ESTABLISHMENT OF AN IN VITRO MICROPROPAGATION PROTOCOL FOR FARMER PREFERED COCOYAM [Colocasia esculenta (L) Schott] and [Xanthosoma sagittifolium (L) Schott] CULTIVARS GROWN IN KENYA

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A thesis submitted in partial fulfillment of the requirements for the award of the Degree of Master of Science in Biotechnology in the School of Pure and Applied Sciences of Kenyatta University

SEPTEMBER 2012
DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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This work is dedicated to little Daisy Muthoni who had to endure long hours without the one person she needed most at the time of carrying out the project, her dear mother.
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>x</td>
</tr>
<tr>
<td>ABBREVIATIONS AND ACRONYMS</td>
<td>xi</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>1</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Background information</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Problem statement</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Justification</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Research questions</td>
<td>5</td>
</tr>
<tr>
<td>1.5 Null hypothesis</td>
<td>5</td>
</tr>
<tr>
<td>1.6 Objectives</td>
<td>5</td>
</tr>
<tr>
<td>1.6.1 Broad objective</td>
<td>5</td>
</tr>
<tr>
<td>1.6.2 Specific objectives</td>
<td>5</td>
</tr>
<tr>
<td>1.7 Significance of the study</td>
<td>6</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>7</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>7</td>
</tr>
<tr>
<td>2.1 Origin and distribution of cocoyam</td>
<td>7</td>
</tr>
<tr>
<td>2.2 Cocoyam</td>
<td>8</td>
</tr>
</tbody>
</table>
2.3 Economic importance of cocoyam
2.4 Field management of cocoyam
2.5 Constraints to cocoyam production
2.6 Cocoyam improvement through biotechnology
2.7 Plant tissue culture systems
  2.7.1 Shoot cultures
  2.7.2 Meristem and shoot-tip cultures
  2.7.3 Organogenesis
2.8 Plant nutrition and media
  2.8.1 Inorganic mineral elements
  2.8.2 Organic compounds and vitamins
  2.8.3 Plant growth regulators
2.9 Tissue culture gaseous environment
  2.9.2 Carbon dioxide
  2.9.3 The influence of light and temperature on tissue culture
  2.9.4 Effects of pH on cultures
  2.9.5 Sterility/asceptic conditions
  2.9.6 Acclimatization of micropropagated plants

CHAPTER THREE
MATERIALS AND METHODS
  3.1 Collection and pre-treatment of cocoyam varieties
  3.2 Experimental design
  3.3 Tissue culture reagents and chemicals
  3.4 Media preparation and sterilization
APPENDIX II: Media for \textit{in vitro} establishment weighed using an analytical balance

.............................................................................................................................................. 62

APPENDIX III:.............................................................................................................................................. 63

(A) ANOVA results for shoots formed across all concentrations ......................... 63
(B) Number of cultured explants with shoot at varying concentrations (ratio) of
PGR.................................................................................................................................................. 63

APPENDIX IV .............................................................................................................................................. 64

(A) Results for leaves formation .................................................................................. 64
(B) Number of cultured explants with leaves at varying concentrations (ratio) of
PGR.................................................................................................................................................. 64

APPENDIX V .............................................................................................................................................. 65

(A) Result for formation of roots.................................................................................. 65
(B) Number of cultured plantlets with roots at varying concentrations (ratio) of
PGR across varieties .............................................................................................................. 65
(C) Number of rooted plantlets across concentrations (ratio) of PGR in meristem
and petiole explants .............................................................................................................. 66

APPENDIX VI .............................................................................................................................................. 67

(A) Results for the surviving plantlets in all varieties across all the concentrations
.................................................................................................................................................. 67
(B) Number of cultured plantlets that survived after potting from varying
concentration (ratio) across varieties .................................................................................. 67

APPENDIX VII .............................................................................................................................................. 68

(A) Results for the formation of corms across all varieties in all concentrations... 68
(B) Number of cultured plantlets from varying concentrations (ratio) that yielded
corms across varieties .............................................................................................................. 68
LIST OF TABLES

Table 3.1  Observations for the experimental design ........................................... 24
Table 4.1A: Shoot formation across all Concentrations at 7 Days.......................31
Table 4.1B: Shoot formation across all concentrations at 42 days .....................34
Table 4.2A: Formation of leaves in all concentrations ......................................... 35
Table 4.2B: Leaf formation across all concentrations at 42 days ....................... 37
Table 4.3: Formation of rooted plantlets in all varieties across the concentrations ....38
Table 4.4: Surviving plantlets in all varieties across all the concentrations .......... 40
Table 4.5: Formation of corms across all varieties in all concentrations ............... 42
LIST OF PLATES

Plate 3.1: Samples reduced in size after removal of leaves and corms

Plate 3.2: Surface sterilized explants in A) magenta bottles and B) petri dishes ready for inoculation into media

Plate 3.3: Sub-culturing of cocoyam showing A) micro-propagated explants and B) sub-cultured explants into Magenta bottles

Plate 4.1: Samples of collected cocoyam for the varieties

Plate 4.2: Corms produced from A-Githungu (X. sagittifolium), B-Ngirigacha (C. esculenta), and C-Kigoi (C. esculenta) variety

Plate 4.3: Tissue culture production system of Cocoyam
## ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABVC</td>
<td>Alomae-Bobone Virus Complex</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BAP</td>
<td>Benzyl-amino Purine</td>
</tr>
<tr>
<td>CaCl₂ H₂O</td>
<td>Calcium Chloride</td>
</tr>
<tr>
<td>CBDV</td>
<td>Colocasia Bobone Disease Virus</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon Dioxide</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>Cobalt Chloride</td>
</tr>
<tr>
<td>CRD</td>
<td>Completely Randomized Design</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>Double Distilled Water</td>
</tr>
<tr>
<td>DMV</td>
<td>Dasheen Mosaic Virus</td>
</tr>
<tr>
<td>EAH</td>
<td>East African Herbarium</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic Acid</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric Acid</td>
</tr>
<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Air</td>
</tr>
<tr>
<td>H₃O₃</td>
<td>Boric acid</td>
</tr>
<tr>
<td>IAA</td>
<td>Indole-acetic Acid</td>
</tr>
<tr>
<td>KARI</td>
<td>Kenya Agricultural Research Institute</td>
</tr>
<tr>
<td>KNO₃</td>
<td>Potassium Nitrate</td>
</tr>
<tr>
<td>KOH</td>
<td>Potassium Hydroxide</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>Magnesium Sulphate</td>
</tr>
<tr>
<td>Mg/l</td>
<td>Milligrams / litre</td>
</tr>
<tr>
<td>MS</td>
<td>Murashige and Skoog</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>Ammonium Nitrate</td>
</tr>
<tr>
<td>NMK</td>
<td>National Museums of Kenya</td>
</tr>
<tr>
<td>PGRs</td>
<td>Plant Growth Regulators</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl Chloride</td>
</tr>
<tr>
<td>RAPD</td>
<td>Random Amplified Polymorphic</td>
</tr>
<tr>
<td>TaBV</td>
<td>Taro Bacilliforum Virus</td>
</tr>
<tr>
<td>WPM</td>
<td>Woody Plant Medium</td>
</tr>
</tbody>
</table>
ABSTRACT

Most of the world’s food poor people live in sub Saharan Africa. For example in Kenya in 2004, 48.4% of Kenya’s population was considered as food poor. To meet this shortfall, a 55% increase in food production in Africa will have to come from intensification of production from land under cultivation. Several abiotic and biotic stresses limit crop production in Kenya. Exploitation of indigenous leafy vegetables adapted to the local environment will not only overcome both abiotic and biotic stresses but also improve food security, nutrition, and health of the rural poor. Cocoyam (Colocasia esculenta spp. and Xanthosoma sagittifolium spp) (L.) Schott) is an ancient crop grown throughout the humid tropics of Africa, the West Indies, the Pacific region and Asia for its edible corms and leaves, as well as for its traditional uses and is an important staple food for these developing countries as well as a principal root crop with great promise in generating income within the rural communities. Although cocoyam is an important staple in Africa, its production is constrained by diseases caused by fungi, bacteria, viruses and other pathogens. Tissue culture systems have assumed considerable importance as methods of producing disease-free plants. In vitro techniques offer an alternative, reliable method for the production of planting material, and the rates of multiplication have generally been modest. Currently there is no tissue culture system in place for cocoyam propagation for Kenyan cultivars. There is need therefore to develop a tissue culture system for regeneration of Kenyan cocoyam varieties. In this study applied tissue culture techniques to three Cocoyam varieties collected from major growing areas using their petioles and meristems as explants, that is, Kigoi (Colocassia esculenta L. Schott), Githungu (Xanthosoma sagittifolium L. Schott) and Ngirigacha (Colocassia esculenta L. Schott). The resultant plantlets were then exposed to greenhouse and field conditions and performed well. Five variations of plant growth regulators namely (BAP and IAA) in combinations of 0.0 mg/L and 0.0 mg/L IAA; 2.0 mg/L BAP and 0.5 mg/L IAA; 4.0 mg/L BAP and 1.0 mg/L IAA; 6.0 mg/L BAP and 2.0 mg/L IAA and; 8.0 mg/L BAP and 3.0 mg/L IAA were titrated into Murashige and Skoog (MS) medium. Analysis for shoot and leaf formation, rooting, survival and corm production parameters illustrated that the highest yields were from meristems explants for BAP/IAA (mg/L) concentration (ratios) whereby Kigoi (6.0: 2.0) led in shoot formation, Githungu (without growth regulators PGR) in leaf formation and Ngirigacha (2.0:0.5) in root formation, plantlet survival and corm production. These concentrations were optimum for in vitro growth and production of corms in the three varieties. The micro-propagation protocol established in this study can be applied to laboratories in the regions efficiently, whereby it is hoped that the study findings will help to address the issue of food security.
CHAPTER ONE
INTRODUCTION

1.1 Background information

Cocoyam is a herbaceous plant belonging to the *Araceae* family (Reyes-Castro *et al.*, 2005). The *Araceae* is a large family, comprising some several hundred genera and more than fifteen-hundred species. Mostly tropical or subtropical plants, the aroids grow mainly in moist or shady habitats. Some are terrestrial plants while others are vines, creepers, or climbers (Lee, 1999). The major edible aroids are classified in two tribes and five genera; Lasioideae (*Cyrtosperma* and *Amorphophallus*) and Colocasiodeae (*Alocasia, Colocasia,* and *Xanthosoma*) (Lee, 1999). In some locations, the name cocoyam refers to plants in both the *Xanthosoma* and *Colocasia* genera (Volin and Beale, 1981). *Colocasia esculenta* (L. Schott) is the scientific name of “old” cocoyam and *Xanthosoma* spp. refers to “new” cocoyam (Karikari, 1971; Onwueme, 1978; Lyonga, 1979). Other common names of cocoyam include Taro (*Colocasia* spp.), Dasheen, Eddoe, Old cocoyam and Malanga (*Xanthosoma* spp.) (Kay, 1978).

*Colocasia* and *Xanthosoma* are the most important of the edible genera (Lee, 1999). The two, *C. esculentum* and *X. sagittifolium* (L) Schott are commonly referred to as Taro and Tannia, respectively (Lewu *et al.*, 2010). These are mirror root crops erroneously referred to as arrowroot, a West Indian name of *Maranta arundinacea* (Accland, 1971). Although there are about 30-40 cocoyam species that grow randomly throughout the tropics, only five or six are important sources of edible products (National Academy of Science, 1975). Despite their adaptability, acceptance, and commercial food value, cocoyams have received little attention by researchers. Consequently, their potential is not being realized, and their use was reported as declining (Ayensu *et al.*, 1975).
The total world production of cocoyams (*Colocasia* spp. and *Xanthosoma* spp.) was estimated at $5 \times 10^6$ t in 1983, with more than half of that production ($3.4 \times 10^6$ t) from Africa (Lyonga and Nzietchueng, 1986). However, Onwueeme (1991) estimated the global average cocoyam yield to be 6,000 kg/ha. Recently, the annual production of *Colocasia* was estimated at 170,000 tons from an area of 31,000 ha (FAO, 2001) and is now cultivated in more than 65 countries worldwide (USDA, 2001). Current yield levels of cocoyam production are low; on a worldwide basis, the crop yield only about 6,000 Kg ha$^{-1}$ compared with 14,746 kg ha$^{-1}$ for potato (*Solanum Tuberosum* L) and 13,628 kg ha$^{-1}$ for sweet potato *Ipomoea Batatas* L (FAO, 2007) On a global basis the growth in root and tuber projections averaged 1.3% p.a., 1.8% p.a. and 1.6% p.a. (FAO 2000), (FAO 2010) and (IFPRI 2020) studies respectively. In Kenya 75.8% of farmers have less than 0.5 hectares (1 acre) of land of which 25% (0.1 hectare) is allocated to cocoyam production and in urban areas where commercial value is associated with the crop, involvement of men in production was 70% (Serem et al., 2008).

1.2 Problem statement

Sub-Saharan Africa is considered one of the world’s food insecure regions, for example in 2004, 48.4% of Kenya’s population was considered as food poor (ROK, 2004). The available FAO statistics indicates a worsening scenario in which Africa’s overall food production capacity is said to be increasing at the rate of only 1.4% while its population is expanding at about 2.4% per year (FAO 2000). The continuing decline in food production will have to be reversed if massive food insecurity, poverty, social and political instability are to be averted. Several abiotic and biotic stresses do limit crop production in Kenya. These range from drought to salinity and from pests to diseases. Exploitation of indigenous leafy vegetables adapted to the local environment will not only overcome these stresses but also improve food
security, nutrition and health of the rural poor (Okeno et al., 2008). Area expansion and irrigation are estimated to account for 45% of the increase expected while the remainder 55% will have to come from intensification of production from land under cultivation (Kitch et al., 2002)

Cocoyam has been a principal root crop with great promise in generating income within the rural communities and as a staple crop. Despite the adaptability, acceptance, and commercial food value of cocoyam, it has received little attention from researchers (Engel and Julien, 1975) and consequently its potential as an economic and food security crop is not realized. Progress has been made in the adequate production of cocoyam amongst smallholder farmers particularly through vegetative propagation used as the conventional cultivation method for the field. Many (or most) cocoyam plants are infected by virus. The virus is found in the corms and cormels, so transplanting them transmit the virus (Vietmeyer, 2006). Low cocoyam yields in Africa are mainly attributed to disease (Arene and Okpala, 1980). The major diseases are cocoyam disease (*Corticium rolfsii*) and a fungal root rot disease of *xanthosoma* (*Phythium myriotylum*) and both reduce yield by 90% (Engel and Julien, 1975).

Traditional cultivation techniques, in which suckers are planted at depths of up to 30 cm, also tend to give a higher corm yield at the expense of sucker production (Malamug et al., 1992). However, there has been limited availability of proper planting material which has posed problems in the small-holder farming systems. This is due to the weakness and susceptibility of the raw planting material to pathological agents which have spread amongst farmers’ fields (Ko et al., 2008). A number of problems, such as the inevitable use of a considerable percentage of cocoyam harvested, as ‘seed’ for the next season (Okonkwo, 1987) have led to the development of several tissue culture techniques for its propagation. Additionally,
cocoyam diseases caused by fungi, bacteria, viruses or other pathogens, result not only in subsequent reduction in vigor, quality and yield of the crop, but also constitute a barrier to international exchange of its germplasm. Unlike fungal and bacterial diseases of cocoyam, viral diseases are not curable with any commercially available treatment (Wagih, 1997). There was need therefore for developing tissue culture methods for cocoyam varieties.

1.3 Justification

Tissue culture has assumed considerable importance as a method of producing disease-free cocoyam plants. In general fungal and bacterial pathogens as contaminants are routinely eradicated. Viruses, however, are much more difficult to eliminate. In vitro meristem-tip culture technique alone, or with the aid of thermal or chemical therapy or a combination of both, has frequently been used to eliminate systemic viruses from various crop plants (Wagih et al., 1995).

Micropropagation of cocoyam plants has been reported through protocorm-like bodies (Sabapathy and Nair, 1992), callus culture (Yam et al., 1990) and subsequent regeneration of adventitious plantlets. In vitro techniques offer an alternative, reliable method for the production of planting material, and the rates of multiplication have generally been modest (Malamug et al., 1992). However, tissue culture requirements of cocoyam cultivars vary (Sabapathy and Nair, 1992), and therefore new or modified procedures are needed for the micropropagation of cocoyam cultivars in Kenya. To achieve this, there is need to adopt an efficient and rapid system of in vitro micropropagation of local cocoyam cultivars in order to make adequate, clean planting material available to the subsistence farmers. Developing a rapid and sustainable micropropagation system by shoot multiplication of cocoyam will contribute to the food production systems of the rural communities which depend on
cocoyam as a staple root crop. This study therefore aimed to investigate the in vitro responses of local cocoyam cultivars in Kenya, and establish a rapid and sustainable mass propagation protocol for adequate clean and healthy cocoyam planting material in a relatively short time. The cultivars to be studied were Kigoi (Colocassia esculenta L. Schott), Githungu (Xanthosoma sagittifolium L. Schott) and Ngirigacha (Colocassia esculenta L. Schott), which are the main farmer preferred varieties.

1.4 Research questions

i. Is it possible to propagate Kenyan cocoyam varieties via in vitro tissue culture?

ii. Would Kenyan cocoyam respond to growth regulators?

iii. Would invitrally grown plantlets mimic vegetatively cultivated cocoyams in response to the natural environment?

1.5 Null hypothesis

Kenyan cocoyam varieties cannot be propagated via in vitro tissue culture.

1.6 Objectives

The study had both a broad and a specific objective.

1.6.1 Broad objective

To develop a tissue culture protocol for in vitro micropropagation of Kenyan cocoyam.

1.6.2 Specific objectives

i. To identify the best explants cultivar for use in regeneration of Cocoyam
ii. To determine the optimal BAP and IAA concentration to promote shooting and rooting of the explants cultivar of cocoyams

iii. To assess the performance of the regeneration in the greenhouse environment and in the field.

1.7 Significance of the study

This study will go towards improving the food security status and the nutrition of rural households in Kenya and within the region. There will be readily available seedlings for planting to increase the income levels of farmers. Additionally, the study will go towards realizing the potential of cocoyam as an economic crop and toward its conservation since it is an endangered species classified as an orphan crop (Morren and Hyndman, 1987). This will also facilitate the establishment of a cocoyam tissue culture database in the country.
CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and distribution of cocoyam

*Colocasia* spp. originated in South East Asia and was introduced to Africa through Egypt. It was then spread along the East Coast of Africa and across the continent to West Africa (Plucknett *et al.*, 1970). *Xanthosoma* spp. originated in tropical America and was introduced to West Africa by Indian missionaries around 1840 (Purseglove, 1972; Doku, 1980). From its centre of origin, taro spread eastward to the rest of South East Asia, and to China, Japan and the Pacific Islands (FAO, 2009). From Asia, taro spread westward to Arabia and the Mediterranean region. By 100 B.C., it was being grown in China and in Egypt (FAO, 2009). It arrived on the east coast of Africa over 2,000 years ago (FAO, 2009), finally spreading into East and West Africa and thereby being an introduced crop in East Africa (Ki-zerbo, 1990).

*Colocasia* is one of the most important economic crops for starch food in the eastern Carribean (Archibald 2002). However, the largest area of cultivation is in West Africa, which therefore accounts for the greatest quantity of production (FAO, 2007). It is an important food security crop in Nigeria and commonly grown by women (Okoye *et al.*, 2008). Nigeria is currently the world’s largest producer of cocoyam accounting for 37% of total world output in 2007 (FAO, 2007), prior to which it accounted for about 40% of total production (Eze and Okorji, 2003). *Xanthosoma* is cultivated in the Caribbean, Central and Tropical America, West and Central Africa, South East Asia, Oceania and New Caledonia (Giacometti and Leon, 1994).

In Tanzania and Uganda, cocoyam is occasionally grown in areas with well distributed rainfall such as the highlands and to the North and Western shores of Lake Victoria (Accland,
In Kenya, it is widely grown in central province, and to a lesser extent in Embu, Meru, Machakos, Nandi and Kakamega Counties where it is cultivated on upland valley bottoms and also where waste water collects (Maundu et al., 1999). However, there is very little information available on varieties found in Kenya and identified cocoyams in the East African Herbarium (EAH) found at the National Museums of Kenya (NMK) (Personal communication, 2008). This study availed samples to the EAH, for identification and labeling through literature searches and photography.

2.2 Cocoyam

Colocasia genus is herbaceous often with large leaves (Lee, 1999) and grows to a height of 1-2 meters (Miyasaka, 1979). The leaf is peltate, and the root system is fibrous, lying in the top one meter of the soil (Miyasaka, 1979). The corm is a nutrient storage organ with an abundance of periderm, food storage in large, thin-walled parenchymatous cells, poorly developed few vascular bundles and with presence of latex cells and mucilage latex (Miyasaka, 1979). The surface of the leaf laminae is glabrous and marked by a pinnate venation pattern with three major veins that extend through the length of the lamina and through the basal lobes (Lee, 1999).

Xanthosoma produces a subterranean stem called corm, from which smaller offshoots, termed cormels arise (ReyesCastro, 2005). The plants are vegetatively propagated through pieces of the main corm or whole, small cormel and thus guarantees the genetic stability of the material (ReyesCastro, 2005) and reproduced asexually since the plant seldom flowers (Waters et al., 1992). The leaf morphology of Xanthosoma is sagittate (Mbouobda et al., 2007). There are three types of Xanthosoma ("white", "red" or "pink and yellow") with distinctive features in texture and color of the inside of the cormels and corms, as well as the petiole color and
shape (Oghenekome et al., 1992). Salazar et al., (1985) and Monge et al., (1987) named the white cultivar *X. sagittifolium* and the red one *X. violatum* respectively, while, Lyonga et al. (1979) proposed that the yellow cultivar could be *X. atrovirens*. The inflorescence and floral organization between the yellow and the other two cultivars are different and there exists a pollen sterility coupled with stamina indehiscence in the yellow cultivar (Ngouo et al., 1989).

### 2.3 Economic importance of cocoyam

*Colocasia* is an ancient crop grown and cultivated throughout the humid tropical and subtropical countries for its edible corms and leaves, as well as for its traditional uses (Lee, 1999). It is an important group of tropical and sub-tropical root crops produced and consumed as a staple food by about 200 million people (Lyonga and Nzietcheung, 1986), being one of the few major staple foods where both the leaf and underground parts are important in the human diet (Lee, 1999). It forms the staple diet of many people throughout the South Pacific region, Asia and Africa (Sabapathy and Nair, 1992). The corms and cormels may be eaten boiled, mashed, pounded, alone or mixed with other starchy staples, or grated and incorporated into soups and stews (Lyonga and Nzietcheung, 1986). Many varieties of *Colocasia* spp. and *Xanthosoma* spp. are valuable because most parts of the plant may be used for food. The tubers provide easily digested starch, and the leaves are consumed as green vegetables.

Cocoyam leaves have the same nutritional value as that of spinach; have a high thiamine and potassium content and vitamin B-complex more than that of whole milk (Miller, 1971; Morton, 1972) which is an advantage over modern diets where a lot of refined carbohydrate is consumed (Lyonga and Nzietcheung, 1986). Large servings of cocoyam corms become a significant source of dietary protein, especially if taken more than once a day (Miller, 1971).
Nutritionally, cocoyam is superior to cassava and yam in the possession of higher protein, mineral and vitamin contents in addition to having a more digestible starch (Splitoesser et al., 1973; Parkinson, 1984). It is highly recommended for diabetic patients, the aged, children with allergy and for persons with intestinal disorders. It can also be used as an industrial raw material in the manufacture of alcohol and drugs (Onyenweaku and Okoye, 2007).

In East Africa, cocoyams has traditionally been steamed and eaten as a snack alongside tea or a beverage (Serem, 2008). The leaves are also used as vegetables as a side dish (mboga), and for mashing with traditional food (Kienyeji) (Maundu et al., 1999). Many “modern” restaurants and hotels in Nairobi have added indigenous food to their menu, such as cocoyam, sweet potato, mashed maize, potatoes, and cocoyam leaves (kienyeji), and maize and beans mixture (githeri) (Daily Nation, 2008). Occasionally, the corms are roasted in fire and eaten without peeling (Accland, 1971). The *Colocasia* leaves contain higher levels of protein and are also an excellent source of carotene, potassium, calcium, phosphorous, iron, riboflavin, thiamine, niacin, vitamin A, vitamin C and dietary fibre (Onwueme, 1978; Lambert, 1982; Hanson and Imamuddin, 1983). The corm contains greater amounts of vitamin B-complex than whole milk (Lee, 1999) and is higher in protein than that of tannia (new cocoyam; *Xanthosoma sagittifolium*) and all other nutrients except oil (Deo et al., 2009).

2.4 Field management of cocoyam

Cocoyam is vegetatively propagated (Strauss et al., 1979), however, it may reproduce sexually (Ivancic, 1992). New cultivars can be generated with improved qualities through sexual hybridization (Strauss et al., 1979). For the production of better yields, using about 1 cm of the corm tip with 15-20 cm petiole is preferable than planting the corm alone.
(Masalkar and Keskar, 1998). Harvesting is done when the plant reaches maturity in approximately 200 days (Waters et al., 1992) and when leaves have yellowed and almost died. Tubers can be left in the soil until needed, unless there is a possibility of frost (Larkcom, 1991). Maintaining the crop is easy because the large leaves shade the soil and prevent vigorous weed growth (Accland, 1971).

### 2.5 Constraints to cocoyam production

Although cocoyam has been identified as an important staple root crop (Reyes Castro, 2005; Mbouobda et al., 2007; Lewu et al., 2008), it is being replaced by other root crops largely due to pests and disease problems (Ivancic, 1992). Its production has been declining over the years in countries such as Cameroon (Onokpise, O.U, 1999) and other cocoyam-producing countries largely due to a root rot disease complex whose main causal agent is *Pythium myriotylium* (Steiner, 1981; Adams et al., 1988; Agueguia and Onokpise, 1991). Currently, Cocoyam is being grown in artificial environments where a polyvinyl chloride (PVC) paper is spread in a dug ground and covered with soil to aid in water retention hence providing a conducive environment for the growth of cocoyam (Personal communication, 2010). The vegetative propagation of cocoyam makes it sensitive to viruses and bacteria, transmitted through the propagation material. Such viruses include the Dasheen Mosaic virus (DMV) (Zettler and Hartman, 1987), The *Taro bacilliform* virus (TaBV)Brunt et al., 1990; Pearson et al., 1999 and the Alomae-Bobone virus complex (ABVC) which contributes to the dwindling production of cocoyam (Sar et al., 1998). The symptoms exhibited in DMV include “feathering”, mosaic and distortion on the leaves (Zettler and Hartman, 1987) The *Taro bacilliform* virus (TaBV) is a *badnavirus* that results in a range of mild symptoms including stunting, mosaic and down curling of the leaf blades, while, Colocasia bobone disease virus (CBDV) is a *cytorhabdovirus* which causes alomae disease in complex with
TaBV (Deo et al., 2009). The resulting symptoms include feathery mosaic on the leaves; the lamina and veins becoming thick, and crinkling of the young leaves (Deo et al., 2009).

Among the pests, the taro beetle belonging to the genus *Papuana* is of great concern. It feeds on the growing corm leaving large holes that reduce the market quality (Deo et al., 2009). Others include the taro leafhopper (*Tarophagus Proserpina*), that transmits viruses and may cause wilting and death of the plant in heavy infestations (Deo et al., 2009). Additionally, there is limited availability of proper cocoyam planting material and thus an emerging problem in generating income within the rural communities (Ko et al., 2008). It is therefore important to come up with ways of increasing planting materials and possibly drought resistant plants.

### 2.6 Cocoyam improvement through biotechnology

The improvement of cocoyam has been applied using genetic markers, which have offered breeders an opportunity to improve the efficiency of selection for crop improvement (Heun and Helentjaris, 1993). Markers have been used to study the genetic diversity and population structure of cocoyam accessions with a view to generating the necessary information for breeding programs (Offei et al., 2004). *In vitro* storage and conservation of cocoyam has been carried out (Zandvoort et al., 1994) including cryopreservation of embryoids (Jayos et al., 1994). Additionally, cocoyam cormels have been utilized as simple solid substrates to produce protein enriched products through fermentation procedures (Duru and Uma, 2003).

Regeneration of taro plants from protoplasts has been reported in *C. esculenta* (Murakami et al., 1995) with low frequencies and lengthy processes. Induction of organogenic callus has been reported in *C. esculenta* by (Yam et al., 1991) and somatic embryogenesis procedures
(Verma et al., 2004). (Deo 2008) has described the effective transformation system in *C. esculenta* using both *Agrobacterium tumefaciens* and microprojectile bombardment of regenerable embryogenic suspension cultures. Intensive clonal propagation of axenic and disease-free colocosia through tissue culture (Jackson et al., 1977) involving excision of apical and auxiliary buds, decontamination and culturing them *in vitro* in sterile nutrient medium has also been described (Deo et al., 2009). Efforts to produce a stable micropropagation system for quality plants have been done through achieving a simple, economical and rapid multiplication protocol for the mass propagation of cocoyam (Ko et al., 2008). Micropropagation protocols for cocoyams have been achieved in the Asian world (Paul and Bari, 2007; Ko et al., 2008), however there is no information available on tissue culture for the local Kenyan cultivars.

2.7 Plant tissue culture systems

Plant tissue culture techniques are a part of a large group of plant biotechnologies which involve the aseptic growth of cells, tissues, organs and *in vitro* regeneration of plants (Brown and Thorpe, 1995). Plant tissue culture is used in the micropropagation and mass production of many valuable crops (Levin et al., 1998; Burne, 2006). It is useful when producing genetically modified plants since the genetic content of one cell can be replicated throughout the entire plant (Burne, 2006). There are three methods used for micropropagation: (1) enhancing auxiliary-bud breaking; (2) production of adventitious buds; and (3) somatic embryogenesis (Brown and Thorpe, 1995).

Additionally, plant tissue culture has been successfully used in the production of synthetic or artificial seed (Brown and Thorpe, 1995); disease elimination by heat therapy and meristem culture; higher rates of multiplication of virus-tested plantlets by micropropagation, and the

### 2.7.1 Shoot cultures

Shoot cultures have been established in numerous species of higher plants (Vasil and Vasil, 1984). Shoot tip cultures describe rootless sprouts growing on media, and are also widely used for clonal propagation (Binding and Krumbiegel-Schroeren, 1984). Shoot cultures are initiated from explants which include meristems (embryos, seeds, or shoot tips and nodes of plants) or other parts which exhibit other types of organization (Binding and Krumbiegel-Schroeren, 1984). Shoot tips or primary shoot apices excised from healthy corms have been used as explants (Arditi and Strauss, 1979) for initiation into tissue culture.

The constituents of all apical meristems are less differentiated and are genetically more stable than those of other mature tissues (D'Amato, 1952; Partanen *et al.*, 1955) with the result that the progeny regenerated from *in vitro* cultured meristem exhibit greater genetic stability (Kartha, 1984). Meristem-tip culture of cocoyam has commonly been used for the production of virus-free clones (Strauss and Arditti, 1980). This technique can equally be applied in the local Kenyan cocoyam cultivars to enhance the quality and production of cocoyam and hence elevate the incomes of rural communities as well as improve the national food security status.

### 2.7.2 Meristem and shoot-tip cultures

Meristem and shoot-tip culture are techniques performed to accomplish objectives as described by Styer and Chin (1983) including: propagation of genetically uniform plants; production of pathogen-indexed plants and preservation of germplasm (Styer and Chin,
1983). These techniques require the axenic excision of the meristematic region of a shoot, its transfer to a sterile growth medium, and the manipulation of the culture conditions that result in the formation of complete plantlets. Additionally, the benefits of meristem-tip and shoot tip culture include the rapid vegetative propagation of economically important horticultural plants, especially plant that are difficult to propagate conventionally, such as orchids (Styer and Chin, 1983).

### 2.7.3 Organogenesis

This is a process of differentiation by which plant organs are formed from pre-existing structures whereby cells are forced to undergo changes that lead to the production of unipolar structures, namely root and shoot primordium that are connected to the parent tissue (Bohovora *et al.*, 1995). Organogenesis relies on the production of organs, either directly from an explant or from a callus culture. The methods of organogenesis depend on adventitious organs arising either from a callus culture or directly from explants (SBP2, 2003). Organogenesis relies on the inherent plasticity of plant tissues, and is regulated by altering the components of the medium. In particular, it is the auxin to cytokinin ratio of the medium that determines which developmental pathway the regenerating tissue will take (SBP2, 2003). Hossain (2009) established a suitable organogenesis protocol for *in vitro* maintenance of cocoyam. This can be adopted for the local Kenyan cocoyam varieties.

### 2.8 Plant nutrition and media

Tissue culture media consists of 95% water, macro-elements and micro-nutrients, plant growth regulators (PGRs), vitamins, sugars and amino acids. The different media differ in the concentrations of the different constituents and the source of certain minerals (Dodds and Roberts, 1985).
The basic nutritional requirements of cultured plant cells are very similar to those normally utilized by whole plants. Many plant tissue culture media are in the market, Murashige and Skoog (MS) 1962 is the most suitable and most commonly used basic tissue culture media for plant regeneration from tissues and callus. It was originally developed for tobacco. Others include Linsmaier and Skoog media (1965), Lloyd and McCown (1980), which is usually referred to as the woody plant media (WPM) and Gamborg’s B5 medium (Gamborg et al., 1968). Most of these media are available commercially but they can also be prepared in the laboratory as stock solutions. However, the nutrient media which are used successfully for cells, tissues, and organs were devised to meet particular requirements (Murashige and Skoog, 1962; Gamborg et al., 1968; Schenck and Hildebrandt, 1972).

Murashige and Skoog (MS) (1962) or Linsmaier and Skoog (LS) (1965) salt composition is used very widely, particularly if the desired objective is plant regeneration. The B5 medium, or its derivatives, have been valuable for cell and protoplast culture (Gamborg et al., 1981; Kao, 1977). The type of tissue culture media selected depends on the species to be cultured. Some species are sensitive to high salts or have different requirements for plant growth regulators (PGRS). The type of organ cultured is important, for example roots require thiamine (Roberts et al., 1990). The nutrient media generally consist of inorganic nutrients, carbon source(s), vitamins, growth regulator(s), possible organic supplements, and a variety of salts to supply the needed macro- and micronutrients (Gamborg, 1984). These components have been used in the tissue culture of a wide range of crops, ornamental plants and trees including roots and tubers, bananas and plantains, cereals, flowers, medicinal and industrial plants and eucalyptus (Tideman and Hawker, 1982; Taylor et al., 2004; Ali et al., 2007; Jones et al., 2009). The components of the nutrient media are explained in the following subsections.
2.8.1 Inorganic mineral elements

Plants grown *in vitro* differ markedly from those grown *in vivo*. The root system of *in vivo*-grown plants provides a large surface area and hence a greater potential for mineral uptake. Plants grown *in vitro* often lack root systems and rely solely on unspecialized cells for nutrient uptake (Leifert *et al.*, 1995). In addition, the natural environment provides a certain amount of replenishment through mineralization and ion exchange. Therefore, *in vivo* plants have a relatively continuous supply of mineral nutrients, while *in vitro* culture media contain a defined level of mineral nutrients that are not usually replaced unless the plant is subcultured onto fresh media. Though there exists a number of fundamental differences between *in vivo* and *in vitro* grown plants, the underlying principles of mineral uptake and transport appear to be the same (reviewed by Leifert *et al.*, 1995; Williams, 1995).

The choice of macro- and micro-salts and their concentrations is species-dependent (Dodds and Roberts, 1985). The macro-elements are classified as inorganic mineral elements and include nitrogen, potassium, magnesium, calcium, phosphorus and sulfur (Dodds and Roberts, 1985). Microelements include cobalt (Co), iron (Fe), manganese (Mn), molybdenum (Mo), copper (Cu) and zinc (Zn). Macro and microelements are supplied in the medium in the form of salts (Schaeffer, 1981).

2.8.2 Organic compounds and vitamins

The organic compounds used for tissue culture include sugar, myo-inositol, glycine, nicotinic acid, pyridoxine, thiamine and biotin. Sugar is a very important part of any nutrient medium and its addition is essential for *in vitro* growth and development of culture (Murashige and Skoog, 1962). Most plant cultures are unable to photosynthesize effectively for a variety of reasons including; insufficiently organized cellular and tissue development, lack of
chlorophyll, limited gas exchange in the tissue culture vessels and less than optimum conditions such as low weight (Skoog and Miller, 1957). A concentration of 20–60 g/l sucrose is often used as a carbon or energy source, since the sugar is also synthesized and transported naturally by the plant. Other sugars that may be used include glucose, fructose, sorbitol and maltose. The sugar concentration chosen is dependent on the type and age of the explant in culture (Caponetti, 2000). For carbon and energy source, sucrose in the medium is rapidly converted to glucose and fructose (Schaeffer, 1981).

Vitamins are also organic substances required in the medium. Of the vitamins, only thiamine is essential in culture as it is involved in carbohydrate metabolism and the biosynthesis of some amino acids. Other commonly used vitamins in tissue culture media include pyridoxine, folic acid, tocopherol, riboflavin and ascorbic acid (Caponetti, 2000). A sugar alcohol (inositol) is also incorporated and is important in the synthesis of phospholipids and cell wall pectins (Schenk and Hildebrandt, 1972). Complex organics are also sometimes added, and examples which include casein hydrolysate, coconut milk, orange juice and banana puree. Such compounds are often used when no other combinations of known defined components produces the desired growth or development (Fosket, 1994).

Amino acids such as L-glutamine, asparagine, serine and proline are also used in cell culture media. Plant cells in culture have a requirement for thiamine. There are beneficial effects achieved by addition of nicotinic acid, pyridoxine, pantothenate, biotin, and folate (Gamborg et al., 1981); and enzymatic hydrolysates of proteins such as casein (Gamborg, 1984).
2.8.3 Plant growth regulators

Plant growth regulators (PGRs) also known as plant hormones or phytohormones regulate and integrate the overall growth, development and reproduction in plants. This involves the regulation at the level of size of individual parts, integration of their function and ultimately the generation of the final form. The morphogenesis devolves basically around the processes of cell division; cell elongation and cell differentiation and hormones are involved in regulating all of them (Johri and Mitra, 2001). The most important PGRs used in tissue culture are auxin and cytokinins (Gaspar et al., 1996). Auxin was among the first hormone to be discovered and subsequently gibberellins, cytokinins, abscisic acid and ethylene were identified. These five hormones are often referred to as the classical five and represent five distinct groups (Johri and Mitra, 2001). The PGRs exert dramatic effects at low concentrations (0.001 to 10.000 μM) (Beyl, 2000). They regulate the initiation and development of shoots and roots on explants and embryos in semisolid or in liquid medium cultures. Sometimes, a tissue or an explant is autotrophic and can produce its own supply of PGRs, but usually, they are supplied in the medium for growth and development of the culture (Beyl, 2000).

2.9 Tissue culture gaseous environment

The gaseous environment of the tissue culture includes both oxygen and carbon dioxide gases.

2.9.1 Oxygen

Though a small flow of gas into and out of cultured plant tissues will be caused by fluctuations in temperature and atmospheric pressure of the growth room, most of the exchange of O₂ and other gases is due to diffusion (Jackson et al., 1987). The available oxygen within culture and plant tissues is therefore influenced by the concentration of the
gases in the ambient atmosphere, its rate of diffusion into the culture vessel and its rate of diffusion into the culture cells or tissues. The optimum level of the lowest tolerable level may vary according to plant species and the type of culture being grown.

2.9.2 Carbon dioxide

All cultured plant tissues produce carbon dioxide on which their metabolism and growth depend, during respiration. Respiration of non-photosynthetic plant tissues (or tissues capable of photosynthesis maintained in the dark) can cause carbon dioxide in gaseous phase of culture vessels to reach as much as 30% CO$_2$ by volume in tightly sealed containers (Rhight et al., 1988). A maximum rate of photosynthesis occurs when the requirement for both carbon dioxide and light is saturated (Kozai 1991). There are several reports (Kozai et al., 1987; 1988; Kozai and Iwanami; 1988 Arai et al., 1989; Kozai, 1991) of shoot or node culture or plantlet growth under high light and carbon dioxide enrichment, growing more rapidly and surviving better than those grown under mixotrophic conditions.

2.9.3 The influence of light and temperature on tissue culture

Tissues of plants grow better in continuous light or in a regular photoperiod (Helgeson, 1980; Mukund et al., 1988). *In vitro* cultures require a light intensity of 3000 - 10000 lux for adequate growth but some plants may require light intensity of above or below this limit while some plants may require low light intensity for the first stage. Usually a regime of 16 hours light and 8 hours dark is ideal, but the regime may change depending on the plant or stage of growth (Skoog and Miller, 1957). Light is best provided by ‘cool white’ TL fluorescent tubes.

In their natural environment, plants usually experience temperatures which fluctuate widely, especially between day and night. Such variation is not essential and in many tissues culture
laboratories, the growth rooms are maintained at the same temperatures by night and day. However Shoot cultures of temperate plants are maintained at 22 °C and tropical species at 28 °C (Stimart, 1986).

2.9.4 Effects of pH on cultures

The pH of a culture medium must be such that it will not disrupt the function of plant cell membrane or the buffered pH of the cytoplasm (George, 1993). Within these physiologically acceptable limits it also governs whether the salt will remain in a soluble form, influences the uptake of medium ingredients and plant growth regulator additives and affects the gelling efficiency of the agar and therefore the effective range of pH is restricted. Uptake of nitrates ions by plants cells leads to a drift towards an alkaline pH, while NH₄⁺ uptake result in more rapid shift toward acidity (Street, 1969; Behrend and Mateles, 1975; Hydman et al., 1982).

2.9.5 Sterility/aseptic conditions

Inadequate sterilization of instruments can lead to a worker picking up a contaminant and spreading it to many subsequent cultures (Boxus and Terrzi, 1987). Plant organs and tissues are moved into culture or sub-cultured on an aseptic bench or lamina chambers. Transfer of explants to the tissue culture medium is done in a laminar flow cabinet. Such a hood is equipped with positive pressure ventilation and bacteria-proof, high-efficiency particulate air (HEPA) filter (Vasil, 1984). The constant flow of bacteria and fungal spore-free filtered air prevents particles from settling on the working area, which must be kept clean and disinfected (Kurtz et al., 1991). The air flow through transfer chambers should be turned on at least 20 minutes before the bench is used. Working surfaces should be disinfected with
70% V/V ethanol or hypochlorite solution. De fossard and Bourne (1977) recommended autoclaved petri dishes containing ca.10 filter papers.

2.9.6 Acclimatization of micropropagated plants

Acclimatization is a process controlled to adapt an organism to an environmental change (Brainerd and Fuchigami, 1981). This is necessary because in vitro plant material is not adapted for in vivo conditions (Dunstan and Turner, 1984). The waxy cuticle and stomata on leaves of in vitro grown plants are inadequate or inoperative (Breinerd and Fuchigami, 1982; Wetzstein and Sommer, 1982). Such leaves are incapable of preventing or reducing the water loss that can occur in the variable humidity of the in vivo environment (Dunstan and Turner, 1984).
CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and pre-treatment of cocoyam varieties

Farmer preferred cocoyam varieties were sourced from three major growing areas in Kenya namely Maragua, Gatundu and Gatanga districts. Whole plants were placed in labeled polythene bags and transferred to the Kenya Agricultural Research Institute (KARI), Thika. The leaves and corms of the plants were chopped off leaving a small section of the corm and were subsequently planted in polythene bags. These were regularly watered for a period of 2 to 3 months to maturity prior to the commencement of the study.

3.2 Experimental design

The experimental design used was a completely randomized design (CRD) with five treatments. The 5 treatment levels had varied concentration (ratios) of Plant Growth Regulators (PGRs) BAP and IAA which were: - 0.0 mg/L BAP and 0.0 mg/L IAA; 2.0 mg/L BAP and 0.5 mg/L IAA; 4.0 mg/L BAP and 1.0 mg/L IAA; 6 mg/L BAP and 2 mg/L IAA and; 8 mg/L BAP and 3 mg/L IAA. The PGRs were to be used on two types of explants, namely the meristem and petiole with each treatment consisting of five replicates. The micropropagated explants were to be sub-cultured into their respective media concentrations.

The observation schedule is further illustrated in Table 3.1.
Table 3.1 Observations for the experimental design

<table>
<thead>
<tr>
<th>Conc. (ratio)</th>
<th>Explant</th>
<th>Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/l BAP:IAA</td>
<td>Replicates (n=5)</td>
<td></td>
</tr>
<tr>
<td>0.0:0.0</td>
<td>Meristem Githungu Kigoi Ngirigacha</td>
<td></td>
</tr>
<tr>
<td>2.0:0.5</td>
<td>Petiole Githungu Kigoi Ngirigacha</td>
<td></td>
</tr>
<tr>
<td>4.0:1.0</td>
<td>Meristem Githungu Kigoi Ngirigacha</td>
<td></td>
</tr>
<tr>
<td>6.0:2.0</td>
<td>Petiole Githungu Kigoi Ngirigacha</td>
<td></td>
</tr>
<tr>
<td>8.0:3.0</td>
<td>Petiole Githungu Kigoi Ngirigacha</td>
<td></td>
</tr>
</tbody>
</table>

The parameters that were considered for observation in each level were: the number of shoots, the number of plants with leaves, the number of rooted plants, the number of surviving plantlets and the number of corms produced. All the cultures were randomly placed in the growth room and observed.

3.3 Tissue culture reagents and chemicals

The types and sources of tissue cultural reagents and chemicals used in this study are given in Appendix I. All the weighing was done using analytical balance model Sartorius maximum 220 gm ± 0.5 mg no. BP 221S manufactured by (B4 1970 Germany. ISO 9001).
3.4 Media preparation and sterilization

The nutrient media used in this study contained organic and inorganic constituents in accordance with Murashige and Skoog (1962). The macronutrients concentration in g/L x 10 stock solutions were as follows: NH₄NO₃ (16.5 g/L), KNO₃ (19.0 g/L), CaCl₂·H₂O (4.4 g/L), and MgSO₄, 7H₂O (3.7 g/L), KH₂PO₄ (1.7 g/L). The micronutrient concentration in g/L x 100 stock solutions contained combinations of K (0.083 g), CuSO₄ 5H₂O (0.0025g) g), MnSO₄ 5H₂O (1.56 g), H₃B0₃ (0.620g) and CoCl₂ 6(H₂O) (0.0025 g). All these were weighed and dissolved in 1 L of ddH₂O. The chemical ferrous EDTA (concentration x 100 g/L) of 5.00 g was weighed and dissolved in a separate 1 L bottle. Vitamins (concentration x 100 g/L) which included myo-inositol (1.00 g/L), nicotinic acid (0.05 g/L), pyrodoxine HCl (0.05 g/L) and thiamine (0.01 g/L) were all dissolved in a 1 L bottle using ddH₂O (Appendix I). All the stock solutions were stored in the refrigerator at 4 °C for subsequent media preparations. Prior to inoculation, 3% sucrose was added to the media and subsequently the pH of the media was adjusted to 5.8 after which 2 g of gerite was added. The media was then brought just to boiling, dispensed into the universal bottles, autoclaved at 121 °C for 15 minutes and allowed to cool overnight.

3.5 Preparation of material for inoculation

The harvested corm plants were thoroughly washed to remove soil and debris under running water before removing the small, developed corm. The corms were excised leaving a small section of the corm intact while leaves were removed leaving the petiole holding (plate 3.1). The explants were transferred to the lab in labeled containers and thoroughly washed under running water. They were further trimmed and transferred into lamina flow in labeled magenta bottles (Plate. 3.2 A). Consequently the explants were swabbed for 5 min using 70% of ethanol to surface sterilize them and rinsed 3 to 4 times with ddH₂O. Commercial bleach
(Jik) containing 0.96% sodium hypochlorite (w/v) was added together with 3 drops of Tween-20. The contents were thoroughly shaken for 15 minutes before draining and subsequently rinsed 3 to 4 times with ddH₂O. The explants were further trimmed to pieces of between 15-20 mm for both meristems and petioles (Plate 3.2 B). The explants were further sterilized with commercial bleach (jik) that contained 0.19% sodium hypochlorite and further rinsed 3 to 4 times prior to aseptically inoculating them in the universal bottles containing the medium. These were subsequently transferred to the growthroom for incubation, aseptic conditions were observed throughout the incubation period.
Plate 3.1: Samples reduced in size after removal of leaves and corms

Plate 3.2: Surface sterilized explants in A) magenta bottles and B) petri dishes ready for inoculation into media

3.6 In vitro plant sub-culturing

The meristem explants were micropropagated (Plate 3.3 A) two weeks after inoculation into tissue culture and subsequently further micropropagated 4 weeks later. The four week duration before the second micropropagation was necessary to allow the explants time to recover and as such there was no noticeable change in growth within this duration.
Preparation of the meristem explants was done in a laminar flow under aseptic conditions. The shoot was excised leaving 2 cm long explants with the small section of the corm intact. A thin layer of the corm was removed. The stem was dissected twice in transverse section and explants sub-cultured into magenta bottles containing the respective hormone concentrations (Plate. 3.3 B). The explants were transferred to the growth room chambers for incubation at 28 °C with a photo-period of 16/8 h (day/night) and growth observed for proliferation for another 4 weeks. In both steps, the cultures were inoculated into their respective media concentrations in magenta bottles and subsequently incubated in the growth chamber and their growth observed to formation of whole rooted plants.

Plate 3.3: Sub-culturing of cocoyam showing A) micro-propagated explants and B) sub-cultured explants into Magenta bottles

3.7 Plant acclimatization and hardening

The rooted plantlets were removed from the media and excesses media washed off with tap water. The roots were deeped into a fungicide (Kocide DF-3.75g/l). The plantlets were then transferred to the greenhouse and potted in polythene bags containing sterile soil and very fine ballast at a ratio of 4:1. The plantlets were covered with a polythene paper and watered gently once a week. After one week the polythene cover was cut open at one edge and on the
second week, the second edge was cut open. On the third week, the top most part was opened up and the surviving plantlets were counted and recorded. After 4 weeks the remaining piece of cover was removed and watering continued for 92 days. The plants were subsequently transferred to the field for growth into full plant maturity.

3.8 Data analysis

Data was progressively collected from the initiated cultures which included; the number of shoots produced from each explant per media concentration; the number of plants with leaves; number of rooted plants; number of surviving plantlets in the greenhouse and number of plants with corms produced from each concentration. Data analysis was done using both descriptive and inferential techniques whereby analysis of variance was conducted using the Anova test at an alpha level of 0.05. The data was analyzed using the Statistical package for Social Sciences (SPSS 2003).
CHAPTER FOUR
RESULTS AND DISCUSSIONS

4.1 Identification of cocoyam varieties

The varieties collected from Gatundu, Gatanga and Maragua districts were Kigoi (*Colocassia esculenta* L. Schott), Githungu (*Xanthosoma sagittifolium* L. Schott) and Ngirigacha (*Colocassia esculenta* L. Schott). (Plate 4.1 A, B, C). These were identified at the East African Haberium, and specimens left there for documentation.

Plate 4.1: Samples of collected cocoyam for the varieties

A) Kigoi (*C.esculenta* L. Schott) B) Githungu (*X.sagittifolium* L.Schott) and C) Ngirigacha (*C. Esculenta* L. Schott)

4.2 Effect of plant growth regulators on the three cocoyam varieties

The study intended to verify the effect growth regulators had on the three cocoyam varieties in terms of formation of shoots, leaves and roots, on the survival of plantlets as well as on the formation of corms.
4.2.1 Formation of shoots at seven days

The levels of shoot formation across the concentrations of the study at seven days are presented in Table 4.1A.

Table 4.1A: Shoot formation across all Concentrations at 7 Days

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BAP:IAA 0.0:0.0</td>
<td>BAP:IAA 2.0:0.5</td>
</tr>
<tr>
<td>Githungu</td>
<td>Meristem 4/5(80%)</td>
<td>5/5(100%)</td>
</tr>
<tr>
<td></td>
<td>Petiole 0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Kigoi</td>
<td>Meristem 5/5(100%)</td>
<td>5/5(100%)</td>
</tr>
<tr>
<td></td>
<td>Petiole 0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Ngirigacha</td>
<td>Meristem 5/5(100%)</td>
<td>4/5(80%)</td>
</tr>
<tr>
<td></td>
<td>Petiole 0(0%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

Percentage of explants with shoots within 7 days of culturing the explants

As shown by Table 4.1A, explants survived in all the varieties as evidenced from their enlargement and manifested by an elongation of the shoot tip in meristem only. At the same time, most cultures changed from the initial creamy color to green coloration for both meristem and petiole. The Table shows the percentage of meristem and petiole explants that produced shoots in all concentrations BAP mg/l/IAA mg/l (ratios) within seven days. Kigoi yielded the highest 100% at four optimal concentrations without PGRs; 2.0: BAP ml/L and 0.5 IAA mg/L, 4.0; BAP ml/L and 1.0 IAA mg/L; 6.0: BAP ml/L and 2.0 IAA mg/L respectively. Githungu yielded 100% in three optimal concentrations 2.0:BAP mg/L and 0.5
IAA mg/L; 4.0 BAP mg/l:1.0 IAA mg/L, 6.0 BAP mg/L: 2.0 IAA mg/ L. Ngirigacha also yielded three optimum concentrations of 100% at BAP mg/l/IAA mg/L without PGRs, 4.0 BAP mg/l:1.0 IAA mg/L and 6.0 BAP mg/l:2.0 IAA mg/L. The lowest shoot yield was for Kigoi at 60% at concentration 8.0 BAP mg/l: 3.0 IAA mg/L while the remaining concentrations had a yield of 80% each. None of the petiole explants produced any shoot. For all the varieties however, it appears that concentrations beyond 6.0 BAP mg/L: 2.0 IAA mg/L are not conducive to shoot formation. For the first seven days, the two concentration (ratios) of 4.0 BAP mg/l: 1.0 IAA mg/l and 6.0 BAP mg/l: 2.0 IAA mg/L appeared to perform best in shoot production with both having a mean score of 100%.

Analysis of variance for shoot versus concentration at p<0.05 indicated that there was a significant difference in shoot yield by concentration (ratio), indicating that indeed, concentration (ratio) was an important deciding factor in shoot yield in the early stages. Thus the two concentrations of 4.0 BAP mg/L: 1.0 IAA mg/L and 6.0 BAP mg/l: 2.0 IAA mg/L which both yielded the highest mean score of 100% were the best performing in shoot production in the first 7 days. Analysis of variance for shoot versus variety at p>0.05 however, indicated that there was no significant difference for the three varieties in shoot yield for the same period, hence any perceived difference was due to random error.

During the next seven days there was no measurable change in shoot yield except that a few shoots dried up sporadically.

The results for shoots formed in this experiment were similar to those found by several workers who obtained C. esculenta shoots from mass propagation on MS medium containing plant hormones BA and IBA. In their experiments, shoots were formed and turned green after one week of incubation in tissue culture from the meristem tips (Malamug et al., 1992;
Valverde *et al.*, 1997; Nhut *et al.*, 2004; Ko *et al.*, 2008). The work carried out by Ko *et al* (2008) found cultures yielded shoot formation between 30.5% and 99.5% from their explants. For *Xanthosoma spp.*, the percentage of shoot formation ranged between 80% and 100 %, and was consistent with the results of Zandvoort *et al* (1994).

Additionally, there was a high regeneration from shoots recorded by Paul and Bari (2007) with a percentage of 65 on cultures grown on MS media supplemented with 0.5 mg/L BAP and 1.0 mg/L IAA. In their work, they also recorded 89% of shoot regeneration across 1.0 mg/L BAP and 2.0 mg/L NAA. This study, with a score range of between 60% and 100 % yield for *C. esculenta* was therefore consistent with other studies.
4.2.2 Formation of shoots at forty two days

The results for the formation of shoots across all the concentrations at forty two days are illustrated by Table 4.1B.

Table 4.1B: Shoot formation across all concentrations at 42 days

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 0.0:0.0</td>
<td>BAP:IAA 2.0:0.5</td>
<td>BAP:IAA 4.0:1.0</td>
<td>BAP:IAA 6.0:2.0</td>
<td>BAP:IAA 8.0:3.0</td>
</tr>
<tr>
<td>Githungu</td>
<td>Meristem</td>
<td>10/4(250%)</td>
<td>11/5(220%)</td>
<td>0/5(0%)</td>
<td>2/5(40%)</td>
<td>3/4(75%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Kigoi</td>
<td>Meristem</td>
<td>6/5(120%)</td>
<td>0/5(0%)</td>
<td>11/5(220%)</td>
<td>18/5(360%)</td>
<td>0/3(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Ngirigacha</td>
<td>Meristem</td>
<td>1/5(20%)</td>
<td>2/4(50%)</td>
<td>7/5(140%)</td>
<td>0/0(0%)</td>
<td>0/4(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

Percentage of explants with shoots after 42 days of culturing.

After 42 days of culturing, Table 4.1B shows that the highest shoot yield was for Kigoi variety at concentration (6.0/2.0) BAP mg/l/IAA mg/l with a score of 360% shoot yield. Further, results for Analysis of variance at 42 days (F [8,420] =3.95, P=0.001)) for concentration versus variety in number of shoots yielded, indicated a statistically significant interaction effect between concentrations and varieties, unlike in the early stages, implying that in the overall result, concentration had an effect on the shoot yield of the different varieties. Thus concentration (ratio) 6.0 BAP mg/l: 2.0 IAA mg/L, which had the highest score at 42 days proved to be the best concentration ratio for shooting. Appendix III(B)
shows that there was a statistical difference between the varieties in shooting with Kigoi being the best performing.

4.2.3 Formation of leaves at fourteen days

The formation of leaves within fourteen days across all concentrations is shown by Table 4.2A.

Table 4.2A: Formation of leaves in all concentrations

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explanants</th>
<th>Concentration mg/l</th>
<th>BAP:IAA</th>
<th>BAP:IAA</th>
<th>BAP:IAA</th>
<th>BAP:IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.0:0.0</td>
<td>2.0:0.5</td>
<td>4.0:1.0</td>
<td>6.0:2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Githungu</td>
<td>Meristem</td>
<td>1/4(25%)</td>
<td>3/5(60%)</td>
<td>2/5(40%)</td>
<td>2/5(40%)</td>
<td>2/4(50%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Kigoi</td>
<td>Meristem</td>
<td>3/5(60%)</td>
<td>3/5(60%)</td>
<td>2/5(40%)</td>
<td>1/5(20%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Ngirigacha</td>
<td>Meristem</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

Percentage of explants with leaves within 14 days of culturing the explants

Within 14 days most of the cultures from the meristem explants had already produced healthy leaves except for Ngirigacha which had not leafed at all (Table 4.2A), while some Githungu explants yielded leaves within 8 days. However nearly all the explants had grown to the top of the universal bottles while the bottom of the explants had swelled. Githungu yielded the best with a mean of 43% and had leaves in all concentrations, with an optimum concentration of BAP ml/L and IAA mg/L (ratio) of 2.0 BAP ml/L:0.5 IAA mg/L, while Kigoi had two
optimum concentration ratios that is without PGR; 2.0:BAP ml/L and 0.5 IAA mg/L. The optimum concentration for leaf formation appeared to be 2.0: BAP ml/L and 0.5 IAA mg/L at a mean of 40 % leaf formation for meristems. However the petiole explants had only acquired a green coloration with no leaves formed.

Analysis of variance for leaf formation versus concentration at day 14 indicated that there was no significant difference in leaf formation between the concentration (ratios) at p>0.5, thus refuting the apparent advantage of the 2.0: BAP ml/L and 0.5 IAA mg/L concentration (ratio). Thus no concentration was actually more productive in leaf yield and any perceived difference in yield was due to random error and not the concentration (ratio). However analysis of variance for leaf formation versus variety at day 14 with a score of p<0.5 indicated that there was a significant difference in leaf yield. This meant that during the first 14 days, the variety of cocoyam was a factor in leaf yield.

Apparently no leaves were formed in Ngirigacha as was the case of Ko et al (2008) and Nhut et al (2004) who micropropagated C.esculenta shoots on nutrient media containing BA and IAA obtaining leaves towards full plant maturation.
4.2.4 Formation of leaves at forty two days

The formation of leaves by the forty second day was also of interest to the study and the findings are reflected in Table 4.2B.

Table 4.2B: Leaf formation across all concentrations at 42 days

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
<th>0.0:0.0</th>
<th>2.0:0.5</th>
<th>4.0:1.0</th>
<th>6.0:2.0</th>
<th>8.0:3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Githungu</td>
<td>Meristem</td>
<td></td>
<td>4/10(40%)</td>
<td>3/11(27%)</td>
<td>0/0(0%)</td>
<td>0/2(0%)</td>
<td>3/11(27%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td></td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Kigoi</td>
<td>Meristem</td>
<td></td>
<td>4/6(67%)</td>
<td>0/0(0%)</td>
<td>4/11(36%)</td>
<td>0/1(0%)</td>
<td>0/0(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td></td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>Ngirigacha</td>
<td>Meristem</td>
<td></td>
<td>0/1(0%)</td>
<td>0/2(0%)</td>
<td>0/7(0%)</td>
<td>0/0(0%)</td>
<td>0/8(0%)</td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td></td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
</tbody>
</table>

After micropropagation, readings on the 42\textsuperscript{nd} day tallied with the results for seven days, and as such indicated a generally low leaf yield for all concentrations as shown by Table 4.2B above, with the highest being 67\% for the concentration without PGR for the Kigoi meristem. Ngirigacha had still not yielded any leaves.

Analysis' of variance however (Appendix IV (A) (F [8,420] =2.209, P<0.05)), illustrated that there was a significant difference of concentrations for different varieties in leaf yield. Thus concentration (ratio) affected leaf production among the different varieties. The best performing concentration for leaf formation was without PGR (Appendix IV (B)), with Githungu (meristem) being the best.
4.2.5 Formation of roots

The results for the formation of roots across all the concentrations in the study is illustrated in Table 4.3 A.

Table 4.3: Formation of rooted plantlets in all varieties across the concentrations

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
<th>Percentage of rooted explants within 70 days (Kigoi and Ngirigacha) and 84 days (Githungu).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 0.0:0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 2.0:0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 4.0:1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 6.0:2.0</td>
<td></td>
</tr>
<tr>
<td>Githungu</td>
<td>Meristem</td>
<td>8/10(80%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11/11(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/12(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/12(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Kigoi</td>
<td>Meristem</td>
<td>6/6(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4/4(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/18(66%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>12/18(66%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/2(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Ngirigacha</td>
<td>Meristem</td>
<td>11/11(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18/18(100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14/14(100%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/8(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0/0(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Petiole</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0(0%)</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of rooted explants within 70 days (Kigoi and Ngirigacha) and 84 days (Githungu).

Root formation of the micropropagated plantlets was not uniform across the varieties as indicated (Table 4.3A) with Kigoi and Ngirigacha varieties both having rooted by at least 60% of the explants per variety by the 70th day while Githungu had not yielded any roots. Both Kigoi and Ngirigacha had three optimum concentration (ratios) of 100 %, that is, without PGR; 2.0 mg/L BAP mg/l and 0.5mg/L IAA for both and 4.0 mg/L BAP mg/l and 10mg/L IAA for Ngirigacha; 8.0 mg/L BAP mg/l and 3.0mg/L IAA for Kigoi. By the 84th day Githungu had rooted and had three optimum concentration (ratios) of 100% at 2.0 mg/L BAP mg/l and 0.5mg/L IAA; 4.0 mg/L BAP mg/l and 1.0mg/L IAA; and 6.0 mg/L BAP mg/l and 2.0mg/L IAA. This was also similar to the work of Mix –Wagner (1993) in Hossain
(2009), who reported a growth generation frequency ranging between 80% to 110% in the concentrations of 2.0 mg/l and 1.0 mg/l NAA using meristem cultures of MS Media. Most of the explants that formed roots were subsequently sub-cultured in vitro for plantlets formation, a phenomenon that was also realized by Chand et al (1999). Paul and Bari (2007) found highest percentage of root induction and proliferation in a media containing 0.4 mg/l of IAA and established rooted plantlets that were well acclimatized with good growth performance.

In this study, analysis of variance with Anova (Appendix V (A)) (F [8,120] =13.449, P<0.001) of plantlets) revealed a significant difference between the concentration (ratio) and variety that had rooted, with Ngirigacha performing best (Appendix V (B). Thus concentration (ratio) caused a difference in rooting performance of the plantlets with concentration (ratio) 2.0 mg/L BAP mg/l: 0.5mg/L IAA yielding the best. Further analysis of variance (Appendix V (A)) (F [4,120] =19.101, P<0.001) indicated a significant interaction difference between concentration (ratio) and explants with the petioles not yielding at all (Appendix V (C)), and 2.0 mg/L BAP mg/l: 0.5mg/L IAA being the best for meristem.
4.2.6 Surviving plantlets

The rate of plantlet survival across all the concentrations of the study in the three varieties is presented in Table 4.4A.

Table 4.4: Surviving plantlets in all varieties across all the concentrations

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BAP:IAA 0.0:0.0</td>
<td>BAP:IAA 2.0:0.5</td>
<td>BAP:IAA 4.0:1.0</td>
<td>BAP:IAA 6.0:2.0</td>
<td>BAP:IAA 8.0:3.0</td>
</tr>
<tr>
<td>Githungu Meristem</td>
<td>8/8(100%)</td>
<td>9/11(81%)</td>
<td>0/0(0%)</td>
<td>9/12(75%)</td>
<td>0/0(0%)</td>
<td></td>
</tr>
<tr>
<td>Githungu Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Kigoi Meristem</td>
<td>5/6(83%)</td>
<td>4/4(100%)</td>
<td>3/3(100%)</td>
<td>9/12(75%)</td>
<td>0/2(0%)</td>
<td></td>
</tr>
<tr>
<td>Kigoi Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Ngirigacha Meristem</td>
<td>3/5(60%)</td>
<td>17/18(94%)</td>
<td>13/14(92%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>Ngirigacha Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
</tbody>
</table>

Percentage of surviving plantlets 21 days after potting.

The survival rate for the explants after micropropagation, rooting and potting (in sterile sand and ballast) showed variations across the different concentrations 21 days after potting (Table 4.4A). Ngirigacha though having low percentage survival yield with a percentage of 94% at an optimum concentration ratio of 2.0 mg/L BAP mg/l and 0.5mg/L IAA had the highest number of surviving plantlets in given concentration (ratios). Kigoi had an optimum yield of 100% at concentration (ratios) of 2.0 mg/L BAP mg/l and 0.5mg/L IAA; 4.0 mg/L BAP mg/l and 1.0mg/L IAA. Githungu yielded an optimum 100% plantlet survival yield for the concentration (ratios) without PGRs. The survival rate in this study concurs with 100% survival rates for plantlets grown on soil for Nhut et al., (2004) while Ko et al. (2008)
micropropagated *C. esculenta* from shoot apices to full plant maturity on nutrient MS media supplemented with BA and IAA and reported a survival rate frequency of 86.2 % and 96.1%.

The optimum concentration ratio of plantlet survival by mean score was 2.0 mg/L BAP mg/L and 0.5mg/L IAA at 91.7%. Percentage analysis of variance of concentration versus plantlet survival at p<0.05 indicated that there was a significant difference and therefore the concentration ratio had an effect on the survival performance of the plantlets. This survival score was further supported by analysis of variance for concentration (ratio) versus variety which showed a significant difference (Appendix VI (A)) F [8,120] =16.461, P<0.001) in mean number of surviving plantlets. The concentration (ratio) 2.0 mg/L BAP mg/L and 0.5mg/L IAA which had the highest survival mean score (Appendix VI (B)) proved to be the best concentration (ratio) for plant survival, with Ngirigacha performing best.
4.2.7 Formation of corms

Table 4.5A illustrates the results obtained for the formation of corms in all concentrations for the three varieties involved in the study.

Table 4.5: Formation of corms across all varieties in all concentrations

<table>
<thead>
<tr>
<th>Variety</th>
<th>Explants</th>
<th>Concentration mg/l</th>
<th>BAP:IAA 0.0:0.0</th>
<th>BAP:IAA 2.0:0.5</th>
<th>BAP:IAA 4.0:1.0</th>
<th>BAP:IAA 6.0:2.0</th>
<th>BAP:IAA 8.0:3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Githungu Meristem</td>
<td>8/8(100%)</td>
<td>9/9(100%)</td>
<td>0(0%)</td>
<td>9/9(100%)</td>
<td>0(0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
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</tr>
<tr>
<td>Kigoi Meristem</td>
<td>5/5(100%)</td>
<td>0/4(0%)</td>
<td>8/9(88.8%)</td>
<td>8/9(88.8%)</td>
<td>0(0%)</td>
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</tr>
<tr>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
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<tr>
<td>Ngirigacha Meristem</td>
<td>3/3(100%)</td>
<td>14/17(82.3%)</td>
<td>9/13(69%)</td>
<td>0(0%)</td>
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</tr>
<tr>
<td>Petiole</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
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</table>

Corm production after five months (Ngirigacha and Githungu) and six months (Kigoi)

After exposure to the field environment for five months (Ngirigacha and Githungu) and six months (Kigoi), corm yield proved to be optimal without PGR for all varieties at a 100% plantlet corm yield as indicated in Table 4.5A above. However, Githungu variety had another two optimum concentration (ratios) of 100% plantlet corm yield at a concentration ratio of 2.0 mg/L BAP mg/l 0.5: mg/L IAA and 6.0 mg/L BAP mg/l : 2.0mg/L IAA thereby making it appear to be the best variety for corm production. It also produced multiple cormels per plantlet (Plate 4.2 A) unlike the other two varieties which only produced one corm per plantlet, making it necessary to consider the number of plantlets that yielded corms rather
than the actual number of corms yielded per plantlet in order to measure success at corm production by the plantlets.

The success at corm production for plantlets that survived was relatively high across the concentration (ratios), with most of the concentrations (ratios) having success rates of above 82% of plantlets that produced corms. The exceptions were Ngirigacha at concentration (ratio) 4.0 mg/L BAP mg/L: 1.0mg/L IAA with 69% plantlets that produced corms and Kigoi at concentration 2.0 mg/L BAP mg/L 0.5: mg/L IAA with no corm yield. Corms were produced in all the varieties as illustrated by the samples (Plate 4.2 A, B, C). The formation of corms in this work are consistent with the work carried out by Ko et al (2008) who harvested *C. esculenta* corms from their tissue cultured plantlets.

Analysis of variance for plants with corms versus concentration (ratio) at p < 0.05 showed a significant difference in yield of plantlets with corm across the different concentration (ratios), indicating that concentration (ratio) affected the ability of plantlets to produce corms. Further analysis of variance (appendix VII (A)) between concentrations for different varieties showed that there was a significant difference (F [8,116] =8.601, P<0.001) between the varieties for each concentration (ratio) in the number of plantlets that yielded corms. This meant that each concentration (ratio) affected each plantlet variety differently. In this regard, the most successful concentration (ratio) was 2.0 mg/L BAP mg/L 0.5: mg/L IAA for Ngirigacha, 2.0 mg/L BAP mg/L: 0.5mg/L IAA and 6.0 mg/L BAP mg/L: 2.0 mg/L IAA for Githungu and 6.0 mg/L BAP mg/L: 2.0mg/L IAA and 4.0 mg/L BAP mg/L: 1.0 mg/L IAA for Kigoi

Analysis of variance for explants versus plantlets producing corms (appendix VII (A)) (F [4,116] =9.895, P<0.001)) showed a significant difference between the two types of explants.
in producing corm. The result reflected the fact that petioles did not proliferate or even yield shoots. Thus the meristems proved to be the explants that could be successfully propagated.

*Cocoyam samples produced in the study from tissue culture plantlets.*

Plate 4.2: Corms produced from A-Githungu (*X. sagittifolium*), B-Ngirigacha (*C. esculenta*), and C-Kigoi (*C. esculenta*) variety
In vitro establishment of cocoyam material

Plate 4.3: Tissue culture production system of Cocoyam

(A) Inoculated explants, (B) whole plantlet in Magenta bottle, (C) Plantlet with roots, (D) Potted Plantlets covered with polythene bags for acclimatization, (E) Uncovered acclimatized plantlets, (F) Uniform production of micropropagated plantlets, (G) Githungu (X. sagittifolium) variety, Kigoi (C. esculenta) variety and (I) Ngirigacha (C. esculenta) variety.
CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The findings from this study indicated that micropropagation of the three main farmer preferred cocoyam varieties grown in Kenya is possible. After the experimental findings in this study, the researcher rejected the null hypothesis (Ho) “Kenyan cocoyam varieties cannot be propagated via *in vitro* tissue culture” and adopted the alternative H1, “It is possible to propagate three Kenyan cocoyam varieties (*C. esculenta*, “Ngirigacha”, *C. esculenta*, “Kigoi” and *X. sagittifolium*, “Githungu”) through tissue culture”. The process of Tissue culture in plant propagation itself is relatively expensive, but nevertheless, the study established that without the growth regulators, it was possible to regenerate *X. sagittifolium* (Githungu). Also *C. esculenta* (Ngiringacha) was able to respond at a relatively low concentration than *C. esculenta* (Kigoi).

It was established that the best explants to use for *in vitro* micropropagation of cocoyam material in tissue culture is the plant meristem. Shoot formation from the meristem explant was realized 7 days after culturing in all the three varieties with Kigoi yielding the highest at an optimum concentration of 6.0mg/L BAP and 2.0mg/l IAA.

Leaf formation within 14 days after culturing the explants was only realized in two varieties, Githungu and Kigoi, with Githungu yielding the highest at an optimum concentration without PGR. All the varieties were able to yield roots *in vitro* with Ngirigacha (*C. esculenta*) yielding the highest mean number of rooted explants at an optimum concentration of 2.0 mg/L BAP; 0.5 mg/L IAA.

Survival on potting of the *in vitro* plantlets was recorded in all varieties with Ngirigacha (*C.
(esculenta) yielding the highest at an optimum concentration of 2.0 mg/L BAP; 0.5 mg/L IAA. Corm production was recorded in all the varieties with Githungu (X. sagittifolium) yielding the highest number of corms at an optimum concentration without PGR. However, Ngirigacha (C. esculenta) had the most successful yield of plantlets that yielded corms at a given concentration (ratio) with 14 out of 17 plantlets yielding corms in the concentration (ratio) 2.0 mg/L BAP mg/L: 1.0 mg/L IAA.

5.1 Recommendations

5.1.1 For this study

Beside the farmer known conventional methods of propagating cocoyams, the tissue culture protocol established in this research for three Kenyan cocoyam varieties can be used by the to supplement the farmers methods. The preferred explants should be from plant meristem. This study recommends that the protocol be adopted to produce clean seedlings for farmers sensitize them about the planting materials, and conservation of endangered species and rare cultivars.

5.1.2 For further work

i. To investigate why the petiole explants did not yield shoot formation, leaves and root growth to whole plant maturity including formation of corms. It is recommended to alter the plant hormones and utilize those such as Thiadizuron (TDZ) or 1-naphthalene acetic acid (NAA), which has been used elsewhere in the in vitro micro propagation of cocoyam.

ii. To investigate why leaf formation was not enhanced in the variety Ngirigacha despite having high yield in shoot formation in the earlier developmental stage.
iii. To investigate the performance of the multiplied cocoyams in the field and response to viral diseases.

iv. Investigate the social and economic impact of introduction of the *in vitro* plantlets to the small holder farming systems.

v. Further research may establish whether it is possible to produce transgenic cocoyam with a prolonged shelf life.
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Tujiwaiza, K., Kozai and Watanabei, I. 1987. Funclame metal studies on environments in plant tissue culture vessels containing tissue culleved plantlets and estmates of net photosynthetic rates of the plantlets Journal *Agricultural method 43; 21-30.*


Underexploited tropical plants.
# APPENDICES

## APPENDIX I: Sources of tissue culture reagents and chemicals

<table>
<thead>
<tr>
<th>Reagent/chemical</th>
<th>Manufacturer</th>
<th>Local supplier (Kenya)</th>
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<td>Duchefa Biocemic</td>
<td>Medispan suppliers</td>
</tr>
<tr>
<td>Indoacetic acid</td>
<td>Duchefa Biocemic</td>
<td>Medispan suppliers</td>
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<td>Uchumi Supermarket Ltd</td>
</tr>
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<td>Household bleach (Jik)</td>
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<td>Uchumi Supermarket Ltd</td>
</tr>
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<td>Dettol</td>
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<td>Sudi Teepol</td>
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<td>Uchumi Supermarket Ltd</td>
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<tr>
<td>Koicide</td>
<td>Farmchem Industries LTD</td>
<td>Githurai Agrovet</td>
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APPENDIX II: Media for *in vitro* establishment weighed using an analytical balance

Murashige and Skoog Medium (milligrams per litre)

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<tr>
<td>CaCl$_2$2H$_2$O</td>
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<td>MgSO$_4$7H$_2$O</td>
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<td>KHO$_3$</td>
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<tr>
<td>KH$_2$PO$_4$</td>
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<td>FeSO$_4$.7H$_2$O</td>
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APPENDIX III:

(A) ANOVA results for shoots formed across all concentrations

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<th>P-value</th>
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<td>0.48</td>
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<td>0.48</td>
<td>1.19</td>
<td>0.305</td>
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<tr>
<td>Variety * Conc</td>
<td>12.81</td>
<td>8</td>
<td>1.60</td>
<td>3.95</td>
<td>0.001</td>
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<tr>
<td>Explant * Conc</td>
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<td>0.50</td>
<td>1.24</td>
<td>0.292</td>
</tr>
<tr>
<td>Variety * Explant * Conc</td>
<td>12.81</td>
<td>8</td>
<td>1.60</td>
<td>3.95</td>
<td>0.001</td>
</tr>
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<td>Error</td>
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<td>Total</td>
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(B) Number of cultured explants with shoot at varying concentrations (ratio) of PGR

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- The number of plants with shoot within 42 days of culturing the meristem explants
APPENDIX IV

(A) Results for leaves formation

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(B) Number of cultured explants with leaves at varying concentrations (ratio) of PGR

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- The number of plants with leaves within 42 days of culturing the meristem explants.
APPENDIX V

(A) Result for formation of roots

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(B) Number of cultured plantlets with roots at varying concentrations (ratio) of PGR across varieties

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- Number of plantlets with roots after 70 days (Kigoi and Ngirigacha) and 84 days (Githungu)
(C) Number of rooted plantlets across concentrations (ratio) of PGR in meristem and petiole explants

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- All petiole explants dried 21 days after culturing.
APPENDIX VI

(A) Results for the surviving plantlets in all varieties across all the concentrations

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<td>0.41</td>
<td>2.392</td>
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<td>4.48</td>
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<td>Variety * Explant * Conc</td>
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<td>8</td>
<td>2.80</td>
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<tr>
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(B) Number of cultured plantlets that survived after potting from varying concentration (ratio) across varieties.

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- Number of cocoyam surviving in the greenhouse after three weeks transfer from growth room
APPENDIX VII

(A) Results for the formation of corms across all varieties in all concentrations

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<td>.591</td>
<td>.555</td>
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<td>4</td>
<td>2.790</td>
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<td>2</td>
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<tr>
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</table>

Corrected Total

133.493 149

(B) Number of cultured plantlets from varying concentrations (ratio) that yielded corms across varieties.

![Graph showing the number of cultured plantlets from varying concentrations (ratio) that yielded corms across varieties.]

- Error bars denote least significant difference (LSD) at 95% confidence interval.
- Corm production after five months (Ngirigacha and Githungu) and six months (Kigoi...