production on LCM 2 compared to Tainung and vice versa on LCM 1 during initiation. Acclimatization of cassava was best on vermiculite while for sweet potato it was on a mixture of rice husks and red soil in the ratio 1:2. This study has shown that it is possible to reduce the cost of cassava and sweet potato tissue culture by adopting alternative nutrient sources. However, the differential responses among varieties calls for further investigation.

CHAPTER ONE

INTRODUCTION

1.1 Background

Increasing agricultural productivity is one way of poverty alleviation in Kenya and Africa. This can go a long way in improving food security and at the same time earn farmers high incomes. Food security has been a priority objective of the Kenyan government since independence. However, high population growth and subdivision of land in high potential regions, considered as the food basket areas of the country, has led to increased land scarcity (Jayne *et al*., 1998; Rees *et al.*, 1999). Drought has also played a significant role in increased food shortage. As a result, farmers are being encouraged to grow crops such as cassava and sweet potato which are relatively drought tolerant and take a shorter time to mature compared to crops like maize.

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub with an edible root, which grows in tropical and subtropical areas of the world. It has the ability to grow on marginal lands where cereals and other crops do not do well. It offers the cheapest source of food calories and the highest yield per unit area and ranks fourth among staple crops, with a global production of about 160 million tons per year (Nhassico *et al.*, 2008). Most production occurs in three regions, i.e. West Africa, tropical South America and South-East Asia (Lincoln and John, 2003). Africa produces more than half of the world's cassava. However, the continent's average yield of 10 tons per hectare is quite low in comparison to other regions (Whingwiri, 2004).

Sweet potato (*Ipomoea batatas* (L) Lam.) is a dicotyledonous plant that belongs to the family Convolvulaceae. It is a herbaceous perennial vine that is cultivated as an annual food crop in the world (Mutegi, 2005). It is among the world's most important crops but is largely underexploited. It is commonly referred to as subsistence, food security or famine relief crop because of its ability to withstand drought conditions that other crops cannot hence one of the few sources of food at this times. Its uses have diversified considerably in developing countries for example, apart from being boiled, it can be ground into flour for bread making (Scott and Maldinado, 1999). There is need to introduce clones that are high yielding and have high vitamin content to the farming community to improve their nutrition and income generation (Lusweti *et al*., 1999).

Lack of healthy planting materials of farmer-preferred varieties, produced locally and at a low-cost is one of the major constraints to the expansion of cassava and sweet potato production. Biotechnological approaches such as TC offer an important solution to this problem. TC technology largely depends on use of Murashige and Skoog (MS) nutrient medium (Murashige and Skoog, 1962) that comprises of macronutrients, micronutrients, vitamins and growth regulators (Table 1.1). A carbon source and gelling agent such as agar are also required.

Table 1.1: General composition of the MS medium used in TC of various crops.

(Adopted from Sigma-Aldrich, 2011)

Common Name	**Chemical formula**	**Concentration per litre of medium (mg/l)**
Macronutrients		
Ammonium nitrate	NH_4NO_3	1,650
Calcium chloride	$CaCl_2 \cdot 2H_2O$	440
Potassium nitrate	KNO_3	1,900
Potassium dihydrogen phosphate	KH_2PO_4	170
Magnesium sulphate	$MgSO_4 \cdot 7H_2O$	370
Micronutrients		
Cobalt chloride	$CoCl_2 \cdot 6H_2O$	0.025
Potassium iodide	KI	0.83
Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.25
Copper sulphate	$CuSO_4 \cdot 5H_2O$	0.025
Boric acid	H_3BO_3	6.2
Manganese sulphate	$MnSO_4 \cdot 4H_2O$	22.3
Zinc sulphate	$ZnSO_4 \cdot 7H_2O$	8.6
Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	27.8
Sodium ethylenediamine tetraacetic acid	$C_{10}H_{12}N_2Na_4O_{8.}2H_2O$	37.2
Common organic additives		
Myo-Inositol	-	100
Pyridoxine HCl	-	0.5
Thiamine HCl	-	0.1
Glycine (re-crystallized)	-	2000
Sucrose	-	20000
Agar	-	10000

Mass propagation of economically important plants depends on the optimization of the media ingredients and other culture parameters. Conventional media components and equipment such as autoclaves and the laminar hood used in TC are very costly and this cost is usually passed on to farmers, most of whom are resource-challenged, thus putting TC seedlings out of reach. To enhance adoption of this technology for commercial purposes it is necessary to identify, develop and use less costly alternatives. This will

significantly reduce the cost of TC planting materials and correspondingly increase their uptake by smallholder farmers, leading to increased agricultural productivity.

1.2 Problem statement and justification

Food security plays a major role in the development of all nations, however, agricultural productivity, especially in sub-Sahara Africa, is threatened by climate change. There is a need for farmers to shift focus to crops such as cassava and sweet potato that are resilient to adverse conditions such as drought resulting from climatic changes. However, their production is poor due to ineffective seed systems for delivering healthy planting materials as compared to crops like maize. Unavailability of clean planting materials and use of infected cuttings is the major cause of the drastic decline in cassava and sweet potato production experienced in Kenya in the recent past (Lusweti *et al.*, 1999). The application of TC technology to address these constraints has been proposed as an appropriate approach to ensure timely availability of clean planting materials and help alleviate the increasing hunger and poverty. The sterile operational nature of TC procedures excludes fungi, bacteria and pests from the production system resulting in production of large quantities of disease free, superior quality planting materials in form of plantlets as opposed to use of cuttings.

Although TC technology is now a routine method in plant propagation in most high technology laboratories in Kenya, majority of the farmers cannot access adequate planting materials to establish viable commercial units due to the high cost of plantlets. The high cost of TC technology has restricted its use to high resource institutions, e.g.

universities, research institutions and a number of private companies such as Mimea International Limited. Key limitations are in the cost of equipment, nutrient media, sterilizing agents, facilities, shortage of trained personnel and complex protocols. There has always been difficulties in selling tissue culture products because traditional planting materials are much cheaper (Savangikar, 2002). There is therefore need to develop low cost protocols so that farmers can get access to healthy planting materials affordably. This requires alternatives that lower the input costs of equipment, chemicals, energy, labor and capital. The aim of this study was to establish low-cost procedures for micropropagation of cassava and sweet potato without compromising the quality of the plantlets. This will contribute to the success of emerging commercial TC laboratories.

1.3 Research questions

1. Are low cost TC media as effective as the conventional medium in the regeneration of cassava and sweet potato?
2. Is there a difference in terms of cost between alternative and conventional tissue culture media nutrient sources?
3. What are the varietal differences in response of cassava and sweet potato plantlets produced through low cost TC technology compared to those produced through conventional TC?

1.4 Hypotheses

1. Low cost TC media are as effective as the conventional medium in the regeneration of cassava and sweet potato.

2. Alternative tissue culture media ingredients are significantly cheaper compared to conventional ones.
3. There are no varietal differences in response between sweet potato and cassava plantlets micropropagated through low cost tissue culture technology compared to those micropropagated through conventional tissue culture.

1.5 Objectives

1.5.1 General objective

To evaluate low cost tissue culture options for selected cassava and sweet potato varieties.

1.5.2 Specific objectives

1. To evaluate regeneration efficiency of selected cassava and sweet potato varieties on low cost media compared to the conventional TC media.
2. To determine the cost efficiency of regenerating selected cassava and sweet potato varieties using the alternative low cost TC medium compared to conventional TC medium.
3. To assess varietal differences in response of selected sweet potato and cassava varieties when cultured on the developed alternative low cost media and on the conventional MS medium.

1.6 Significance of the study

This study generated low cost tissue culture protocols for cassava and sweet potato which will help reduce the cost of production of seedlings significantly thus making them

affordable to resource-challenged smallholder farmers. Adoption of findings of this work will help in the establishment of clean seed delivery systems for the two crops which is paramount in increasing agricultural productivity for the attainment of Vision 2030 and the Millennium Development Goals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of the Kenyan agricultural sector

Agriculture is the single most important sector in the economy, contributing approximately 25% of the GDP and employing 75% of the national labor force (Republic of Kenya, 2005). Over 80% of the Kenyan population live in the rural areas and derive their livelihoods, directly or indirectly from agriculture. Development of the sector is therefore vital in the development of the whole economy.

A decline in agriculture has far reaching implications in terms of employment and income inequality as well as food security for the country (UNDP, 2002). Only about 17% of the country is high and medium potential agricultural land where most intensive crop and livestock production take place (Republic of Kenya, 2007). This means that increasing agricultural production will have to come from intensification of land use in the high and medium potential lands through utilization of advanced technologies such as tissue culture.

The country needs to diversify to other crops such as cassava and sweet potato which have a potential of thriving under limited water supply. This would improve and stabilize agricultural output, productivity, incomes, significantly check famine and thus food insecurity (Smith and Karuga, 2004). However, adoption of advanced technologies is needed to enhance production of these crops.

2.2 Conventional and low cost tissue culture technology

Tissue culture refers to growing and multiplication of cells, tissues and organs on defined nutrient medium under aseptic and controlled environmental conditions. The technology is used for the production of doubled haploids, cryopreservation, propagating new plant varieties, conserving rare and endangered plants, difficult-to-propagate plants, production of secondary metabolites and transgenic plants (Ahloowalia *et al.,* 2002). The production of high quality planting materials of crop plants and fruit trees, propagated from vegetative parts, has created new opportunities in global trade, benefited growers, farmers, nursery owners and improved rural employment. Biotechnology offers the possibility of expanding and optimizing the use of crops by means of genetic engineering. However, in order to develop new transgenic crops, robust transformation and regeneration methods must be in place (Monica *et al*., 2009).

Tissue culture conditions need to be optimized for obtaining vigorous shoot growth coupled with modifications in the nutrient medium to reduce the cost (Sood and Chauhan, 2009). A conventional plant tissue culture medium usually contains a basal solution with major and minor mineral elements, a source of carbon (normally sucrose), vitamins, growth regulators, a gelling agent for semi-solid media and water (Trigiano and Gray, 2000).

Low-cost tissue culture technology should adopt practices, use alternative equipment and chemicals that reduce the unit cost of micropropagule production. There are reports on tissue culture of other plant species such as banana, vetiver grass and irish potato where

the conventional sources of some components of the nutrient medium have been replaced with low cost substitutes such as sucrose with table sugar (Kaur *et al*., 2005), omission of agar (Mehrotra *et al*., 2007) and use of sunlight and tubular skylight instead of light from fluorescent tubes (Kodym and Francisco, 2004). Cassava flour has been used as a substitute for agar (Maliro and Lameck, 2004). It has been reported that the 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). Every crop has its specific nutrient requirements hence the low cost TC media designed so far are specific to target crops. In Kenya, media have not been developed for micropropagation of cassava and sweet potato using low cost nutrient sources. This study aimed to fill this gap. The design of plant tissue culture medium as a cost reduction strategy to produce affordable planting materials must guarantee high quality and well developed plants that can easily be adapted to green house and field conditions.

2.3 Cassava (*Manihot esculenta* Crantz)

2.3.1 Biology of cassava

Cassava (*Manihot esculenta* Crantz) belongs to the family Euphorbiaceae. The genus *Manihot* consists of at least 98 species (Hershey, 1993) with four centers of diversity that lie in Central Brazil, Western Mexico, Northeast Brazil and Eastern Bolivia (Nasser, 1978). Cassava is a perennial shrub with a height of 1-5 m, palmate leaves with 5-9 leaf lobes and it is grown for its starch containing tuberized roots which are harvested often after 6 months of planting although in some places it may take up to one year to mature.

Cassava has 2n=36 chromosomes and is considered to be a diploid, based on regular meiosis.

2.3.2 Origin, distribution and importance

Cassava was first domesticated between 5000 and 7000 B.C. In the 15th century, it was cultivated throughout the Americas and the Caribbean. In the 16th century, it was introduced to Africa. The introduction to Asia is less well documented, but probably it did not occur until the 18th century (Hershey, 1993).

Cassava is grown between 30° North and South of the equator and the altitudinal limit is approximately 2000 m above sea level. It tolerates practically any hot climate but it is not grown in areas where the mean annual temperature is below 20°C. It can be grown in areas with an annual rainfall of 500-8000 mm. The crop can survive up to six months of drought by reducing new leaf formation and abscission of old ones. During periods of drought, the crop becomes essentially dormant and after rainfall the root reserves are used to produce new leaves. Excess water represents a severe restriction for cassava production. The crop has no specific soil requirements, only salinity and alkalinity represent severe restrictions (Lazano *et al*., 1980). It is one of the cheapest sources of calories in the tropical lowlands with the highest potential production per hectare per year.

Cassava is mostly used for human consumption but it is also fed to animals and is a source of starch for industrial uses. It can be left in the soil for a long period and

harvested when needed. However, harvested fresh cassava roots are subject to deterioration which takes place in two phases. Primary deterioration is characterized by vascular discoloration, accumulation of phenolic compounds and increased oxidase activity. In the second phase the deteriorating roots are infested by bacteria and fungi, followed by rotting. Another disadvantage is the fact that fresh roots of some varieties contain cyanogenic glycosides, linamarin and lotaustralin. When the roots are damaged, enzymes release cyanide from these compounds, which is highly toxic. Intake of insufficiently processed cassava can lead to neurological disorders.

2.3.3 Conventional practices for propagation

There is absence of flowering in some varieties and in many varieties special conditions are required to stimulate flowering. In addition, there is low production of seeds often associated with irregular germination (Mussio *et al*., 1998). Therefore, lignified stem cuttings are used in traditional cassava multiplication systems. Lignification occurs after the plant has matured, which usually takes place after about 5-8 months of growth. In this way, up to four hundred, 50 cm long, cuttings are produced in one year from one plant (Cock, 1985). This traditional technique has been refined into two effective rapid propagation techniques, multiple shoot technique and auxiliary bud proliferation technique (Cock, 1985). In the multiple shoot technique, lignified cuttings with two nodes are cut from a healthy mature plant and grown in sterilized soil. Sprouts are formed within three weeks. When they have a length of 5-10 cm, the upper part is cut off and rooted in liquid medium after which they are transferred to the field. The auxiliary buds of the lower part of the shoot of the lignified cutting will sprout again and the procedure

can be repeated. With this technique, up to 24,000 commercial cuttings can be produced in one year from a single plant (Hershey, 1993).

In the auxiliary bud proliferation technique, stem pieces with a leaf and the accompanying bud are grown in the soil. At least 100 single cuttings can be obtained from one plant. Five months later, these plants can be used to repeat this process. With this technique, up to 300,000 commercial cuttings can be produced in one year from a single mother plant (Cock, 1985).

Multiplication by stem cuttings is often a slow process compared to seed multiplication in grain crops (Santana *et al*., 2009). Diseases such as cassava mosaic disease also often accumulate in the stem cuttings resulting in infected plants and low yields. Small-scale farmers acquire planting materials from neighbours or as volunteer plants left in fallow (Mutegi, 2009). This contributes to pest and disease accumulation and dissemination. Other challenges with the use of cuttings include high perishability since they dry up within a few days, high handling and transport costs and bulk of the material (Escobar *et al.,* 2006).

The cultivation and utilization of cassava presents several problems such as high cyanide content, low protein content and rapid deterioration. The feasibility of overcoming these problems by classical plant breeding is hampered by the high degree of heterozygosity, low fertility, poor seed set and low rates of seed germination (Bai, 1987).

2.3.4 Tissue culture of cassava

Plant tissue culture technology has been used extensively to propagate cassava in many parts of the world. This is because it has been found to be the best method of producing a large number of disease-free cassava planting materials. Through tissue culture, challenges such as diseases often associated with conventional practices can be overcome. This makes tissue culture an important technology in setting up cassava seed systems. Tissue culture is also important in cassava transformation. Efforts have been made to develop genetic modification procedures for cassava improvement, for example increased resistance to cassava mosaic virus (Schopke *et al*., 1993). For this to succeed, efficient regeneration methods are a prerequisite.

Tissue culture has effectively been applied in elimination of viruses and other systemic diseases from cassava vegetative materials allowing exchange and conservation of rejuvenated propagation materials with higher yields (Jorge *et al.*, 2000). However, the cost of *in vitro* plant production is an obstacle to its access by smallholder farmers. Thus, efforts to develop low-cost technologies for smallholder farmers are necessary (Thro *et al.*, 1999).

2.4 Sweet potato (*Ipomoea batatas* (L) Lam.)

2.4.1 Biology of sweet potato

Sweet potato is a dicotyledonous root tuber crop belonging to the family Convolvulaceae. It has an extended storage root which accumulates more edible component than the Irish potato (*Solanum tuberosum*). The colour of the stem and leaves varies from green to

totally purple due to the presence of anthocyanin pigmentation (Laurie and Niederwieser, 2004). The general leaf outline varies from round to almost divided with some varieties having deeply lobed margins. The shape and size of the storage root can be between round and long, irregular or curved depending on the variety and environmental factors (Woolfe, 1992). The colour of the storage root skin ranges from white to dark purple and the colour of the fleshy roots in the field vary from white to orange in various distributions.

2.4.2 Origin, distribution and importance

The exact origin of sweet potato is still not well documented. However, historical evidence suggests that it originated from Central or South American lowlands. South American indigenous communities have probably cultivated the crop since 3000 B.C. Therefore, it is believed to have originated from the region between Yucatan Peninsula of Mexico and Orinoco river in Venezuela and spread to the rest of the world by explorers (Zhang *et al*., 2004) and introduced to Europe and Asia and spread to Africa by the 16th century (Allemann *et al*., 2004).

The wild progenitors of sweet potato have not been documented. It is believed that the cultivated varieties of sweet potato, which are hexaploid, are as a result of hybridization between tetraploid primitive and diploid weedy sweet potatoes (Sauer, 1993). It is possible that wild hexaploids are available but according to history, cultivars were independently domesticated in different regions.

Sweet potato is the only economically important food crop of the family Convolvulaceae and ranks seventh in production globally after wheat, rice, maize, Irish potato, barley and cassava (International Potato Centre, 2008). Among root crops it ranks third in acreage (9.1 million ha), behind Irish potato and cassava. It is an important crop worldwide, with an estimated 126 million metric tons produced annually (FAOSTAT, 2008). In Eastern and Southern Africa, it is third to cassava and Irish potato among the major food root crops, both in cultivation and consumption and thus plays an important role in food security and nutrition in Africa (Ewell and Mutuura, 2004). In sub-Saharan Africa it is considered a food security crop which provides a compliant source of food before maturity of other crops. It is largely grown by small-scale farmers for household consumption and income generation (Mutegi, 2005). Sub-Saharan Africa produces more than 7 million tons of sweet potato annually, which constitutes 5% of global production (Ewell, 2002). Increasing importance of sweet potato in Eastern Africa has been due to the constraints affecting the production of other food crops. Sweet potato is an important crop in Kenya both in terms of food security and income generation with an annual production of 750,000 tonnes/hectare (Njagi, 2004). It is one of the most widely consumed root crops in Kenya.

It is easily propagated and cultivated with good productivity in both high and low agricultural systems. Due to its ease of cultivation, low fertilizer requirements and high adaptability, it is considered a major staple food crop in subsistence and rural economies. It has a significant potential to contribute to the alleviation of widespread food shortages in many developing countries and can be cultivated by farmers with limited resources

because it requires a few inputs and takes 3 months to mature (Mwanga, 2002). The storage roots can be harvested and stored over a long period of time (Karyeija *et al*., 1998). Besides its direct use as table and feed-stock, sweet potato is also a candidate for the production of renewable plant products such as ethanol, high-grade starches, stable natural dyes and vitamin precursors (beta-carotene for vitamin A) (Woolfe, 1992). About 80% of the dry matter in sweet potato storage roots consists of carbohydrates, which are principally type C starches that are quite susceptible to α-amylase digestion (Shin *et al.*, 2005). The high percent of fermentable biomass in the sweet potato roots makes it a potential energy crop for ethanol production (Wilson *et al*., 2007). The orange-fleshed sweet potato has high levels of beta-carotene which is a forerunner of vitamin A, an important nutrient especially for children.

Sweet potato production in sub-Sahara Africa is on the decline due to constraints that include poor varieties and diseases such as sweet potato mottle feathery disease (Karyeija *et al*., 2000; Qaim, 2001; Odame *et al*., 2002). Through tissue culture, clean planting materials can be produced and viruses indexed by Enzyme Linked Immuno-Sorbent Assay (ELISA) or other methods (Oggema *et al*., 2007).

2.4.3 Tissue culture of sweet potatoes

Sweet potato has long been considered a recalcitrant species for plant regeneration (Sihachakr *et al.*, 1997). Nevertheless, a variety of methods for *in vitro* regeneration of diverse sweet potato genotypes have been developed (Liu *et al*., 2001). Different tissues have been used as explants, which undergo either direct shoot organogenesis or

embryogenesis. Shoot tips are generally preferred for embryogenic procedures (Padmanabhan *et al*., 1998) but leaves, petioles, roots and stems have also been used (Cheng and Yeh, 2003; Gong *et al.*, 2005). Rapid shoot-organogenesis has been obtained from leaf explants (Garcia *et al*., 1999). Since the invent of *in vitro* techniques, a lot of interest has been generated in the rapid multiplication of virus free-plants through apical meristems (Naz *et al*., 2009).

Biotechnological approaches are of potential significance in sweet potato improvement. Plant tissue culture and regeneration techniques are useful in the production of somaclonal variants, the development of transgenic plants and are valuable tools in understanding basic plant biology (Xiansong, 2010). The extent to which tissue-cultured sweet potatoes are used by smallholder farmers varies from country to country (Mutandwa, 2008).

Sweet potato is obstinate to regenerate producing adventitious plants from nonmeristematic tissue (Prakash, 1994). However, it is easy to micropropagate producing a higher number of plantlets within a short time compared to conventional mode of propagation. Traditional propagation by use of cuttings is time consuming and laborious. Tissue culture is considered the system with highest potential to achieve the goal of producing quality plantlets (Nandwani and Tudela, 2010). Tissue culture has enabled mass propagation of pathogen-free sweet potato plants (Mevart, 2007). The technology has high fecundity, with production of thousands of sweet potato propagules in the same time it would take the conventional technique to produce tens or hundreds (ASARECA,

2008). Despite the capability of tissue culture to produce a high number of planting materials, its application is constrained by the fact that TC derived sweet potato plants are extremely sensitive to acclimatization *ex vitro* (Oggema *et al.*, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant materials

Cuttings of two varieties of sweet potato (Tainung and KEMB 36) and two cassava (KME 1 and Muchericheri) varieties were obtained from KARI research station in Embu. The two varieties of each crop were selected based on farmer preferences in terms of yield and maturity period. Both cassava varieties take 365-420 days to mature with average yields of 35000 kg/ha (Jaetzold *et al.*, 2007). KEMB 36 sweet potato variety takes 5-6 months to mature with average yields of 8645 kg/ha while Tainung takes 3-4 months to mature with average yields of 19760-22230 kg/ha (INFONET-BIOVISION, 2007). Some of the cuttings were grown in polythene pots in a net house at KU and others in the field to establish the mother stock plants.

3.2 Media formulation

Two low cost media, LCM 1 and LCM 2, were formulated and tested. Modified conventional MS medium for cassava and sweet potato propagation was used as the control.

3.2.1 Low Cost Medium 1 (LCM 1)

Easygro® vegetative fertilizer from OCIL was used at 2g/l as an alternative source for MS basal salts supplemented with table sugar (30g/l) and agar (9g/l). Easygro® is a water-soluble fertilizer with chelated macroelements, microelements and bio-stimulants (Appendix 1) for foliar feeding.

3.2.2 Low Cost Medium 2 (LCM 2)

In LCM 2, the conventional sources of four MS macronutrients (potassium dihydrogen phosphate, potassium nitrate, ammonium nitrate and magnesium sulphate) were substituted with locally available fertilizers while Stanes iodised microfood® (Appendix 2) was used as a substitute source for the micronutrients. These salts were obtained from Osho Chemical Industries Limited in Nairobi. The conventional source of calcium chloride was not substituted due to shortage of an alternative source. The conventional source of sucrose was substituted with table sugar obtained from shops locally. The concentrations of the macronutrients, micronutrients and supplements which were used in the micropropagation of cassava are shown in table 3.1 while those used for sweet potato are in table 3.2.

Table 3.1: Composition of LCM 2 used in the micropropagation of cassava.

Component	**Weight per litre of the stock solution (g/l)**	**Weight per litre of the medium (g/l)**	**Amount of the stock solution used per litre of the medium (ml/l)**
Macronutrients			
Calcium chloride (conventional)	11	0.44	
Monopotassium phosphate (MKP)	4.25	0.17	40
Potassium nitrate fertilizer	47.5	1.9	
Epsom salt	9.25	0.37	
Ammonium nitrate (quarry explosive)	41.25	1.65	
Micronutrients			
Stanes iodised microfood®	-	0.2 [α]	-
Carbon source			
Table sugar	-	30 [α]	-
Gelling agent			
Gelrite	-	3 [α]	-

[α] Were added during the preparation of the culture medium.

Table 3.2: Composition of LCM 2 used in the micropropagation of sweet potato.

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

α Were added during the preparation of the culture medium.

3.2.3 Modified conventional MS medium

Modified MS medium adopted from Yona *et al*. (2010) was used as the conventional medium for tissue culture of cassava (Table 3.3). A protocol developed by scientists at the International Potato Centre (CIP) and comprising of modified MS salts was adopted as the conventional medium in the tissue culture of sweet potato (Rolando *et al*., 1992) (Table 3.4).

Table 3.3: Composition of CM used in the tissue culture of cassava (Adopted from Yona *et al.,* 2010).

Essential elements	**Concentration in stock solution (g/l)**	**Concentration in a litre of medium (g/l)**	**Amount of the stock solution used per litre of medium (ml/l)**
MS Macroelements			
NH_4NO_3	41.25	1.65	
KNO_3	47.5	1.9	
$MgSO_4.7H_2O$	9.25	0.37	40
KH_2PO_4	4.25	0.17	
$CaCl_2$	11	0.44	
MS Microelements			
H_3BO_3	0.62	0.0031	
$MnSO_4$	1.69	0.0085	5
$ZnSO_4.7H_2O$	0.86	0.0043	
KI	83	0.083	1
$Na_2MoO_4.2H_2O$	2.5	0.0025	1
$CuSO4.5H_2O$	2.5	0.0025	1
$CoCl_2.6H_2O$	2.5	0.0025	1
MS Iron sources			
$C_{10}H_{12}N_2Na_4O_8.2H_2O$	7.44	0.0372	
$FeSO_4.7H_2O$	5.56	0.0278	5
MS Vitamins			
D-biotin	0.2	0.0002	
Glycine free base	4	0.004	
Myo-inositol	10	0.01	
Nicotinic acid free acid	0.1	0.0001	1
Pyridoxine hydrochloric acid	0.1	0.0001	
Thiamine hydrochloric acid	1	0.001	
Carbon source			
Table sugar	-	30 $^{\alpha}$	-
Gelling agent			
Gelrite	-	3 $^{\alpha}$	-

$^{\alpha}$ Were added during the preparation of the culture medium.

Table 3.4: Composition of the CM used in the tissue culture of sweet potato (Adopted from Rolando *et al*., 1992).

Essential elements	Concentration in stock solution (g/l)	Concentration in a litre of medium (g/l)	Amount of the stock solution used per litre of medium (ml/l)
Stock A			
NH_4NO_3	35	3.5	
KNO_3	40	4	
$CaCl_2.2H_2O$	9	0.9	
KH_2PO_4	3.5	0.35	
H_3BO_3	0.1	0.01	100
$MnSO_4.4H_2O$	0.5	0.05	
$ZnSO_4.7H_2O$	0.2	0.02	
KI	0.02	0.002	
$Na_2MoO_4.2H_2O$	0.005	5×10^{-4}	
$CuSO4.5H_2O$	5×10^{-4}	5×10^{-5}	
$CoCl_2.6H_2O$	5×10^{-4}	5×10^{-5}	
Stock B (Magnesium sulphate)			
$MgSO_4$	37	0.37	10
Stock C (Iron sources)			
$C_{10}H_{12}N_2Na_4O_8.2H_2O$	7.5	0.0375	5
$FeSO_4.7H_2O$	5.5	0.0275	
Stock D (Vitamins)			
Thiamine hydrochloric acid	0.04	4×10^{-4}	
Glycine	0.2	0.02	1
Nicotinic acid	0.05	5×10^{-4}	
Pyridoxine hydrochloric acid	0.05	5×10^{-4}	
Carbon source			
Table sugar	-	30 $^{\alpha}$	-
Gelling agent			
Gelrite	-	3 $^{\alpha}$	-

$^{\alpha}$ Were added during the preparation of the culture medium.

3.2.4 Sterilization of the media

The pH of each media was adjusted to 5.6, using 1N NaOH and 1N HCL, and the media dispensed into glass bottles. The bottles containing media were then sterilized by pressurized steam at a temperature of 121°C and 15 pounds of pressure per square inch

for 15 minutes in a pressure cooker. The sterile media were kept in the transfer room under sterile conditions until use.

3.3 *In vitro* initiation of cassava and sweet potato explants

3.3.1 Preparation of explants

Healthy and vigorous plants were collected from the net house, leaves excised and stems cut into about 10 cm long pieces. They were washed thoroughly in running tap water for 5 minutes to remove soil debris. The stem sections were then cut at the internodes to produce nodal cuttings of about 2-3 cm and transferred into a laminar air flow hood.

3.3.1.1 Sweet potato sterilization

The nodal cuttings were immersed in 1.5% sodium hypochlorite with a drop of Tween 20® for 20 minutes. They were then put in 70% ethanol for 6 minutes after which they were rinsed 4 times with sterile distilled water. They were finally put in 0.08% sodium hypochlorite before washing twice with sterile distilled water.

3.3.1.2 Cassava sterilization

The cassava nodal cuttings were put in 70% ethanol for 2 minutes then transferred into 1.5% sodium hypochlorite with a drop of Tween 20® for 15 minutes. They were then washed 4 times in sterile distilled water.

3.3.2 Culturing of explants

Using a sharp sterile scalpel blade a slant cut was made on the damaged parts of each explant. Each explant was then carefully cultured onto the relevant media. The cultures were incubated in the growth room at a temperature of 28±2°C under an illumination of 2000 lux white light (generated by L 18W/765 fluorescent tubes) and a photoperiod of 16 hours light and 8 hours darkness. There were 9 replicates for each variety of the two crops in each medium which were arranged in a completely randomized design. Examination for growth and contamination was carried out after every three days. Any bottle that was found contaminated was discarded. The number of nodes, leaves, roots and plant height were recorded at 7 days interval for cassava and 14 days interval for sweet potato. This was carried out for 5 and 6 weeks for cassava and sweet potato respectively. The experiment was repeated twice.

3.4 Multiplication

Multiplication was achieved through sub-culturing after every 5 weeks for cassava and 6 weeks for sweet potato on media containing same composition as the initiation media. Rooting occurred during multiplication without addition of any auxin. Culture bottles containing plantlets with 4-6 nodes were carefully opened and using sterile forceps, the plantlets pulled out. The plantlets were transferred onto a sterile aluminium paper foil and the stems cut into micro-cuttings with at least one bud. Culture bottles containing fresh multiplication media were opened and the nodal cuttings were cultured onto them. Each culture bottle was sealed and labeled with corresponding line number and date of subculture. The bottles were transferred into the growth room and incubated at a

temperature of 28±2ºC under an illumination of 2000 lux white light (generated by L 18W/765 fluorescent tubes) and a photoperiod of 16 hours light and 8 hours darkness. A completely randomized design with 9 replications per medium for each variety was used. The numbers of leaves, nodes, roots and plant height were recorded after every 7 and 14 days for cassava and sweet potato, respectively. This was carried out for 5 and 6 weeks for cassava and sweet potato respectively. The experiment was repeated twice.

3.5 Acclimatization of plantlets

Plantlets with well-developed root and leaf systems were washed with tap water to remove adhering medium hence discourage mould growth. Sweet potato plantlets were transplanted into the following acclimatization media: (1) vermiculite, (2) red soil mixed with rice husks in the ratio 2:1 and (3) red soil. These media were dispensed in rectangular (20cm by 40cm) trays and plantlets transplanted on them. The trays with plantlets were then put in an acclimatization chamber made using a transparent polythene sheet. The survival rate of the plantlets was evaluated by counting the number of live plants after 21 days of transplanting onto the acclimatization media.

Cassava plantlets were acclimatized on (1) vermiculite, (2) vermiculite mixed with red soil in the ratio 1:1 and (3) red soil mixed with rice husks in the ratio 2:1. For cassava acclimatization, the three acclimatization media were dispensed into 20 litre buckets up to the quarter-mark level. The plantlets were then transplanted on these media and the buckets covered with transparent polythene sheets to retain moisture hence protect the plantlets from desiccation. Small holes were made on the polythene sheets after four days

to adapt the plants to the natural environment. After three weeks the polythene sheets were removed completely and plantlets transplanted onto soil in polythene pots then put in a chamber resembling a green house. The plantlets were regularly sprayed with water to avoid desiccation. Survival rate of plantlets on the three acclimatization media was determined by counting the number of live plants on each medium after 21 days.

3.6 Cost analysis

The current market prices of the conventional and the alternative sources of MS salts were obtained. Based on the quantities used per litre of the medium the cost of each compound was calculated as follows:

$$\frac{\text{(Amount used in culture medium (g/l)} \times \text{Price of amount bought (Kshs)}}{\text{Amount bought (g)}}$$

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

3.7 Data analysis

Data on the effect of the different media on the number of nodes, roots, leaves and plant height was subjected to analysis of variance (ANOVA) using STATA® statistical software version 11. Means were separated using Tukey's test at 5% level.

CHAPTER FOUR

RESULTS

4.1 Performance of formulated low cost media

4.1.1 Cassava growth during culture initiation

Regeneration of cassava plantlets on the two low cost and the modified conventional MS media was successful as shown in plates 4.1 to 4.3 below.

Plate 4.1: Plantlets of Muchericheri (A) and KME 1 (B) cassava varieties regenerated on LCM 1 after five weeks of culture.

Plate 4.2: Plantlets of Muchericheri (A) and KME 1 (B) cassava varieties regenerated on CM after five weeks of culture.

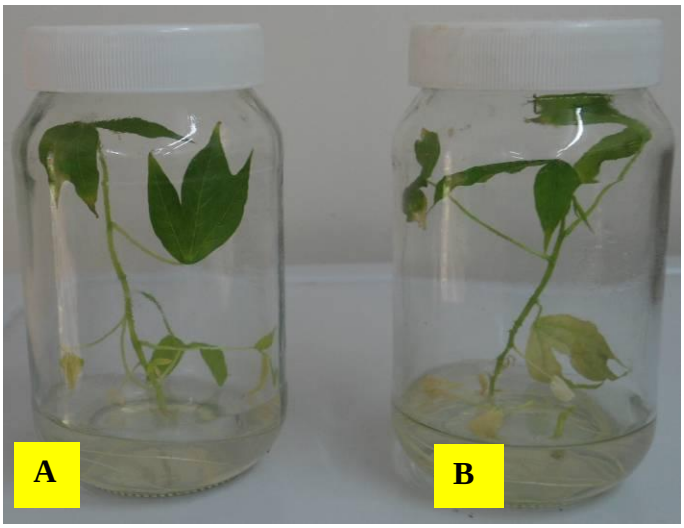

Plate 4.3: Plantlets of Muchericheri (A) and KME 1 (B) cassava varieties regenerated on LCM 2 after five weeks of culture.

4.1.1.1 Number of nodes

Significant differences ($p \leq 0.05$) were noticed between the three media treatments in the number of nodes produced for the two varieties of cassava (KME 1 and Muchericheri). Both varieties produced the highest number of nodes in LCM 1 followed by CM (Fig. 4.1). LCM 2 had the lowest number of nodes formed for both varieties. KME 1 had

means of 5.6, 3.7 and 4.6 nodes on LCM 1, LCM 2 and CM, respectively, while Muchericheri had a mean of 7 nodes on LCM 1, 3.5 on LCM 2 and 6.2 on CM after five weeks of culture (Fig. 4.1). There was a significant difference ($p \leq 0.05$) in the number of nodes produced by the two cassava varieties with variety KME 1 having fewer nodes than Muchericheri in LCM 1 and CM. However, KME 1 produced more nodes in LCM 2 compared to Muchericheri after five weeks of culture but the difference was not significant (Fig. 4.1).

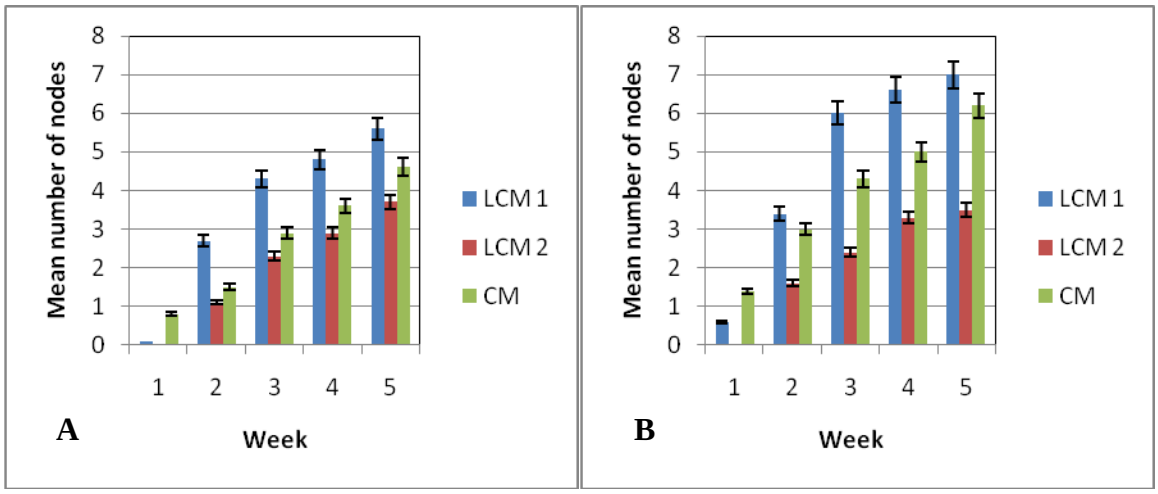

Figure 4.1: Mean number of nodes per week for two cassava varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KME 1 and (B) Muchericheri. Error bars 95% C.I.

After five weeks of culture, the plantlets had a good number of nodes which prompted multiplication. The nodal explants were indistinguishable morphologically from the plants used as the source of explants, showing similar visual performance in terms of proliferation and leaf system development.

4.1.1.2 Number of leaves

There was no significant difference ($p \geq 0.05$) in the number of leaves produced on low cost medium 1 compared to the modified conventional MS medium for the variety KME 1 which produced a mean of 4.8 leaves on LCM 1 and 5.1 leaves on CM after five weeks of culture (Fig. 4.2). Muchericheri variety had a significantly ($p \leq 0.05$) higher number of leaves on CM (7.3) compared to LCM 1 (6.3) and LCM 2 (2.7) at the end of the culture period. Relatively fewer leaves were produced on LCM 2 (with KME 1 producing a mean of 3.4 leaves while Muchericheri had a mean of 2.7 leaves) compared to LCM 1 and CM at the end of the fifth week (Fig. 4.2).

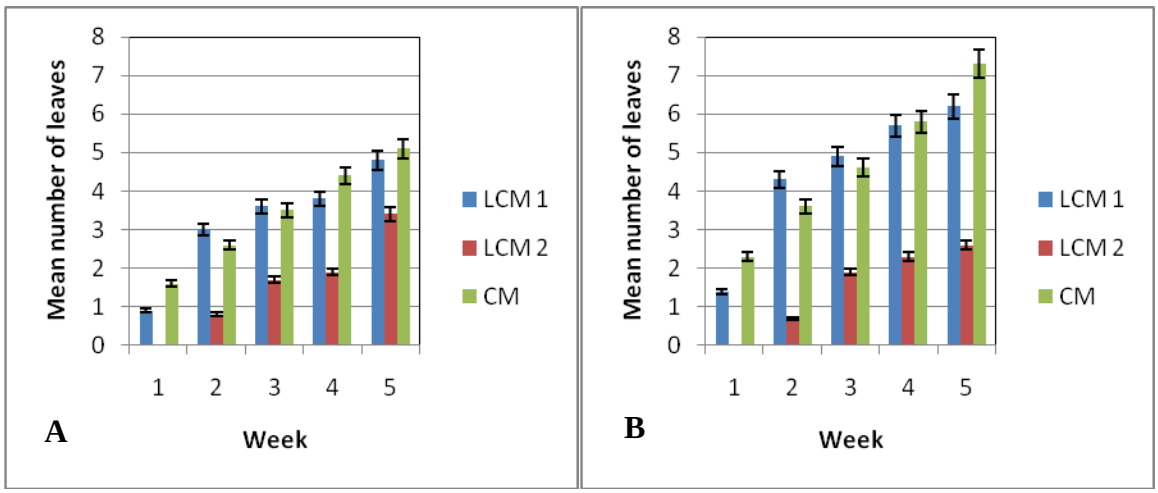

Figure 4.2: Mean number of leaves per week for two cassava varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KME 1 and (B) Muchericheri. Error bars 95% C.I.

Muchericheri variety had significantly ($p \leq 0.05$) higher number of leaves compared to KME 1 in LCM 1 and CM while KME 1 had significantly ($p \leq 0.05$) higher number of leaves in LCM 2 compared to Muchericheri after five weeks of culture.

4.1.1.3 Number of roots

The cassava plantlets developed no roots in the first week of culture. In the second week, only plants cultured on LCM 1 had roots. Plants cultured on LCM 2 and CM developed roots in the third week. The variety KME 1 had an average of 4.0, 3.3 and 3.6 roots in LCM 1, LCM 2 and CM respectively, while Muchericheri had a mean of 4.8 roots in LCM 1, 3.2 on LCM 2 and 4.8 in CM after five weeks of culture (Fig. 4.3). The cultivar KME 1 produced the highest number of roots in LCM 1 after five weeks of culture. Muchericheri produced the same number of roots in LCM 1 and CM at the end of the fifth week (Fig. 4.3).

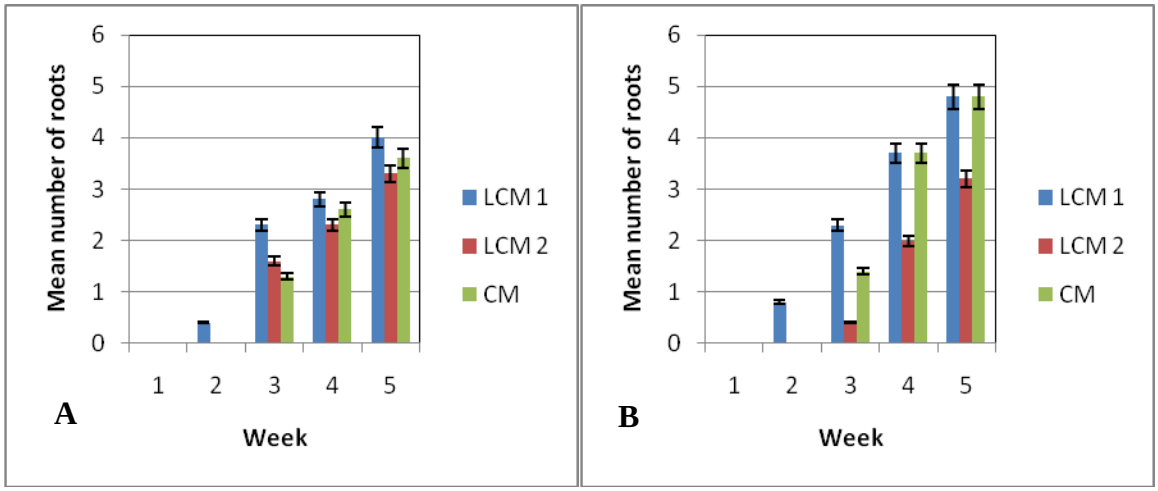

Figure 4.3: Mean number of roots per week for two cassava varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KME 1 and (B) Muchericheri. Error bars 95% C.I.

There was a significant difference ($p \leq 0.05$) in the number of roots produced by the two varieties in LCM 1 and CM with Muchericheri having higher number of roots than KME 1. The two cassava varieties did not show any significant difference ($p \geq 0.05$) in root production on LCM 2 at the end of the culture period.

4.1.1.4 Plant height

Explants cultured on LCM 2 did not produce any shoot during the first week of culture hence no plant height was recorded. Explants cultured on LCM 1 and CM had developed plantlets by the end of the first week with CM having significantly ($p \leq 0.05$) taller plants compared to LCM 1. Explants cultured on LCM 2 produced plantlets in the second week. Plants of the variety KME 1 on LCM 1 were significantly ($p \leq 0.05$) taller (4.7 cm) compared to those on CM (2.7 cm) and LCM 2 (3.2 cm) after five weeks of culture (Fig. 4.4). This variety had significantly ($p \leq 0.05$) taller plants on CM compared to LCM 1 and LCM 2 during the first week of culture. This pattern changed from the second to the fifth week with significantly ($p \leq 0.05$) taller plants being produced on LCM 1 compared to CM and LCM 2. Muchericheri had taller plants in CM compared to LCM 1 and LCM 2 for the first three weeks. This, however, changed in the fourth and fifth weeks where LCM 1 had taller plants compared to LCM 2 and CM.

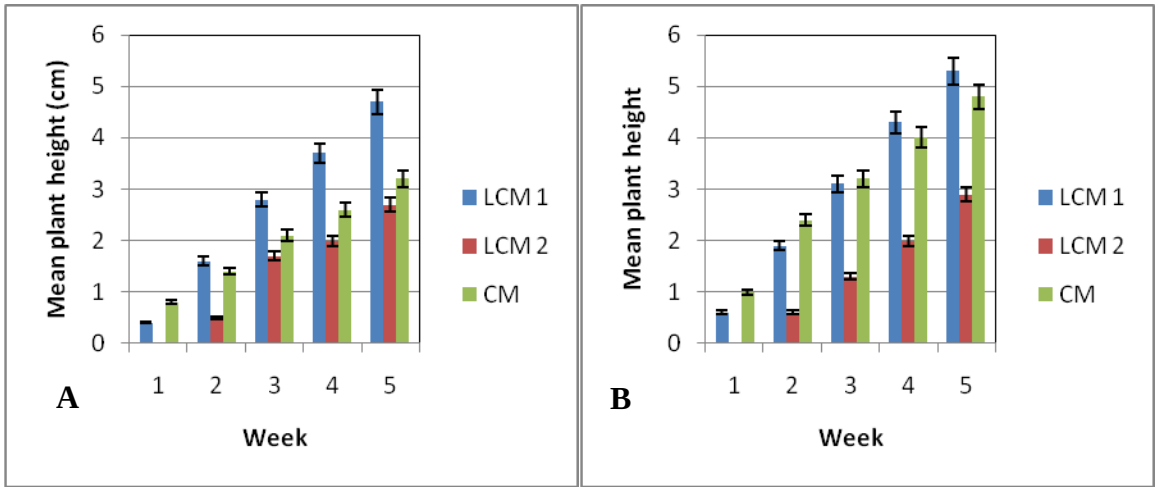

Figure 4.4: Mean plant heights per week for two cassava varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KME 1 and (B) Muchericheri. Error bars 95% C.I.

4.1.2 Cassava growth during multiplication

4.1.2.1 Number of nodes

The two cassava varieties had a better performance on LCM 1 compared to LCM 2 and CM during multiplication (Table 4.1). There were significant differences ($p \leq 0.05$) in the number of nodes produced by the two varieties on the three media. The variety KME 1 had a significantly high mean number of nodes on LCM 1 (5.65) compared to LCM 2 (4.15) and CM (4.45). On average, the variety Muchericheri produced the highest number of nodes on LCM 1 followed by CM and LCM 2. The variety Muchericheri formed significantly ($p \leq 0.05$) more nodes on LCM 1 and CM compared to KME 1 on both occasions of subculture.

Table 4.1: Mean number of nodes for KME 1 and Muchericheri cassava varieties during multiplication.

	Mean number of nodes*					
	KME 1			**Muchericheri**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	5.9 ± 0.26^{cx}	5.4 ± 0.32^{bx}	**5.7 ± 0.25^{cx}**	6.9 ± 0.29^{cy}	6.6 ± 0.37^{cy}	**6.8 ± 0.15^{cy}**
LCM 2	4.0 ± 0.32^{ax}	4.3 ± 0.29^{ax}	**4.2 ± 0.15^{ax}**	3.7 ± 0.29^{ax}	4.1 ± 0.29^{ax}	**3.9 ± 0.20^{ax}**
CM	4.6 ± 0.42^{bx}	4.3 ± 0.37^{ax}	**4.5 ± 0.15^{bx}**	5.6 ± 0.42^{by}	4.9 ± 0.55^{by}	**5.3 ± 0.35^{by}**

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.2.2 Number of leaves

The KME 1 variety produced significantly ($p \leq 0.05$) higher number of leaves on LCM 1 compared to LCM 2 and CM during both subcultures (Table 4.2). The averages of the mean number of leaves for this variety during the two subcultures were 6.05 on LCM 1,

4.65 on LCM 2 and 5.1 on CM. Muchericheri variety also produced significantly higher number of leaves on LCM 1 compared to LCM 2 and CM with averages of 7.4, 4.5 and 6.1, respectively. Muchericheri produced significantly ($p \leq 0.05$) higher number of leaves on LCM 1 compared to KME 1 during the two subcultures. The two varieties showed no significant ($p \geq 0.05$) differences in leaf production on LCM 2 during multiplication.

Table 4.2: Mean number of leaves for KME 1 and Muchericheri cassava varieties during multiplication.

	Mean number of leaves*					
	KME 1			**Muchericheri**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	6.1 ± 0.46^{cx}	6.0 ± 0.38^{bx}	$\mathbf{6.1 \pm 0.05^{cx}}$	7.4 ± 0.46^{cy}	7.4 ± 0.48^{cy}	$\mathbf{7.4 \pm 0.00^{cy}}$
LCM 2	4.3 ± 0.45^{ax}	5.0 ± 0.38^{ax}	$\mathbf{4.7 \pm 0.35^{ax}}$	4.0 ± 0.31^{ax}	5.0 ± 0.27^{ax}	$\mathbf{4.5 \pm 0.50^{ax}}$
CM	5.3 ± 0.45^{bx}	4.9 ± 0.29^{ax}	$\mathbf{5.1 \pm 0.20^{bx}}$	6.4 ± 0.49^{by}	5.8 ± 0.37^{by}	$\mathbf{6.1 \pm 0.30^{by}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.2.3 Number of roots

KME 1 variety had the highest number of roots produced in LCM 1 followed by CM and LCM 2 on both occasions of subculture. On average the mean numbers of roots produced by KME 1 variety per subculture were 3.7, 2.7 and 3.1 in LCM 1, LCM 2 and CM, respectively (Table 4.3). Muchericheri variety had no significant difference ($p \geq 0.05$) in the number of roots produced in LCM 1 and CM during the second subculture but significantly low number of roots were formed in LCM 2 compared to LCM 1 and CM during both occasions of subculture. The variety Muchericheri produced significantly higher number ($p \leq 0.05$) of roots in LCM 1 compared to KME 1 during the first

subculture, however, in the second subculture no significant differences were observed between the two varieties. Muchericheri variety produced significantly higher number of roots in CM compared to KME 1 during the two subcultures. For the variety KME 1, the highest number of roots was recorded in LCM 1 while for Muchericheri it was in CM.

Table 4.3: Mean number of roots for KME 1 and Muchericheri cassava varieties during multiplication.

	Mean number of roots*					
	KME 1			**Muchericheri**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	3.6 ± 0.29^{bx}	3.8 ± 0.25^{bx}	$\mathbf{3.7\pm0.10^{cx}}$	4.1 ± 0.35^{by}	3.7 ± 0.29^{bx}	$\mathbf{3.9\pm0.20^{bx}}$
LCM 2	2.8 ± 0.25^{ax}	2.6 ± 0.29^{ax}	$\mathbf{2.7\pm0.10^{ax}}$	2.9 ± 0.26^{ax}	3.3 ± 0.25^{ay}	$\mathbf{3.1\pm0.20^{ay}}$
CM	3.4 ± 0.32^{bx}	2.8 ± 0.25^{ax}	$\mathbf{3.1\pm 0.30^{bx}}$	4.5 ± 0.19^{cy}	3.8 ± 0.37^{by}	$\mathbf{4.2\pm0.35^{cy}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.2.4 Plant height

In the first round of subculture, KME 1 had significantly ($p\leq0.05$) taller plantlets on LCM 1 compared to CM and LCM 2 after five weeks of culture (Table 4.4). However, significant differences were not detected ($p\geq0.05$) in heights of KME 1 plantlets on LCM 1 and CM during the second subculture. The variety Muchericheri had the same mean plant height (4.9 cm) on LCM 1 and CM during the first subculture but during the second subculture plantlets on LCM 1 were taller (5.3 cm) compared to those on CM (4.8 cm). The two varieties had the shortest plantlets on LCM 2 during both subcultures and with no significant differences ($p\geq0.05$) in height.

Table 4.4: Mean plant height for KME 1 and Muchericheri cassava varieties during multiplication.

	Mean plant height*					
	KME 1			Muchericheri		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	4.6 ± 0.46^{cx}	3.7 ± 0.29^{bx}	$\mathbf{4.2\pm0.45^{cx}}$	4.9 ± 0.29^{by}	5.3 ± 0.29^{cy}	$\mathbf{5.1\pm0.20^{cy}}$
LCM 2	2.9 ± 0.19^{ax}	3.3 ± 0.21^{ax}	$\mathbf{3.1\pm0.20^{ax}}$	3.0 ± 0.26^{ax}	3.3 ± 0.17^{ax}	$\mathbf{3.2\pm0.15^{ax}}$
CM	3.7 ± 0.38^{bx}	3.8 ± 0.26^{cx}	$\mathbf{3.8\pm0.05^{bx}}$	4.9 ± 0.35^{by}	4.8 ± 0.31^{by}	$\mathbf{4.9\pm0.05^{by}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.3 Cassava survival rate during acclimatization

Plantlets of the two cassava varieties acclimatized on vermiculite recorded the highest survival rate, at 80% for KME 1 and 81% for Muchericheri, followed by those acclimatized on a mixture of vermiculite and red soil with 67% of KME 1 plantlets and 66.7% of Muchericheri plantlets surviving (Fig. 4.5). The media comprising of rice husks and red soil had the lowest survival rate with 53.3 and 55.2% of plantlets surviving for KME 1 and Muchericheri, respectively.

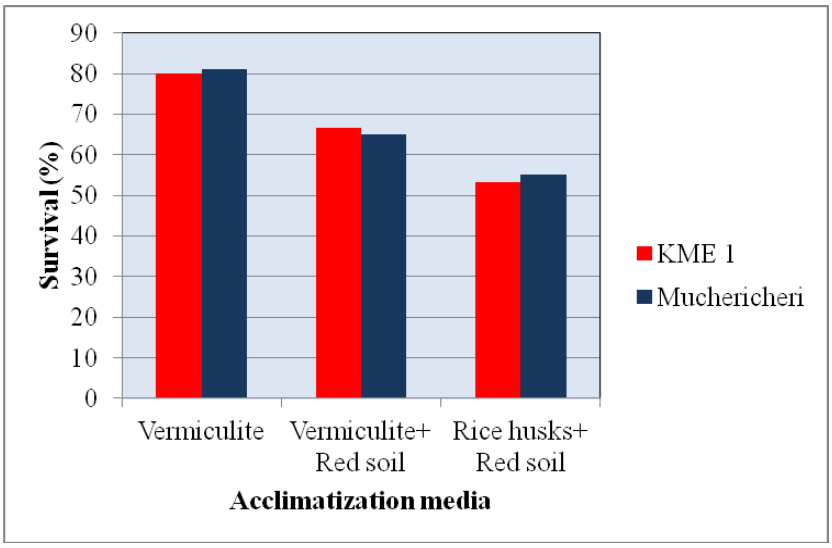

Figure 4.5: Survival rate of plantlets of KME 1 and Muchericheri cassava varieties acclimatized on different media.

The plantlets adapted well to *ex vitro* conditions after transfer from the acclimatization media onto soil (Plates 4.4 and 4.5).

Plate 4.4: Plants of Muchericheri variety two weeks after transfer onto the soil.

Plate 4.5: Plants of KME 1 variety two weeks after transfer onto the soil.

4.1.4 Sweet potato growth during culture initiation

The two sweet potato varieties responded positively to the two low cost media and the modified conventional MS medium in terms of node production, leaf growth, root formation and plant elongation (Plates 4.6 to 4.8).

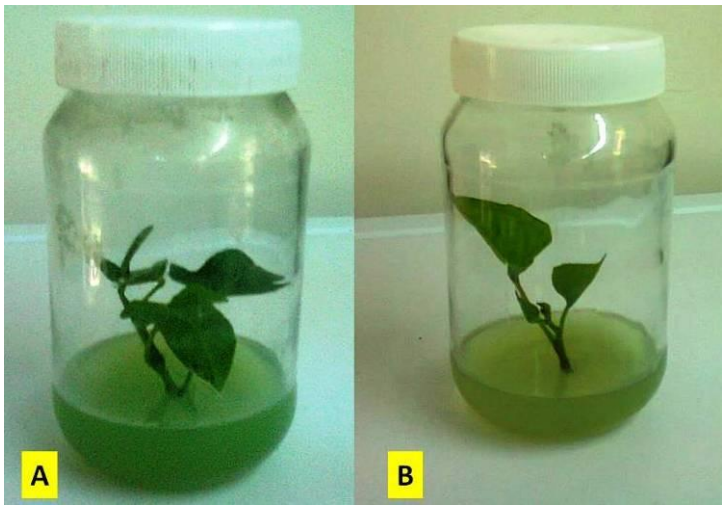

Plate 4.6: Plantlets of KEMB 36 (A) and Tainung (B) sweet potato varieties regenerated on LCM 1 after six weeks of culture.

Plate 4.7: Plantlets of KEMB 36 (A) and Tainung (B) sweet potato varieties regenerated on LCM 2 after six weeks of culture.

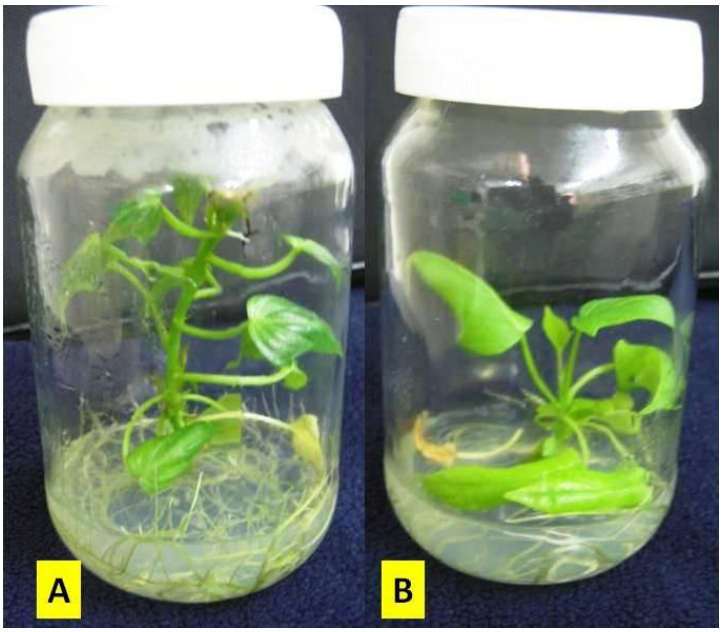

Plate 4.8: Plantlets of KEMB 36 (A) and Tainung (B) sweet potato varieties regenerated on CM after six weeks of culture.

4.1.4.1 Number of nodes

KEMB 36 variety produced a significantly ($p \leq 0.05$) higher number of nodes in LCM 2 (mean of 7.8) compared to LCM 1(mean of 3) and CM (mean of 4.6) after six weeks of culture (Fig. 4.6). Tainung variety had a low response in all the three media compared to KEMB 36. Tainung developed the highest number of nodes in LCM 1 followed by LCM 2 and CM, respectively, in week two. However, this changed in the following weeks with plants cultured on CM producing the highest number of nodes followed by LCM 1 and LCM 2, respectively. The two sweet potato varieties exhibited a slow growth rate hence data had to be recorded at an interval of 14 days instead of 7 days as in cassava.

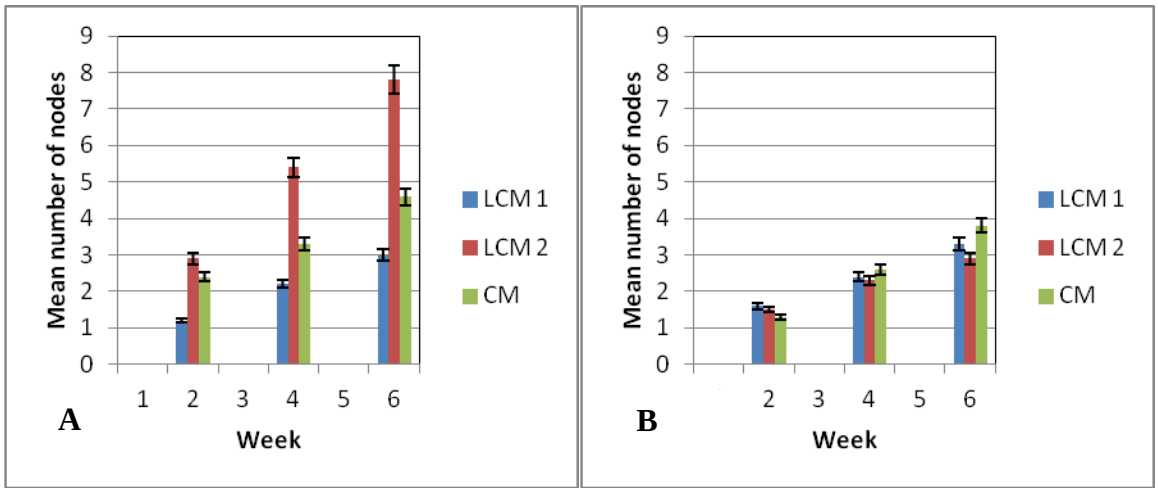

Figure 4.6: Mean number of nodes at two weeks intervals for two sweet potato varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KEMB 36 and (B) Tainung. Error bars 95% C.I.

4.1.4.2 Number of leaves

The two sweet potato varieties, KEMB 36 and Tainung, responded differently to the two low cost media. KEMB 36 had significantly ($p \leq 0.05$) higher number of leaves in LCM 2 (mean of 7.5) compared to LCM 1 (mean of 4) at the end of the sixth week of culture

(Fig. 4.7). The variety Tainung had significantly ($p \leq 0.05$) higher number of leaves in LCM 1 (mean of 4.3) compared to LCM 2 (mean of 2.7) after the sixth week of culture (Fig. 4.7). Plants of the variety KEMB 36 on CM had the second highest number of leaves (mean of 5.3) after six weeks of culture. Tainung had the lowest number of leaves on CM during the second week, however, this changed in the fourth and sixth week with the variety having the second highest number of leaves in CM after the fourth week and the highest number of leaves in the sixth week.

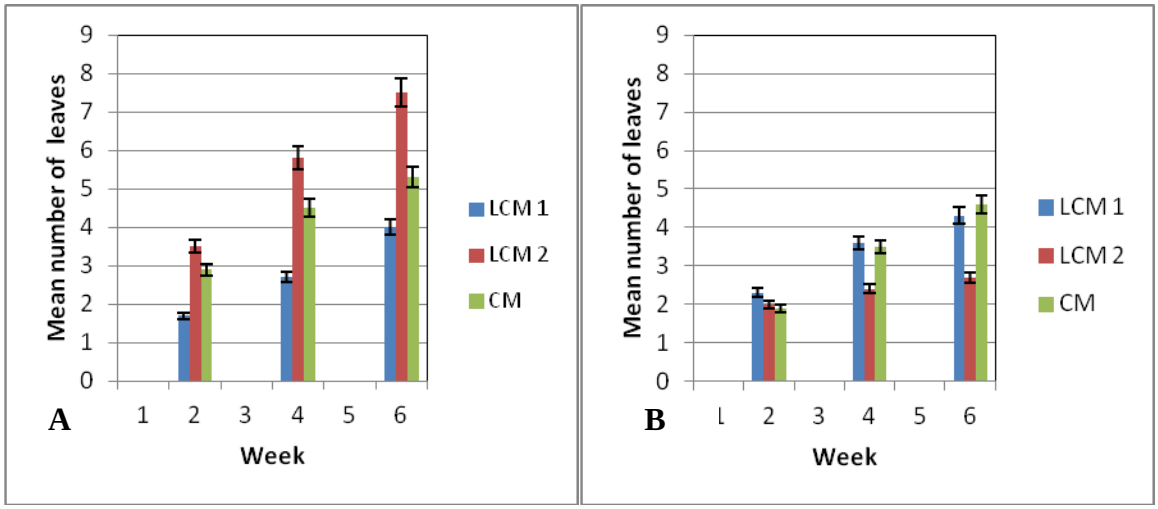

Figure 4.7: Mean number of leaves at two weeks intervals for two sweet potato varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KEMB 36 and (B) Tainung. Error bars 95% C.I.

KEMB 36 variety had the lowest number of leaves in LCM 1 throughout the culture period. There was no significant difference ($p \geq 0.05$) in the number of leaves produced by plants of Tainung variety on LCM 1 and CM. Tainung had the lowest number of leaves on LCM 2 after the sixth week of culture. KEMB 36 had a better response in LCM 2 and CM compared to Tainung. Tainung produced more leaves compared to KEMB 36 in LCM 1 after six weeks of culture (means of 4.3 and 4 respectively) but the difference was not significant ($p \geq 0.05$).

4.1.4.3 Number of roots

KEMB 36 and Tainung produced the highest number of roots in LCM 2 after the 6th week of culture (Fig. 4.8). There was no significant difference ($p \geq 0.05$) in the number of roots produced by plantlets in LCM 1 and CM for both varieties. However, plantlets on CM had slightly higher number of roots compared to those on LCM 1 on the sixth week.

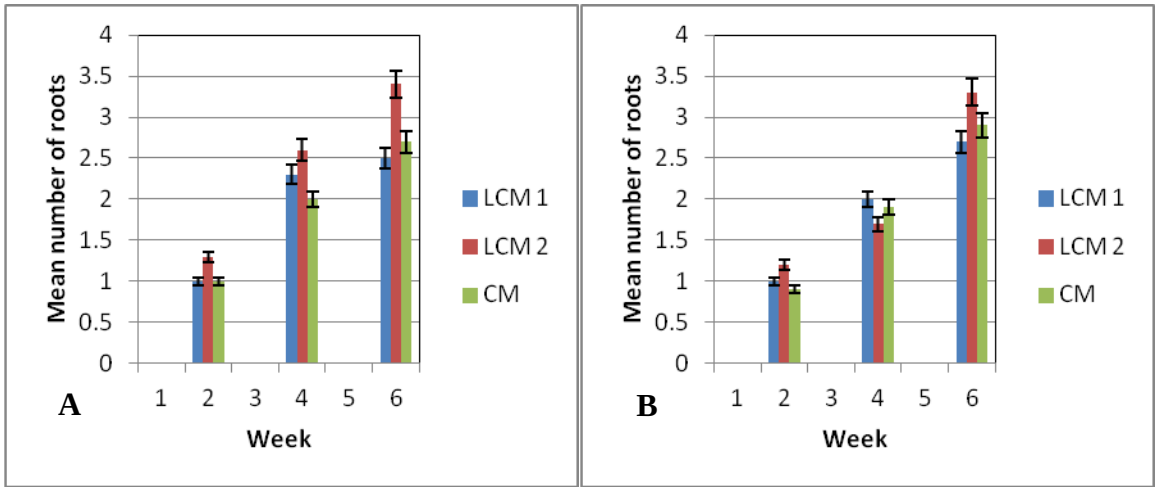

Figure 4.8: Mean number of roots at two weeks intervals for two sweet potato varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KEMB 36 and (B) Tainung. Error bars 95% C.I.

There was no significant ($p \geq 0.05$) difference in the number of roots produced by the two varieties in all the media tested. However, the structure of roots differed between the two varieties with KEMB 36 producing shorter roots with numerous root hairs compared to Tainung which had long roots but with few root hairs (Plate 4.9). Tainung had significantly ($p \leq 0.05$) longer roots with means of 25.2, 24 and 23.7 cm on LCM 2, CM and LCM 1, respectively, compared to KEMB 36 which had means of 23.5, 22 and 20.4 cm on LCM 2, CM and LCM 1 respectively (Fig. 4.9).

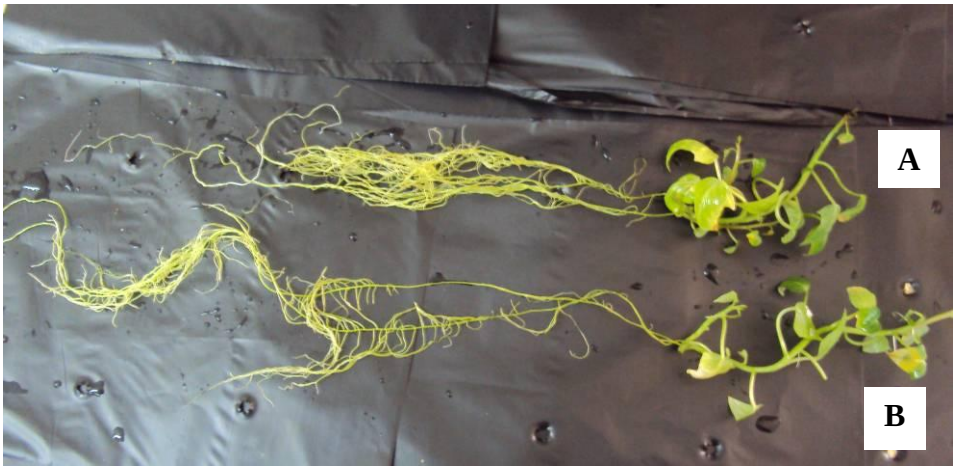

Plate 4.9: Differences in root morphology of plantlets of KEMB 36 (A) and Tainung (B) sweet potato varieties.

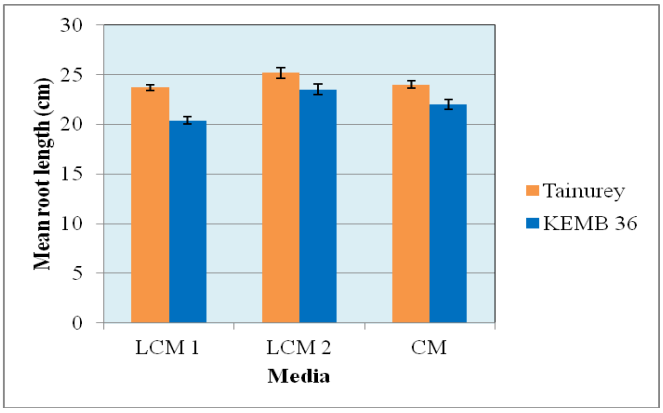

Figure 4.9: Mean root lengths for Tainung and KEMB 36 sweet potato varieties on LCM 1, LCM 2 and CM.

4.1.4.4 Plant height

There was no significant differences ($p \geq 0.05$) in the height of plantlets of the sweet potato varieties, KEMB 36 and Tainung in LCM 1 and LCM 2 after six weeks of culture (Fig. 4.10). However, the variety KEMB 36 produced significantly taller plants on CM compared to Tainung after six weeks of culture. After the sixth week of culture, the mean plant heights for KEMB 36 in LCM 1, LCM 2 and CM were 1.6 cm, 3.9 cm and 3.1 cm, respectively. For the variety Tainung, it was 1.8 cm, 3.5 cm and 2.3 cm in LCM 1, LCM

2 and CM, respectively. Explants cultured on LCM 2 produced the tallest plantlets in both varieties followed by CM and LCM 1 respectively for the two varieties.

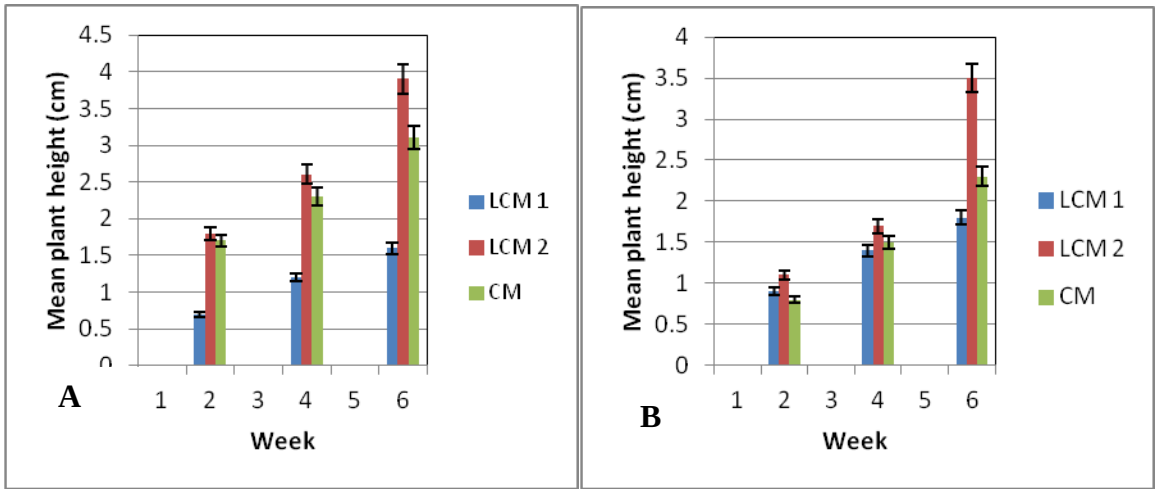

Figure 4.10: Mean plant height at two weeks intervals for two sweet potato varieties regenerated on low cost (LCM 1 and LCM 2) and modified conventional MS (CM) tissue culture media. (A) KEMB 36 and (B) Tainung. Error bars 95% C.I.

4.1.5 Sweet potato growth during multiplication

4.1.5.1 Number of nodes

The variety KEMB 36 had significantly ($p \leq 0.05$) higher number of nodes on LCM 2 compared to LCM 1 and CM at the end of each subculture (Table 4.5). On average, this variety had means of 6.85, 4.15 and 3.75 nodes per plantlet per subculture on LCM 2, CM and LCM 1 respectively. Tainung variety showed no significant ($p \geq 0.05$) differences in the number of nodes produced on the two low cost media during the first subculture but produced significantly more nodes on LCM 1 compared to LCM 2 during the second subculture. Tainung had the highest number of nodes on CM followed by LCM 1 and LCM 2. The number of nodes produced by the two sweet potato varieties on LCM 1 during the first subculture was not significantly different. The variety KEMB 36

produced significantly ($p \leq 0.05$) higher number of nodes on LCM 2 on both occasions of subculture compared to Tainung.

Table 4.5: Mean number of nodes for KEMB 36 and Tainung sweet potato varieties during multiplication after six weeks of culture.

	Mean number of nodes*					
	KEMB 36			**Tainung**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	3.5 ± 0.33^{ax}	4.0 ± 0.31^{by}	$\mathbf{3.8 \pm 0.25^{ax}}$	3.5 ± 0.38^{ax}	3.7 ± 0.42^{bx}	$\mathbf{3.6 \pm 0.20^{bx}}$
LCM 2	7.1 ± 0.41^{cy}	6.6 ± 0.38^{cy}	$\mathbf{6.9 \pm 0.25^{cy}}$	3.4 ± 0.37^{ax}	3.3 ± 0.37^{ax}	$\mathbf{3.4 \pm 0.05^{ax}}$
CM	4.4 ± 0.32^{by}	3.9 ± 0.34^{ax}	$\mathbf{4.2 \pm 0.25^{by}}$	3.8 ± 0.37^{bx}	3.9 ± 0.30^{bx}	$\mathbf{3.9 \pm 0.05^{bx}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.5.2 Mean number of leaves

KEMB 36 variety produced the highest number of leaves on LCM 2 followed by CM and LCM 1 respectively while Tainung variety had the highest number of leaves on CM followed by LCM 1 and LCM 2 on both subcultures (Table 4.6). KEMB 36 had a significantly higher ($p \leq 0.05$) number of leaves on LCM 2 at the end of each subculture period compared to Tainung. Tainung variety produced a significantly ($p \leq 0.05$) higher mean number of leaves on LCM 1 compared to KEMB 36 during the first subculture, however, no significant differences were noted in the number of leaves produced between the two varieties during the second subculture.

Table 4.6: Mean number of leaves for KEMB 36 and Tainung sweet potato varieties during multiplication after six weeks of culture.

	Mean number of leaves*					
	KEMB 36			Tainung		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	3.9 ± 0.29^{ax}	4.4 ± 0.29^{ax}	$\mathbf{4.2\pm0.25^{ax}}$	4.3 ± 0.37^{by}	4.4 ± 0.37^{bx}	$\mathbf{4.4\pm0.05^{bx}}$
LCM 2	8.0 ± 0.54^{cy}	7.5 ± 0.34^{cy}	$\mathbf{7.8\pm0.25^{cy}}$	3.7 ± 0.52^{ax}	4.1 ± 0.39^{ax}	$\mathbf{3.9\pm0.20^{ax}}$
CM	5.4 ± 0.38^{by}	4.7 ± 0.42^{bx}	$\mathbf{5.1\pm0.35^{by}}$	4.4 ± 0.46^{bx}	4.6 ± 0.49^{bx}	$\mathbf{4.5\pm0.10^{bx}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.5.3 Mean number of roots

On average, the variety KEMB 36 had the highest mean number of roots in CM (3.35) followed by LCM 2 (3.1) and LCM 1 (2.55) per subculture (Table 4.7). Significant differences were not detected in the number of roots produced by KEMB 36 in LCM 2 and CM during the second subculture. However, during the first subculture KEMB 36 produced significantly higher number of roots in CM compared to LCM 2. There were no significant differences ($p\geq0.05$) in the number of roots formed in LCM 1 and CM during the first subculture for Tainung. Tainung had a significantly higher number of roots per plantlet in LCM 1 compared to KEMB 36 in both subcultures. Tainung variety produced significantly higher number of roots in LCM 2 compared to KEMB 36 during the first subculture. However, in the second subculture KEMB 36 had a higher mean number of roots per plantlet compared to Tainung in LCM 2 but the difference was not significant ($p\geq0.05$). KEMB 36 variety produced a significantly higher number of roots on CM compared to Tainung.

Table 4.7: Mean number of roots for KEMB 36 and Tainung sweet potato varieties during multiplication after six weeks of culture.

	Mean number of roots*					
	KEMB 36			**Tainung**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	2.4 ± 0.26^{ax}	2.7 ± 0.29^{ax}	$\mathbf{2.6\pm0.15^{ax}}$	3.0 ± 0.27^{ay}	3.3 ± 0.42^{by}	$\mathbf{3.2\pm0.15^{by}}$
LCM 2	2.9 ± 0.51^{bx}	3.3 ± 0.31^{bx}	$\mathbf{3.1\pm0.20^{cx}}$	3.4 ± 0.65^{by}	3.1 ± 0.35^{bx}	$\mathbf{3.3\pm0.15^{bx}}$
CM	3.6 ± 0.26^{cx}	3.1 ± 0.26^{bx}	$\mathbf{3.4\pm0.25^{bx}}$	3.0 ± 0.27^{ay}	2.8 ± 0.31^{ay}	$\mathbf{2.9\pm0.10^{ay}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.5.4 Mean plant height

The two sweet potato varieties, KEMB 36 and Tainung, had significantly ($p \leq 0.05$) taller plantlets on LCM 2 compared to LCM 1 during both subcultures (Table 4.8). KEMB 36 variety produced significantly taller plantlets on LCM 2 compared to Tainung after six weeks of each subculture period.

Table 4.8: Mean plant height for KEMB 36 and Tainung sweet potato varieties after during multiplication after six weeks of culture.

	Mean plant height*					
	KEMB 36			**Tainung**		
Medium	1st subculture	2nd Subculture	Mean	1st subculture	2nd Subculture	Mean
LCM 1	1.5 ± 0.15^{ax}	2.7 ± 0.44^{ax}	$\mathbf{2.1\pm0.60^{ax}}$	2.2 ± 0.38^{ay}	2.6 ± 0.32^{ax}	$\mathbf{2.4\pm0.20^{ay}}$
LCM 2	3.8 ± 0.52^{cy}	4.1 ± 0.41^{cy}	$\mathbf{4.0\pm0.15^{cy}}$	3.1 ± 0.59^{bx}	3.4 ± 0.49^{cx}	$\mathbf{3.3\pm0.15^{cx}}$
CM	3.1 ± 0.23^{bx}	3.0 ± 0.19^{bx}	$\mathbf{3.1\pm0.05^{bx}}$	3.0 ± 0.29^{bx}	3.1 ± 0.31^{bx}	$\mathbf{3.1\pm0.05^{bx}}$

*Values are expressed as mean ± standard error of the mean. Means having the same letters are not significantly different using Tukey's HSD at 5% level. $^{a, b}$ and ^{c} represent comparisons within columns while ^{x} and ^{y} represent comparisons within rows.

4.1.6 Survival rate during acclimatization

Acclimatization of sweet potato plantlets was best on the media containing rice husks and red soil with KEMB 36 having 66.7% of its plants surviving while Tainung had 67% (Fig. 4.11). Plantlets acclimatized on red soil had the second highest survival rate with 56.2% and 53.3% survival rates for Tainung and KEMB 36 varieties, respectively. Vermiculite was the least efficient acclimatization medium with only 38.1% and 40% plants surviving for Tainung and KEMB 36, respectively.

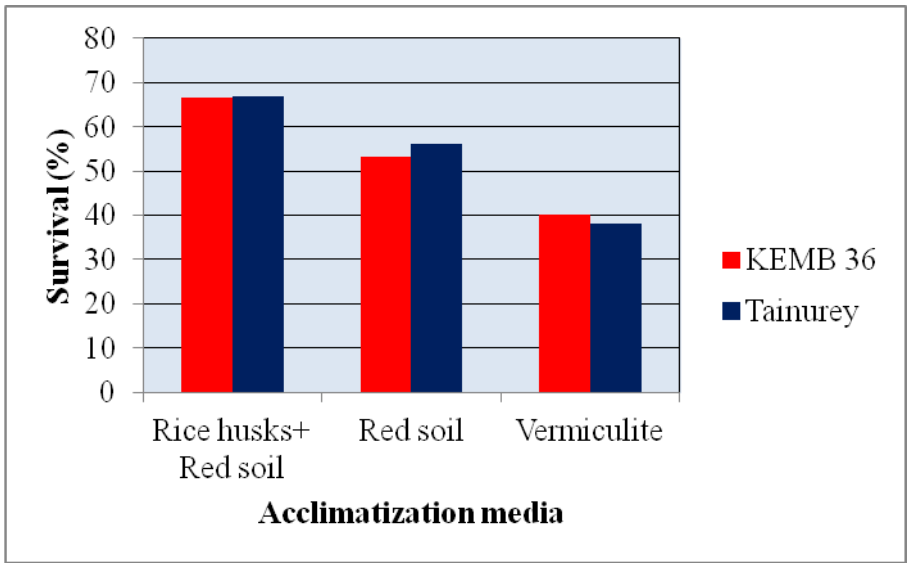

Figure 4.11: Survival rate of plantlets of KEMB 36 and Tainung sweet potato varieties acclimatized on different media.

Transfer of plants of the two sweet potato varieties onto soil was successful with good adaptation to *ex vitro* conditions (Plates 4.10 and 4.11).

Plate 4.10: Plants of KEMB 36 variety two weeks after transfer onto the soil.

Plate 4.11: Plants of Tainung variety two weeks after transfer onto the soil.

4.2 Cost efficiency

4.2.1 Comparison of cost between LCM 1 and CM nutrient sources

The use of Easygro® vegetative fertilizer as an alternative source of both macronutrients and micronutrients in LCM 1 reduced the cost of the two ingredients by 92.2% in the tissue culture of cassava (Table 4.9) and 96.2% in the case of sweet potato (Table 4.10). The use of table sugar as an alternative source for carbon reduced the cost of the carbon source by 97.1%. This led to savings of 96.3 % and 96.9 % in the cost of nutrients and carbon source used in preparing a litre of TC medium for cassava and sweet potato respectively. The same media composition was used both in initiation and in multiplication. Hence, cost reduction realized during initiation was exactly the same as in multiplication.

4.2.2 Comparison of cost between LCM 2 and CM nutrient sources

The use of locally available salts as sources of TC macronutrients in LCM 2 reduced the cost by 87.8% for tissue culture of both cassava and sweet potato (Tables 4.11 and 4.12). The use of Stanes Iodized Microfood® as a source of micronutrients reduced the cost of micronutrients by 28.9% and 68.6% for the tissue culture of cassava and sweet potato respectively. The use of table sugar as the carbon source reduced the cost of the carbon source by 97.1%. Overall, the use of LCM 2 reduced the cost of culture medium nutrients by 95.5% and 94.4% per litre for cassava and sweet potato, respectively.

Table 4.9: Cost analysis of LCM 1 nutrient sources in comparison with modified conventional MS nutrient sources used for the micropropagation of cassava.

Conventional MS nutrient	Low cost substitute	Cost in one litre of the medium (KShs.)		Cost reduction (%)
Macronutrient		**Conventional**	**Low cost**	
$CaCl_2$		1.6		
KH_2PO_4		0.6		
KNO_3		6.8		
$MgSO_4$		1		
NH_4NO_3		10		
Sub-total		**20**		
Micronutrients				
$CoCl_2.6H_2O$	Easygro®	0.0052		
$CuSO_4.5H_2O$	vegetative	0.0045	1.6	
Na_2EDTA	fertilizer	0.154		
$FeSO_4.7H_2O$		0.078		
H_3BO_3		0.102		
KI		0.07		
$MnSO_4.4H_2O$		0.092		
$Na_2MoO_4.2H_2O$		0.0078		
$ZnSO_4.7H_2O$		0.016		
Sub-total		**0.5295**		
TOTAL		**20.5295**	**1.6**	**92.2**
Carbon source				
Sucrose	Table sugar	105	3	97.1
TOTAL		**125.5295**	**4.6**	**96.3**

Table 4.10: Cost analysis of LCM 1 nutrient sources in comparison with modified conventional MS nutrient sources used for the micropropagation of sweet potato.

Conventional MS nutrient	Low cost substitute	Cost in one litre of the medium (KShs.)		Cost reduction (%)
Macronutrient		**Conventional**	**Low cost**	
$CaCl_2$		3.3		
KH_2PO_4		1.2		
KNO_3		14.4		
$MgSO_4$		1		
NH_4NO_3		21		
Sub-total		**40.9**		
Micronutrients				
$CoCl_2.6H_2O$	Easygro®	0.011		
$CuSO_4.5H_2O$	vegetative	0.009	1.6	
Na_2EDTA	fertilizer	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**

Table 4.11: Cost analysis of LCM 2 nutrient sources in comparison with modified conventional MS medium nutrient sources used for the micropropagation of cassava.

Conventional MS nutrient	Low cost substitute	Cost in one litre of the medium (KShs.)		Cost reduction (%)
Macronutrients		**Conventional**	**Low cost**	
$CaCl_2$	-	1.6	-	0
KH_2PO_4	Monopotassium phosphate (MKP)	0.6	0.041	93.2
KNO_3	Potassium fertilizer	6.8	0.342	94.9
$MgSO_4$	Epsom salt	1	0.07	93
NH_4NO_3	Ammonium quarry salt	10	0.381	96.2
Sub-total		**20**	**2.434**	**87.8**
Micronutrients				
$CoCl_2.6H_2O$		0.0052		
$CuSO_4.5H_2O$		0.0045		
Na_2EDTA		0.154		
$FeSO_4.7H_2O$	Stanes iodized microfood®	0.078		
H_3BO_3		0.102	0.24	
KI		0.07		
$MnSO_4.4H_2O$		0.092		
$Na_2MoO_4.2H_2O$		0.0078		
$ZnSO_4.7H_2O$		0.016		
Sub-total		**0.5295**	**0.24**	**28.9**
Carbon source				
Sucrose	Table sugar	105	3	97.1
TOTAL		**125.5295**	**5.674**	**95.5**

Table 4.12: Cost analysis of LCM 2 nutrient sources in comparison with modified conventional MS medium nutrient sources used for the micropropagation of sweet potato.

Conventional MS nutrient	Low cost substitute	Cost in one litre of the medium (KShs.)		Cost reduction (%)
Macronutrients		**Conventional**	**Low cost**	
$CaCl_2$	-	3.3	-	0
KH_2PO_4	Monopotassium phosphate (MKP)	1.2	0.084	93
KNO_3	Potassium fertilizer	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 iodized 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**

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Cassava

5.1.1.1 Initiation

The two cassava varieties, KME 1 and Muchericheri, had a better response in LCM 1 compared to LCM 2 for all the parameters assessed. This is an indication that LCM 1 may have had a better balanced supply of the required nutrients. Easygro® vegetative fertilizer used as an alternative source for macro and microelements in LCM 1 also had a small percentage (0.007%) of amino acids which could have enhanced plant growth. The better performance of Muchericheri variety on LCM 1 and CM compared to KME 1 for all the parameters tested and vice versa for leaf and node production on LCM 2 may be due to genetic variation between the two cassava varieties. The challenge of differential response to tissue culture medium by various plant cultivars remains not only for cassava but also other plants. Dessai *et al*. (1995) reported differential response of 27 sweet potato varieties to *in vitro* propagation techniques in terms of node formation, leaf growth, root development and plant height. Santana *et al*. (2009) reported some differences in node and leaf production of IDEA87 and CM6740-7 cassava varieties to a low cost tissue culture medium developed containing Hydro® Agri´s fertilizer (12-11-18/3 (MgO-EDTA)) and concluded that this may be due to the high diversity of the crop. An ideal tissue culture medium should be able to support a high number of varieties of a species. If this is not achieved then it means developing variety-specific medium, which is expensive.

The regeneration index observed in this study for the two cassava varieties during initiation after five weeks of culture was 3-7 nodes per plantlet. This is similar to the results reported on other cassava varieties by Santana *et al.* (2009). The number of nodes per plantlet is of prime importance since these are regions for shoot development (Mutegi, 2009). *In vitro* multiplication of cassava by direct organogenesis is through nodal cuttings hence a higher number of nodes will lead to a higher number of plantlets. Cassava forms one or more axillary buds on the stem upon sprouting. These buds develop and sequentially form nodal units consisting of a node, a bud, a palmate leaf blade subtended by a long petiole and an inter-node whose length and mass depends on genotype, age of the plant and environment (El-Sharkawy, 2003). Therefore, a good culture medium should be able to supply enough nutrients for maximum node formation.

The two cassava varieties exhibited genotype-dependent leaf production on the two low cost media with Muchericheri producing more leaves on LCM 1 while KME 1 produced more leaves on LCM 2 compared to Muchericheri at the end of the fifth week of culture. This indicates requirement of different nutrient concentrations by the two varieties. Leaves are the major site of food production for the plant and a well developed leaf system is important for survival of TC plantlets during acclimatization. Plantlets with a high number of leaves are more efficient photosynthetically and therefore adapt quickly to natural environment as compared to those with few leaves.

Muchericheri variety produced more roots compared to KME 1 in the hormone-free media. This may be due to genotypic differences between the two varieties. Roots have

an essential role and function in plant life and development, supplying water and nutrients to the plant from the environment (Schiefelbein *et al*., 1997). *In vitro* root development usually enhances transplanting success because functioning roots can create a favorable plant water balance (Diaz-Perez *et al.*, 1995). Micropropagation of cassava has been reported as being relatively straightforward with plantlets rooting without plant growth regulators (Zimmerman *et al.*, 2007; Yona *et al*., 2010).

Muchericheri had taller plants in all the three media tested compared to KME 1. This was a desirable quality during multiplication since plantlets that were tall had higher number of nodes and resulted into many plantlets. The ideal TC medium should produce tall plants with close inter-node length since more nodal cuttings can be obtained leading to a higher multiplication rate.

5.1.1.2 Multiplication

The two cassava varieties had good multiplication rates with 3.3-7.1 nodes per plantlet on all the media. A good multiplication rate for *in vitro* production of cassava planting materials is considered as 1:4 in a cycle (Escobar *et al.*, 2001). The differences in node formation on the two low cost media and the conventional medium can be attributed to differences in the concentration of the different media elements. Small adjustment in the concentrations of the various elements in the media may increase the multiplication rate of the two cassava varieties.

Leaf production was good with plantlets of both varieties producing a good number of leaves which is essential during acclimatization. The genotype-dependent response in leaf production exhibited in LCM 1 and CM can be directly attributed to differences in the genetic make-up of the two cassava varieties. Leaves are the main site for photosynthesis hence of great importance during hardening of plants *ex vitro* (Bell and Bryan, 1993). They are vital in the conversion of light energy into chemical energy. An ideal tissue culture medium should therefore be able to support development of well-structured leaves to ensure *ex vitro* plant survival.

Plantlets of the two cassava varieties developed well-structured root systems on the three media. This is fundamental during transfer onto soil. Genotype-dependent response was also evident in regard to this parameter. Root structure affects survival of plantlets *ex vitro* with plants that have well-developed roots adapting quickly to natural conditions.

Multiplication of *in vitro* cassava plantlets requires good plant height to enable easy splicing into nodal cuttings. The two cassava varieties used here did not show any significant difference in plant height on LCM 2 suggesting that nutrient supply in this medium was optimal. However, differential response on LCM 1 and CM means that these media need adjustment in order to support a wide range of cassava varieties.

5.1.1.3 Acclimatization of cassava plantlets

Adaptation of *in vitro* seedlings to natural conditions is crucial for any protocol to be successful. There are great differences between the artificial culture conditions in the

growth room, the green house conditions and the natural conditions. These include differences in intensity and quality of light; relative humidity; nutrients and medium substrate. Cassava is a delicate plant to harden and huge losses occur during transfer from *in vitro* laboratory to *ex vitro* field conditions (Jorge, 2002). Care and media optimization should therefore be undertaken. The hardening procedure was successful pointing out to the success of this protocol. Vermiculite was the best acclimatization medium followed by the one containing vermiculite and red soil and that containing a mixture of red soil and rice husks. This can be attributed to better aeration in vermiculite as compared to the other two media.

5.1.2 Sweet potato

5.1.2.1 Initiation

The sweet potato variety KEMB 36 had better regeneration efficiency compared to Tainung since it produced significantly higher number of nodes which can be attributed to genotypic differences. Similar differential production of nodes in sweet potato varieties during tissue culture was reported by Dessai *et al*. (1995) noting significant differences in the regeneration frequencies of 27 sweet potato genotypes from a wide geographical distribution. The differential response to tissue culture among different varieties means that *in vitro* regeneration procedures must be developed for each desirable genotype (Monica *et al*., 2009).

The two sweet potato varieties produced differing number of leaves in all the three media. KEMB 36 produced more leaves in LCM 2 and CM compared to Tainung and the

reverse was true in LCM 1. These differences may be due to inherent genotypic differences. A good leaf system is paramount for the survival of the explants *ex vitro*.

Just like cassava, the two sweet potato varieties also produced roots without incorporating any auxin. Tainung produced more roots in LCM 1 and LCM 2 compared to KEMB 36 indicating that the media composition in the two low cost media was more favorable for root formation in Tainung compared to KEMB 36. 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 two sweet potato varieties produced taller plants in LCM 2 and CM compared to LCM 1 after 6 weeks of culture. *In vitro* growth of plantlets of the two varieties was therefore more responsive to LCM 2 and CM compared to LCM 1. Genotype-dependent response to TC was also evident in regard to this parameter with KEMB 36 producing taller plants in LCM 2 and CM compared to Tainung.

5.1.2.2 Multiplication

KEMB 36 variety had a better culture efficiency in terms of node formation compared to Tainung. This points out to genotype-dependent response between the two varieties and means that KEMB 36 is the most suitable for adoption into farm systems since high number of planting materials can be realized. Tissue culture of sweet potato is mainly through direct organogenesis by the use of nodal cuttings thus the higher the number of nodes the higher the number of plantlets.

The two sweet potato varieties exhibited genotype-dependent leaf formation in all the media. 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). 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 (Monica *et al.*, 2009). 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 acclimatization of plantlets. Plantlets with well structured leaves are photosynthetically efficient hence adapt quickly to the natural conditions.

The two sweet potato varieties exhibited varying root formation patterns, with Tainung producing more roots on LCM 1 and LCM 2 compared to CM while KEMB 36 had significantly higher number of roots on CM compared to LCM 1 and LCM 2. Root development is critical for *ex vitro* survival of plantlets. *In vitro* root production enhances acclimatization success because functioning roots are believed to compensate for water loss caused by malfunctioning stomata (Seelye *et al.*, 2003). The physiological status of roots is critical for plant survival during the first few days of acclimatization (Jorge,

2002). Well developed roots with strong connections on the plantlets are desirable for hardening.

The two sweet potato varieties had the tallest plants on LCM 2 which indicates that the nutrient composition of this medium was suitable for plant growth. Genotype-dependent plant elongation was evident in LCM 2 with KEMB 36 producing significantly ($p<0.05$) taller plants compared to Tainung. Plant height is an important parameter when it comes to multiplication. Tall plants with intermediate inter-nodal length produce many nodal cuttings and are easy to excise.

5.1.2.4 Acclimatization of sweet potato plantlets

The acclimatization media containing rice husks and red soil supported the highest number of sweet potato plantlets. This may be due to the fact that this medium was rich in organic nutrients compared to the other two. The benefit of any tissue culture procedure can only be realized after successful transfer of plantlets from *in vitro* culture conditions to *ex vitro* natural conditions (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 and Pruski, 2002).

It is imperative to recognize and comprehend the differences between an *in vitro* and natural environment. By manipulating the *in vitro* environment, leaves that are

photosynthetically competent can be developed as part of the acclimatization process in preparing plantlets for transferring out of culture. Roots formed in culture can be beneficial for enhancing early growth following transfer from the growth room. The optimum growth rate of plantlets *ex vitro* frequently does not occur until new leaves and roots develop in the green-house environment (Seelye *et al.*, 2003).

5.1.3 Cost efficiency

Tissue culture is indispensable as a tool for production of disease-free planting materials and germplasm conservation especially in vegetative crops. TC technology offers an alternative to enhanced rates of multiplication of cassava and sweet potato. Conventional propagation of the two crops is slow and as research in these crops intensifies, it becomes increasingly appropriate to adopt TC for the rapid multiplication and distribution of new varieties or disease-free planting materials of established varieties. Tissue culture technology is, however, costly hence the low adoption rates in developing countries. The design and adoption of cost-efficient TC protocols is therefore paramount to enhance adoption of this technology.

This study has shown that it is possible to use locally available agrochemicals as low cost sources of tissue culture nutrients. This is in agreement with work done by Santana *et al*. (2009) who used different concentrations of Hydro® Agri´s fertilizer to regenerate plantlets of the cassava variety CM6740-7. They reported that higher rates of fertilizer were toxic to the tissues and that intermediate fertilizer use at 2 g/l seemed to be optimal

for cassava propagation. Escobar *et al*. (2006) also tried different kinds of fertilizers at different rates and realized a cost reduction of 24.4 %.

The use of nodal cuttings as explants in the media without growth hormones further reduced the cost of regeneration in this study. This method is based on the principle that the node of an *in vitro* plantlet placed in an appropriate culture medium is induced to develop an axillary bud, resulting in a new *in vitro* plantlet (Rolando *et al*., 1992). This type of propagation promotes the development of a pre-existent morphological structure. The nutritional condition of the medium breaks the dormancy of the axillary bud and promotes its rapid development (Rolando *et al*., 1992).

In the present study table sugar was used as the alternative source of carbon contributing to the reduction in the cost of production. This has also been used in previous studies as a low cost source of carbon in the conservation of turmeric (Tyagi *et al*., 2007) and micropropagation of bananas (Gitonga *et al*., 2010). The source of external fixed carbon during tissue culture process is paramount for the survival of plantlets *in vitro* (Demo *et al*., 2008). According to Kubota *et al*. (2001), the supply of sugar to the culture medium promotes plant growth *in vitro* and compensates for the low or negative net photosynthetic rate as a result of poor photosynthetic ability thus increasing the survival rates of tissue sections cultured *in vitro*. Therefore, plantlets require an initial source of carbon and hence energy from the medium until they are capable of using carbon dioxide as their main source of carbon.

5.2 Conclusion

The two low cost media developed in this study can be used in tissue culture of more cassava and sweet potato varieties since the multiplication rates realized for the varieties used were good. Based on the results, LCM 1 is best suited for cassava regeneration while LCM 2 is best for sweet potato regeneration.

Findings from this study have shown that it is possible to reduce the cost of *in vitro* plantlet production through tissue culture by using locally available alternative sources for MS nutrients. The two low cost media evaluated here are easy to prepare and can be easily adopted in the production of cassava and sweet potato planting materials. This will greatly enhance availability of cassava and sweet potato planting materials to farmers at an affordable cost, which will boost their production.

The type of the variety can influence response to tissue culture. The varieties of the two crops used here had differences in response to the two low cost media and the conventional medium.

5.3 Recommendations

1. The findings of this research can be adopted by commercial tissue culture laboratories in any country since salts similar to those used here to prepare the two low cost media, LCM 1 and LCM 2, can be found at affordable costs globally though under different trade names.

2. The information obtained can be used by scientists as a basis for more research on genotype-media interaction to address variations in varietal response.
3. Based on results LCM 1 is recommended for the production of cassava seedlings while LCM 2 can be adopted for sweet potato.
4. Further research is needed to optimize the media and ensure consistent production of high number of nodes hence consolidate the gains of reduced costs.

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APPENDICES

Appendix 1: Composition of Easygro® vegetative fertilizer

Element/compound	Percentage
N	27
P_2O_5	10
K_2O	16
B	0.03
Cu	0.04
Fe	0.08
Mn	0.04
Mo	0.001
Zn	0.06
Amino acids	0.007

Appendix 2: Alternatives used as sources of macronutrients in Low Cost Medium two (LCM 2)

Low cost source of MS nutrient	Composition
Monopotassium phosphate (MKP)	KH_2PO_4
Potassium fertilizer	KNO_3
Epsom Salt	$MgSO_4$
Ammonium nitrate quarry explosive	NH_4NO_3

Appendix 3: Composition of Stanes iodized microfood®

Element	Percentage
Zn	1.65
Mn	1.22
Mo	0.14
B	2.48
Fe	7.6
Cu	1.0