ASSESSMENT OF REGENERATION POTENTIAL OF SELECTED LOCAL BANANA (*Musa* spp.) CULTIVARS ON DIFFERENT SUBSTRATES

Nicolas Nzeyimana

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE.

MOROGORO, TANZANIA.

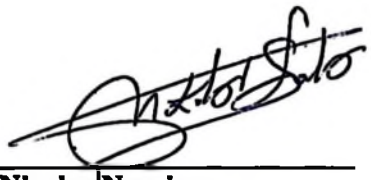
2013

ABSTRACT

Rice husks and saw dusts are traditionally used as initiation media for banana *in vivo* propagation. However, their physical properties have undesirable effect on shoot multiplication and growth. The objective of this study was to evaluate the effect of alternative growth media on the *in vivo* multiplication and shoot establishment in secondary nursery of three selected local banana cultivars. This study was carried out from April to October 2011 at the Horticulture Unit of Sokoine University of Agriculture, in Morogoro, Tanzania. It comprised of experiments established in a split plot under randomized complete block design (RCBD). The main factor was banana cultivars (Bukoba, Mtwike, and Mzuzu), while the sub-factor was the growth media (rice husks as a control, cocus peat and mixed medium). The data were subjected to analysis of variance (ANOVA) and mean separation was done based on Student-Newman-Keuls at $P < 5\%$ using COSTAT6.4 statistical software. Results showed that rice husks and mixed medium resulted in earlier ($P < 0,05$) sucker production at 18.88 and 20.26 days, respectively while cocus peat produced suckers within 23.02 days. Mixed medium (forest soil, farmyard manure and rice husks at 4:2:1 on volume basis) also resulted in the highest ($P < 0,05$) sucker productivity of 4.67 suckers per corm, the biggest sucker with height of 23.64 cm and collar diameter of 1.92 cm, leaf length and width of 24.13 and 13.10 cm, respectively. On the other hand, cv. Mzuzu produced the highest ($P < 0,05$) number of 5.70 suckers per corm compared with cv. Bukoba and Mtwike with 3.83 and 2.73 suckers per corm, respectively. The findings suggest that plantains have the best responce to *in vivo* multiplication while mixed medium is most suitable for enhancing shoot productivity and growth.

DECLARATION

I Nicolas Nzeyimana do hereby declare to the Senate of Sokoine University of Agriculture that this is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.



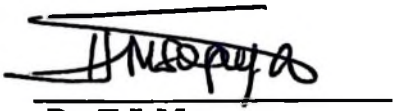
Nicolas Nzeyimana 10-05-2013 **Date**

(MSc. Candidate)

The above declaration is confirmed

Prof. A.P. Maerere 16/05/2013 **Date**

(Supervisor)



Dr. T.J. Msogoya 10-05-2013 **Date**

(Supervisor)

COPYRIGHT

ACKNOWLEDGEMENTS

Thanks to Almighty God for the protection and guidance during the whole period of my studies. I am deeply thankful to my supervisors, Prof. A.P. Maerere and Dr. T.J. Msogoya for their constant technical assistance, guidance, encouragement and advices since the development of the proposal up to completion of this study. I would like to thank the families of Prof. M. Ndabikunze and David Muyenjwa for their research material and social support during my reseach studies, respectively.

I am greatly thankful to the office of Faculty of Agriculture, the Head of Crop Science Department Prof. C.L. Rweyemamu for their administrative support during the study period. I would like to thank the staff and classmates of the Crop Science and Production Department, especially in Horticulture section for their various assistance during the whole period at Sokoine University of Agriculture (SUA). I am grateful to RUFORAM and DeLPHE project for the scholarship granted to me. My sincere gratitude goes to my employer, the Rwanda Ministry of Defense for the release and financial support offered to pursue this higher degree.

Heartfelt thanks to my parents, my brothers, sisters, relatives, neighbors and my friends for their moral support, prayers and encouragement. Allow me also to acknowledge all those people not enumerated who, through their support both material and moral, contributed towards the successful study time. God bless all of you for the kindness.

DEDICATION

To Almighty God my creator; to my mother Kanziga Prudentienne and my father Nzeyimana Revocat, my brothers and sisters whose sacrifice laid the foundation for my education. I also dedicate this work to my wife and our future kids.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

FAO	Food and Agriculture Organization of the United Nations
Fpr	F probability
GA	Gibberellic Acid.
No.	Number
RH	Relative Humidity
RUFORUM	Regional Universities Forum for Capacity Building in Agriculture
Se±	Standard error of mean
SUA	Sokoine University of Agriculture
T	Temperature

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Bananas and plantains (*Musa* spp.) are the fourth most important food crops in developing countries surpassed only by rice, wheat and maize (Karamura *et al.*, 2007). Global production of banana and plantain is about 100 million tonnes per year (FAOStat, 2007). It progresses slowly at a rate of 2 % per year (CIRAD-GRET, 2002). Approximately 25 million tonnes (34 % of annual world production) are produced in Africa (Swennen and Vuylsteke, 2001).

The Great Lakes region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and DRC is the largest producer and consumer of bananas in Africa (Smale, 2006). The *per capita* consumption in the region has been estimated at more than 250 kg being the highest in the world (FAO, 2005). Tanzania occupies the 7th position in the world (4 % of world production) and 2nd in Africa, after Uganda (Maerere *et al.*, 2003).

Bananas and plantains provide a starch staple in some poorest parts of the world, while dessert banana is a major cash crop in many countries (FAOStat, 2007). It is among the most exported fruits in the world ranking second in terms of values (US $ 5.2 billion) after Citrus (FAO, 2007). They present a significant source of income and employment in many tropical countries (Lassoudiere, 2007). In Tanzania, bananas and plantains are staple food for more than 5 million of the 34 million of the population (Maerere et *al.*, 2003).

Banana cultivation in Tanzania occupies 22 % of cultivated land and its production represents 40 % of food production and 15 % of GDP (Ndungo, 1997). Crop production

records show that banana ranks third after maize and cassava in Tanzania (Karamura, 2008). Bananas produced in Tanzania are grouped into traditional bananas (East Africa Highland bananas (AAA-EAHB)); exotic bananas (Cavendish, Gros Michel, Pisang awak, plantains and small yellow sweet) and new bananas introduced since 1997 (FHIA01, FHIA02, FHIA03, FHIA17, FHIA22, FHIA23, FHIA25, Yangambi km5, SH3436-9, Peripita, Cardaba) (Mgenzi *et al.*, 2005). In Kagera region there are found the famous Matooke types, which technically form the East African Highland Banana (Karamura *et al.*, 2007).

The area under banana production fluctuates from 250 000 ha^{-1} to 350 000 ha^{-1} per annum for the past ten years. Kagera Region had the highest banana acreage followed by Kilimanjaro, Mbeya, Tanga, Arusha and Kigoma (MOAC, 2000). Based on the area under bananas, there are five main banana production zone. These zones include Lake (Kagera, Kigoma and Mara regions), Northern (Arusha, Kilimanjaro and Tanga regions), Southern-Highlands (Mbeya region), Eastern zone (Morogoro Region) and the Zanzibar Islands (Karamura *et al.*, 2004). The Lake Zone has the highest banana production followed by Northern Zone and then Southern Zone. In 1997/98 and 1998/99, the Lake Zone produced about 45 percent and 43 percent of the total production of bananas in the country, respectively (MOAC, 2000).

Since the 1970s, reports of low and declining banana yields have been widespread. Some have attributed this phenomenon to constraints such as soil degradation, pests, poor crop husbandry and drought (IITA, 2011). The wild banana contained large seeds but cultivated banana is seedless and is therefore vegetatively propagated by suckers. This traditional method of propagation is slow and over the years has been attributed to a number of

constraints. These include high susceptibility to pests such as the devastating Panama disease (*Fusarium oxysporum* f. *cubense*), weevils (*Cosmopolites sordidus*) and nematodes (Petit, 2010); poor agronomic practices (Dowiya *et al.*, 2009; Robinson *et al.*, 1996) and poor suckering ability (Ndubizu, 1985). Yield reductions of up to 90 % have been reported when using infected suckers on multiple farms (Njukwe *et al.*, 2007). Moreover, conventionally propagated materials are usually in short supply to meet the needs for large-scale plantation (Rasheed, 2003).

The problem of availability of material used for planting banana remains a priority for many farmers. Therefore, urgent need for a large amount of disease-free planting material triggered the development of the banana meristem culture technique for mass production of pest-free planting materials (Ma and Shii, 1974). *In vitro* propagation allows mass propagation of pest-free banana suckers and has several advantages such as reduced cost of disease and pest control, production of crop plants with uniform and vigorous growth, shortened harvesting period and enhanced high yield (Msogoya *et al.*, 2006). Unfortunately, somaclonal variation induced by *in vitro* propagation is undesirable for clonal production of banana planting materials (Msogoya *et al.*, 2008). Also, it is possible for viruses to be transmitted through micropropagated materials (Ma and Shii, 1974). Moreover, this technique is relatively expensive and not readily accessed by the resource-poor farmers (Kwa, 2003).

Njukwe *et al.* (2005) reported that the International Institute for Tropical Agriculture (IITA) has developed an alternative technique for the production of healthy planting material popularly known as *in vivo* multiplication or macropropagation. The technique involves desheathing the corm to expose axillary buds and to remove apical meristems of

suckers so as to suppress apical dominance and stimulate sprouting of axillary buds under modified *in vivo* regeneration (Kwa, 2003). This is a relatively simple technique that can be applied in the field or in propagators by farmers themselves. Macropropagation provides in short period of time mass production of pest-free plantlets for enhanced banana production (Kwa, 2006). Within six to twelve months the method results in the production of nine to fifteen uniform shoots per plant (Singh *et al.*, 2011), and it can also be performed locally at low cost and with little training (Kwa, 2006).

Baiyeri and Aba (2004) reported that either rice husks or saw dust could be used for generating banana plantlets using the *in vivo* propagation technique. They are extremely light in weight, weak in water holding capacity and according to their size of the particle, they resist to decomposition (Dewayne *et al.*, 2003). The physical properties of the growth media especially aeration and water holding capacity have influence on root initiation and growth while chemical properties of growth media, particularly nutrients content have effect on shoot growth (Baiyeri, 2003). On the other hands, Kwa (2003) reported existence of differential cultivar response to *in vivo* multiplication technique. It was reported that AAA banana cv. Grande naine produced fewer suckers than AAB banana plantain types. Tanzania has a large diversity of banana cultivars whose response to *in vivo* macropropagation technique has not yet been evaluated. This study aimed at determining suitable growth media for enhanced *in vivo* multiplication of different banana cultivars.

1.2. Objectives

1.2.1 Main objective

To increase the production of Tanzanian local banana planting material in short period of time and at farmers accessibity.

1.2.2 Specific objectives

(i) To evaluate the effect of growth media on the *in vivo* multiplication of selected local banana cultivars

(ii) To assess the *in vivo* derived suckers establishment in second nursery after transplanting in polybags.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bananas Origin and Diversification

Bananas belong to the genus *Musa* which includes the largest herbaceous plants. It grows to a height of 2 to 8 m depending on the variety and environmental conditions (UNCST, 2007). The precise origin of edible bananas is not known but the generally accepted theory is that Malaysia, a biogeographical region including the Malay Penensula, Indonesia, the Philippines and New Guinea, was the primary centre and India was a secondary centre (Simmonds and Shepherd, 1955). It is likely that dispersal out of Asia was linked entirely to human movement (Daniells *et al.*, 2001). Current varieties of banana and plantain from wild types are present in Southeast Asia which is the primary center of diversification of its kind (CIRAD-GRET, 2002). The introduction of banana in Africa is accredited to Arabs. They introduced the first plants on the coast and gradually spread the plant within the African continent in its natural state (Champion, 1967). There are no wild varieties of banana in Africa (except collections). The great diversity of banana in Africa encountered thus is the result of somatic mutations or somaclonal variations that occurred in Africa and Asia (Swennen and Vuylstake, 2001).

Africa contains two areas of diversification, one for plantains and the other for East African Highland Banana (EAHB). To date, 119 plantain cultivars are identified in West and Central Africa and more than 70 banana cultivars in East Africa, so that these regions can be considered as secondary centres of diversity of these banana groups (Swennen and Vuylstake, 2001).

2.2 Banana Botanical Description

2.2.1 Banana plant morphology

The banana is a herbaceous giant perennial plant that grows to a height of 2 to 8 m. The adult banana or parent plant consists of a pseudostem with leaves, flower or a fruit which became a bunch and suckers (Swennen and Vuylstake, 2001). The aerial shoot formed by the overlapping of leaf sheaths is called a pseudostem (trunk or false stem) which are tightly rolled round each other forming a cylindrical structure (Blomme and Ortiz, 2000).

The underground part of banana plant is called a rhizome. The terminal growing point of the rhizome is a flattened dome from which roots, buds, leaves, and eventually the inflorescence are formed (Fig. 1). The true stem of the banana plant is underground and is commonly referred to as a corm and technically speaking a rhizome (Robinson *et al.*, 2010).

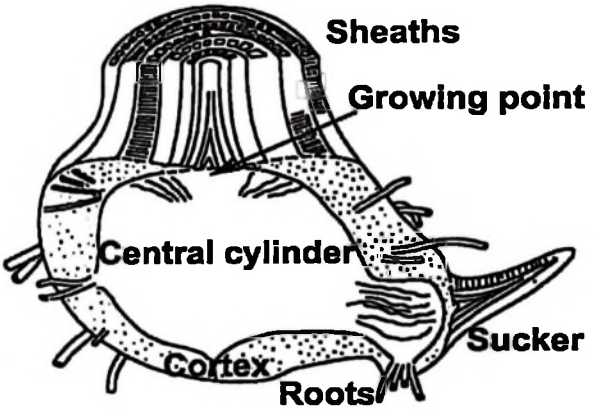


Figure 1: Morphology of banana rhizome (Robinson *et al.*, 2010)

The shoot system consists of supporting stem, photosynthetic leaves and reproductive structures (Buchanan, 1980). Buds are undeveloped shoots that contain potential nodes,

internodes and leaves. Two types of buds occur, namely the terminal bud at the plant apex which is the source of growth in height (Pandey *et al.*, 1995) and the axillary buds are usually dormant but can produce new branches that add to the plant's width (Robinson *et al.*, 2010).

2.2.2 Banana suckering ability

Cultivars vary in the rate and time of suckering (Espino *et al.*, 1992). Banana and plantains have indeterminate growth which is the result of plants having meristems, embryonic tissues composed of unspecialized cells that continue to give rise to new cells (Brand *et al.*, 2007). Apical meristems are found at the root and shoot tips and in axillary buds. They are responsible for the occurrence of apical dominance.

Apical dominance is defined broadly as "the inhibitory control of the shoot apex over the outgrowth of lateral buds" (Cline, 1997). In many plant species, the growth of axillary meristems is inhibited by the primary shoot or primary inflorescence. The plant hormones auxin (Arinaitwe *et* al., 2000) and cytokinin (Cline, 2006) are known to have a major role in controlling the process of apical dominance. Auxin has an inhibitory effect on the growth of axillary buds, whereas cytokinin promotes axillary bud outgrowth (Chao *et al.*, 2006). Apical dominance controls bud growth in the vegetative developmental stages of many herbaceous plants and the juvenile stages of some trees (Cline, 2000).

2.3 Banana Propagation

2.3.1. Conventional banana propagation

The traditional and easiest way to multiply bananas and plantains is to remove suckers from the base of the mother plant and plant them elsewhere (Bonte *et al.*, 1995).

Traditional regeneration of cultivated bananas through suckers is very slow due to hormone-mediated apical dominance of the mother plant (Singh *et al.*, 2011), especially for plantains, which emit less suckers than bananas (Bonta *et al.*, 1995). According to Singh *et al.* (2011), cultivated banana plant produces only 5-20 suckers during its life time of 12-14 months.

Banana is a crop with dual propagation abilities, sexual through seeds and asexual through suckers (Singh *et al.*, 2011). All cultivated bananas are sterile, except for a few AA and AB diploids. They are therefore propagated vegetatively (Samale, 2006). The propagation by suckers is the only natural means of their perpetuation. Artificial methods of propagation include macropropagation and micropropagation (Singh *et al.*, 2011).

There are many types of suckers including sword suckers and water suckers. Sword suckers have a well-developed base, pointed tip and narrow leaf blades, while water suckers are small, less vigorous, broad leaved suckers which emerge in clumps (Singh *et al.*, 2011). Plants derived from sword suckers can progress to inflorescence emergence eight months after development of the first normal leaf, under optimal environmental conditions. Plants derived from water suckers are not well nourished by the parent plant and therefore mature early and show early nutritional deficiency resulting in production of small and uneconomical bunches (Espino *et al.*, 1992; Broadley *et al.*, 2007).

2.3.2 Enhanced *in situ* propagation

Enhanced *in situ* propagation is an induction of sucker multiplication on banana mother plant in the field. It involves several techniques namely ; complete decapitation, folding the mother plant and false decapitation (decortication).

Complete decapitation is a technique which consists of cutting the pseudostem of the old plant to remove the upper trunk with leaves (Bonta *et al.*, 1995). The central meristem is destroyed by using a disinfected knife (Fig. 2). Four to seven suckers, depending on the variety of banana appear three to four weeks after the removal of apical dominance. Emerging shoots are immediately detached at the stage of three to four leaves for direct planting in the field (Njukwe *et al.*, 2005).

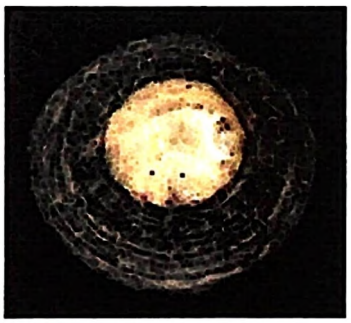


Figure 2: Decapitated sucker

Folding the mother plant is a technique by which the trunks is bent somewhere between its base and its apex so that the leaves lie on the ground. Dying slowly, the trunk will transfer all its water and nutrients to the corm, which will take the opportunity to produce more suckers (Bonta *et al.*, 1995). *In situ* based methods have the disadvantage of being slow to produce suckers. Kwa (2006) states that six to twelve months may be necessary to obtain planting material. On the other hand, many buds formed on the mother in this case remain untapped.

False decapitation (decortication) technique is a logical continuation of complete decapitation (Bonta *et al.*, 1995). This is because it is difficult to completely destroy the apical meristem by inserting a knife at the base of the trunk to view the meristem (Fig. 3). It permits the mother plant continue its photosynthesis and metabolic processes as usual.

Hence all the energy produced can be used for the production of new suckers (Njukwe *et al.*, 2005).

Figure 3: Decorticated sucker

2.3.3 *Ex situ* propagation

a) *In vivo* multiplication

In vivo multiplication involves *in vivo* macropropagation as well as corm cutting and chopping. The *in vivo* macropropagation involves disinfection and desheathing detached corms to expose axillary buds followed by removal of apical meristem to suppress apical dominance and activate the development of axillary buds (Kwa, 2002). The prepared corms are planted in propagator and nursery to produce primary shoots (Auboiron, 1997; Njukwe *et al.*, 2005). Shoots are then scarified to allow the growth of secondary buds and shoots from secondary buds before transferring them to the nursery for rooting. Finally, after acclimatization period, suckers are ready to be transplanted to the field (Njukwe *et al.*, 2005).

The technique of corm cutting consists of dividing a corm into sets after the big bud is excised. These sets are planted in the nursery after treatment with fungicides, insecticides and nematicides (Bonta *et al.*, 1995). This technique allows under special *in vivo* conditions, the regeneration of plantlets from corm parts (Kwa, 2002). Conversely, the technique of corm chopping consists of bud division in four parts (miniset) so that each

miniset contains a part of a bud (Kwa, 2002). Each miniset, raised in the nursery will produce one or more minisets to be carefully separated and reared apart in the nursery or in bags until they reach the size for transplanting (Bonta *et al.*, 1995).

In vivo multiplication techniques are cost effective and can easily be accessible to small producers compared to culture *in vitro* which requires expensive resources (Kwa, 2002). The technique also, facilitates quick multiplication of seedlings for distribution of a new variety. By the end of 140-150 days, a total of 50 - 60 suckers can be produced from a single sucker (Singh *et al.*, 2011). Macropropagation can also be performed locally at low cost and in a relatively short time (Kwa, 2006). However, macropropagation spreads diseases when infected corms are used without disinfection (Njukwe *et al.*, 2005). Macropropagation does not result in mass production of planting materials compared to *in vitro* multiplication.

b) *In vitro* propagation

In vitro propagation or micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants under aseptic conditions using shoot tips, meristems and somatic embryos (Singh *et al.*, 2011). Tissue culture is the most rapid method to produce healthy banana plantlets from selected commercial varieties for distribution to farmers (Kwa, 2000). According to Singh *et al.* (2011), application of micropropagation in banana has several advantages, including production of pest-free suckers, reduced cost of disease and pest control, production of plants with uniform and vigorous growth and shortened harvesting period (Msogoya *et al.*, 2006). Micropropagation results in a high degree of genotypic and phenotypic uniformity of the

progeny plants. However the technique has several limitations such as being sophisticated, high cost and unaffordable for small-scale farmers (Vuylsteke and Talengera, 1998).

2.4 Growth Media for Macropropagation

For optimum nursery performance, the substrate must perform the same functions requisite of good field soil for plant growth (Pertuit *et al.*, 1981; Preece and Read, 1993). Therefore, a good substrate must provide anchorage and support to the plant, serve as source of nutrients, and have a proper balance of water and air available to the plant roots (Raby, 1978). According to Cabrera (2003), the most important physical properties of the growing medium are good aeration, drainage and optimal water retention capacity for the young seedlings. The optimal conditions required for good plant growth are not less than 20 % air content and 20-30 % available water (Manyama, 1999; Sahin *et al.*, 2004). Knowledge of available water of the substrates helps in determining irrigation regime (De Boodt and Verdonck, 1972; Riviere and Nicolas, 1987). Since substrates may have different amounts of water readily available for plant uptake, each substrate should be irrigated according to its limits in water supply, climatic condition and plant requirements (De Boodt and Verdonck, 1972). A suitable substrate should have also adequate nutrients, structural and chemical stability, good nutrient retention, free from pests and a substrate should be available, uniform and consistent overtime (Marschner, 1995). Chemical quality of substrates is of utmost importance especially for substrates in which nutrient supply through irrigation is not possible. The pH should be in the range of between 5.0 and 6.9 to favour most crops in terms of nutrient availability and absence of possibilities of toxicity (Manyama, 1999).

The cation exchange capacity (CEC) is the amount of exchangeable cations per unit weight of dry soil/ substrate expressed in cmol/ kg (Donahue *et al.*, 1990). The CEC is very important as it indicates the fertility status of substrates and increases with organic matter content. Thus, high CEC means good nutrient holding capacity of the substrates and vice versa (Lemaire *et al.*, 1989). Substrates should not be saline. Salinity is most damaging to seedlings and slows down seed germination for several days or may completely inhibit it (Donahue *et al.*, 1990). The carbon: nitrogen (C: N) ratio is also one of the important factors to consider. The C/N decreases during the decomposition process. A low C/N is an indicator that the decomposition process has almost been completed (Manyama, 1999 and Dewayne *et al.*, 2003).

2.4.1 Forest soils

Forest soils contain good proportion of decomposed green leaves and plant roots which provide them with good drainage, water and nutrient holding capacity and thus supporting well the seedlings grown in them (Manyama, 1999). Rodale *et al.* (1960) indicated that forest soils have good provision of potassium (6-7 % K), Iron (18-23 % FeO), Magnesium (3-7.5 % Mg), Phosphoric acid, lime and trace elements. Idnani *et al.* (1971) showed that forest soils had good drainage, aeration, root penetration, retention of moisture and nutrient availability due to decomposed plant leaves and roots. These characteristics make forest soil a suitable substrate for raising nursery seedlings (Manyama, 1999).

2.4.2 Farmyard manure

Tamhane (1978) stated that farmyard manure improves the physical properties and chemical fertility of the growing medium. The physical properties are water holding capacity, permeability, aeration and drainage of the medium (Dewayne *et al.*, 2003). The

chemical properties include nutritional value, cation exchange capacity and buffering capacity of growing mixes. Addition of farmyard manure in the mixes increase the availability of phosphorous and nitrogen (Allison, 1973; Cooke, 1967). Farmyard manure remains of useful value in the mixes for long period of time and helps to reduce fixation of phosphorous in the medium (Sharif *et al.*, 1974).

2.4.3 Rice husks

According to Chovelin (1984), rice husks are obtained as waste product of rice. They are extremely light in weight and are very effective at improving drainage. Rice husks are available in different forms such as fresh, parboiled, carbonized and composted (Dewayne *et al.*, 2003). Nutrient contents in rice husks are estimated at 40 % C, 0.6 % N, 0.1 % P, 1.5 % K and 5 % S and can provide macro nutrients when decomposed (Chovelin, 1984). Fresh rice husks have a pH near neutral, are light in weight and are useful in increasing drainage or aeration. However, parboiled hulls are preferred since any weed or rice seed is killed during the steaming process (Robbins and Evans, 2006). The size of the particle and resistance to decomposition of rice husks and sawdust are very similar. The basic difference however is that N depletion is not as serious as in sawdust (Dewayne *et al.*, 2003). The presence of phenolic acid in rice husks can be hazardous to plant seedlings but can be reduced by complete decomposition of organic matter which constitutes the rice husks (Chov and Chiou, 1979). The easy incorporation of rice husks into media is an advantage for improved drainage and aeration (Wootton *et al.*, 1981). According to Simon (2011), rice husk medium seems to be the best probably due to physical properties which enhance rooting and growth performance. This was in line with Artif riaz *et al.* (2008) findings on effects of soilless media affecting the performance of potted plants.

2.4.4 Mixed growth medium

The mixture of different substrates often consist of forest soil, farmyard manure and aeration materials such as rice husks and coarse sand. Mixed medium characteristics are based on chemical and physical properties of the ingredients (Simon, 2011). These mixed media usually have well balance of nutrients, aeration and water for use by young plants.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Banana cultivars

Three major banana cultivars grown in Tanzania were used during the study, namely Mtwike (AAA - Grande naine), Mzuzu (AAB - Medium French plantain) and Bukoba (AAA - East African Highland banana) (Table 1). Banana cv. Mtwike, cv. Mzuzu and cv. Bukoba constitute the most important dessert banana, plantain and matoke in the country, respectively (Karamura *et al.*, 2004). Therefore, the demand of planting materials for these cultivars is too high to be met by the conventional propagation methods (Rasheed, 2003).

Table 1: Description of plant material used for *in vivo* macropropagation

Local name	Subgroup	Reference name	Genomic group	Use
Mtwike	Cavendish	Grande naine	AAA	Dessert banana
Bukoba	EAHB	Enshakara	AAA	Cooking
Mzuzu	Plantain	Medium French plantain	AAB	Roasting/ frying

Almost equal size sword suckers (height of 70-80 cm, collar diameter of 14-16 cm and 3-5 leaves) of each cultivar were obtained from the Horticulture Unit of the Sokoine University of Agriculture (SUA).

3.1.2 Types of growth media

Rice husks, Cocus peat (coconut peat) and mixed media were used as growth media. The mixed medium was composed of forest soil, farmyard manure and rice husks at 4:2:1 on

volume basis. Rice husk, forest soil and farmyard manure were sourced locally within Morogoro while cocus peat was obtained from Balton Tanzania Limited in Arusha.

3.1.3 Construction of propagators

Propagators of 1.5 m x 2.20 m x 0.3 m dimension were constructed using soft wood timber. They were filled up to three-quarters with steam sterilized growth media and covered with transparent polyethylene sheets and kept in a plastic tunnel (Fig. 4).

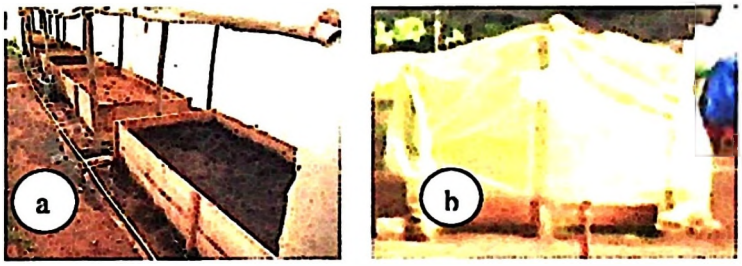


Figure 4: Preparation of propagators [(a) Propagators filled with growth media (b) propagators covered by transparent polyethylene sheets]

3.2 Methods

3.2.1 Corm preparation

Sword suckers were partially peeled and washed to remove all roots and soil debris. Thereafter, they were surface sterilized for between 20 and 30 seconds in boiled water (Faturoti *et al.*, 2002; Kwa, 2002). The sterilized sword suckers were allowed to air dry and cure for 48 hours. Two days after, the corms were desheathed to expose axillary buds followed by excision of the apical meristem using a sterilized sharp knife to remove apical dominance (Fig. 5).

Figure 5: Corm preparation before planting [(a) Sterilizing sword suckers (b) Drying and cure (c) Desheathing banana corm]

3.2.2 Growth media preparation

The growth media were moistened, and steam sterilized for 12 hours in a modified metallic drum. After sterilization, each type of the growth medium was layered and bedded in the propagators, allowed to cool and settle down for three weeks before planting the corms (Fig. 6). Neither fertilizer nor hormones were added into the media before or after corm incubation.

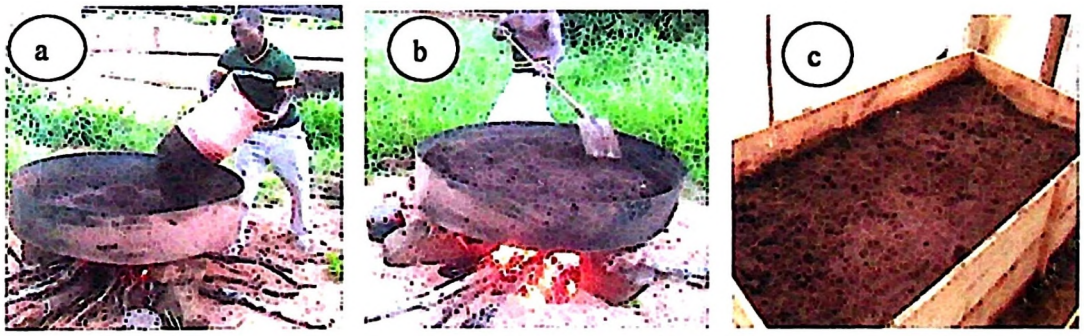


Figure 6: Media preparation [(a) and (b) Forest soil sterilization (c) Cooling and settling down into propagators]

A portion of each growth medium was taken for nutrient analysis. The analyses of the growth media were done in the Department of soil Science Laboratories at SUA. Total Carbon, Nitrogen, Phosphorous and Potassium of the growth media were determined after cooling and settling down. The total organic carbon was determined using the Walkley-black wet oxidation method (Bartlett *et al.*, 1994) and the total nitrogen by using Kjeldahl

method (Kjeldahl, 1883). The extractable phosphorous was determined by using Bray1 method (Bartlett *et al.*, 1994) and the exchangeable potassium by using Amonium Acetate (Barroso *et al.*, 2002). The electric conductivity was determined using Electrometric method (Kahn and Stokes, 1926).

3.3 Experimental Design

3.3.1 Experiment 1

To evaluate the effect of growth media on the *in vivo* multiplication of selected local banana cultivars

1) Planting and management practices

The corms were planted on 22 April, 2011 into propagators at 20 cm interval and covered with 2cm layer of each growth medium (Fig.7). The propagators were watered immediately and thereafter watering was done when necessary.

Figure 7: Planting of corms [(a) Corms planting in cocus peat (b) Corms planting in rice husks]

Relative humidity and temperatures were recorded daily and average monthly temperature and humidity were calculated. The temperature ranged from 24.4 to 31.1 °C while the relative humidity varied from 73 to 80.1 % (Table 2).

Table 2: Mean monthly temperature and relative humidity

Average mean during banana cultivar multiplication and growth						
	Mixed media		Rice husk		Cocus peat	
Month, year	Mean T (°C)	Mean RH (%)	Mean T (°C)	Mean RH (%)	Mean T (°C)	Mean RH (%)
April 2011	26.1	80.1	29.0	76.2	27.4	78.0
May 2011	28.0	79.6	31.1	73.6	29.4	76.6
June 2011	24.4	74.8	27.0	73	25.4	73.9
July 2011	26.5	76.9	27.2	73.6	26.8	78.4

Shoots from first generation at 3-4 leaf stage were excised to promote sprouting of more axillary buds (Fig. 8). Second cycle shoots were also excised at 3-4 leaf stage to further stimulate the 3rd and the next regeneration.

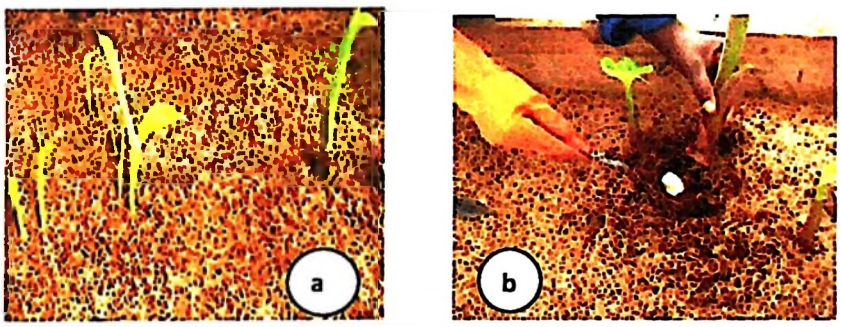


Figure 8: Sprouting of corms and excision of shoots [(a) Shoots sprouting from corm (b)1st excision operation after 5 weeks from sowing]

2) Experimental design

The experiment was established on 19th April, 2011 in a split plot under randomized complete block design (RCBD). The main factors were banana cultivars (cvs. Bukoba, Mtwike, and Mzuzu) and the sub-factor was growth media (rice husks as the control, cocus peat and mixed medium). The treatments combinations were replicated three times and each replication consisted of 10 corms.

3.3.2 Experiment 2

To assess the *in vivo* derived suckers establishment in second nursery after transplanting in polybags.

1) Planting and management practices

Black polyethylene bags of 5 liters were filled with mixed growth media with properties as detailed in Experiment 1. Suckers were detached from the corm while leaves were cut to reduce evapotranspiration. One sucker was planted in each polybag. The polybags were kept in acclimatization area, watered immediately and thereafter watering was done when required (Fig. 9).

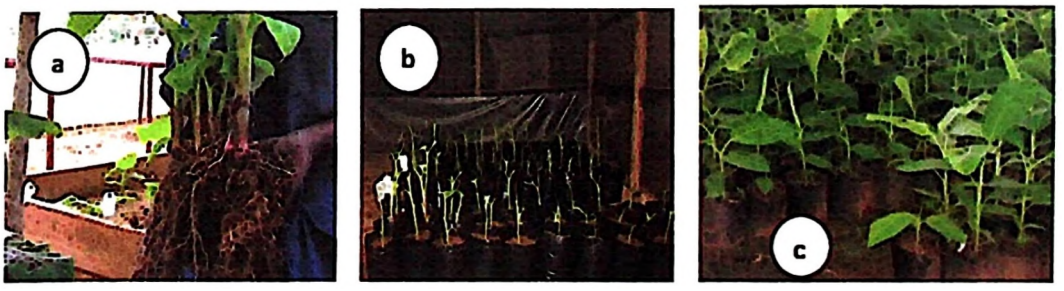


Figure 9: Harvesting, transplanting and growth of suckers [(a) Harvesting of suckers (b) Transplanting of suckers in black polyethylene bags (c) Suckers at 2 months after transplanting]

2) Experimental design

The experiment was established on 22nd July, 2011 in a split plot under randomized complete block design (RCBD). The main factors were banana cultivars (cvs. Bukoba, Mtwike, and Mzuzu) and the sub-factor were initiation media from which the suckers were regenerated (mixed medium, cocus peat and rice husks). The treatments were replicated three times, with each replication having 15 suckers.

3.4 Data Collection

3.4.1 Data collected on shoot multiplication (Experiment 1)

(i) Number of days from corm sowing to first shoot emergence

(ii) Number of corms which survived and produced shoots was counted up to the end of the experiment; (% survival)

(iii) Total number of shoots initiated per corm was counted up to the end of the experiment

(iv) Shoot size:

a. Shoot height was measured using vernier caliper (0-150 mm x 0.05/6"x 1/28", Japan) from root collar up to the base of cigar leaf

b. Shoot collar diameter was measured using vernier caliper (0-150 mm x 0.05/6"x 1/28", Japan) at the root collar

c. Number of leaves per sucker was counted at the end of the experiment

d. Leaf length was measured using a tape measure from the base leaf blade up to its tip

e. Width of widest part of the leaf lamina was measured using a tape measure.

3.4.2 Data collected on shoot establishment in the secondary nursery (Experiment 2)

Data collected included shoot height, shoot collar diameter, number of leaves per shoot, leaf length and leaf width. These data were collected as detailed in data collection under experiment 1.

3.5 Data Analysis

The data collected were analyzed using the Statistical Analysis System (SAS), version 9.0 (SAS Institute, 2002). The Kolmogorov-Smirinov test for normality was performed prior to the Analysis of Variance (ANOVA) as suggested by Kutner *et al.* (2004). Multiple correlations were used to establish the degree of relationship between parameters by using Pearson correlation as described by SAS Inst., 2002. Mean separation was done based on Student-Newman-Keuls at $p < 5$ % using COSTAT6.4 (Cohort Software, Minneapolis, USA, 2006).

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Initiation Media and Banana Cultivars on *in vivo* Multiplication and Growth

4.1.1 Chemical and nutritional characteristics of initiation media

Mixed medium and cocus peat are suitable growth media compared to rice husks medium (Table 3). Mixed medium has highest electric conductivity (5.02 mS/ cm) and exchangeable potassium (19.11 cmol (+)/ Kg) but the lowest organic carbon (9.12 %). The total nitrogen (0.53 %) and extractable phosphorous (254.46 mg/ kg soil) of mixed medium are comparable to those of cocus peat medium. Rice husks medium has the lowest total nitrogen (0.36 %), and extractable phosphorous (38.00 mg/ kg soil), exchangeable potassium (3.38 cmol (+)/ Kg) and electric conductivity (0.26 mS/ cm).

Table 3: Chemical and nutritional characteristics of initiation media

Media characteristics	Mixed medium	Cocus peat	Rice husks
Organic Carbon (%)	9.12	38.42	29.49
Total Nitrogen (%)	0.53	0.53	0.36
Extractable phosphorous (mg/ kg soil)	254.46	220.13	38.00
Exchangeable potassium (cmol (+)/ Kg)	19.11	8.99	3.38
Electric Conductivity (mS/ cm)	5.02	0.35	0.26

Source: SUA Soil Science laboratory - November 2011. **N.B:** 1 cmol (centimole) = 10 meq (milliequivalent) per 1000 g of soil.

4.1.2 Effect of initiation media on *in vivo* shoot multiplication

Growth media had significant ($P < 0.05$) effects on number of days to shoot emergence (Table 4). Corms incubated in rice husks produced suckers earlier (18.88 days) compared to corms incubated in mixed media and cocus peat with 20.02 and 23.02 days, respectively. Growth media had also a significant ($P < 0.05$) effect on shoot multiplication. Mixed medium produced the largest number of shoots per corm (4.67) followed by cocus peat and rice husk with 4.03 and 3.57 shoots per corm, respectively. Corm survival (%) was not affected by growth medium.

Table 4: Effect of initiation media on *in vivo* shoot multiplication

Media	Number of days to shoot emergence	Corm survival (%)	Number of shoots per corm
Mixed media	20.26b	87.78a	4.67a
Rice husks	18.88b	83.33a	3.57c
Cocus peat	23.02a	87.78a	4.03b
Mean	20.76	86.30	4.09
Fpr	*	ns	*
CV (%)	10.10	6.40	5.10
SE±	1.43	4.21	0.72

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant,* = significant ($p < 0.05$).

4.1.3 Effect of banana cultivar on *in vivo* shoot multiplication

Banana cultivar had a highly significant ($P \leq 0.0001$) effect on the number of shoots formed per corm (Table 5). Banana cv. Mzuzu had higher number of shoots of 5.7 compared with cv. Bukoba and cv. Mtwike which formed 3.8 and 2.7 shoots per corm, respectively.

Table 5: Effect of banana cultivars on growth characteristics

Cultivar	Number of days to shoot emergence	Corm survival (%)	Number of shoots per corm
Mtwike	21.92a	80.00b	2.73c
Bukoba	18.55a	84.44ab	3.83b
Mzuzu	21.81a	94.44a	5.70a
Mean	20.76	86.30	4.09
Fpr	ns	ns	***
CV (%)	10.10	6.40	5.10
SE±	1.55	5.05	0.47

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant, ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$)

Banana cultivar-growth media interaction had none significant influence on shoot initiation, corm survival and shoot multiplication (Table 6).

Table 6: Interaction effect of banana cultivar-growth media and shoot multiplication

Cultivar-Media	Number of days to shoots emergence	Corm survival (%)	Number of shoots per corm
Mtwike–Mixed media	20.63a	80.00a	3.07a
Mtwike-Rice husk	18.73a	76.70a	2.53a
Mtwike–Cocus peat	26.40a	83.30a	2.60a
Bukoba–Mixed media	16.77a	83.30a	3.97a
Bukoba–Rice husk	17.13a	80.00a	3.50a
Bukoba–Cocus peat	20.67a	83.30a	4.03a
Mzuzu–Mixed media	22.67a	100.00a	6.97a
Mzuzu–Rice husk	20.77a	93.30a	4.67a
Mzuzu-Cocus peat	22.00a	90.0a	5.47a
Mean	20.76	86.30	4.09
Fpr	ns	ns	ns
CV (%)	14.80	9.70	17.20
SE±	3.03	8.92	0.70

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant.

4.1.4 Correlation between assessed variables for *in vivo* shoot multiplication

The correlation between traits was computed by regressing values of one variable on those of other variables (Table 7). The results showed a significant ($P < 0.05$) correlation between corm survival and days to shoot emergence ($r = 0.40$) and highly significant ($P \leq 0.01$) correlation between number of shoot per corm and corm survival ($r = 0.56$).

Table 7: Correlation coefficients of assessed variables

Variable	Number of days to shoots emergence	Corm survival (%)	Number of shoots per corm
Days to shoots emergence	1.00		
Corm survival (%)	0.40*	1.00	
Number of shoots/ corm	0.12ns	0.56**	1.00

ns = not significant, * = significant ($p < 0.05$), ** = highly significant ($p \leq 0.01$).

4.1.5 Effect of initiation media on shoot growth of different banana cultivars

Initiation media had a high significant ($p \leq 0.001$) influence on shoot growth based on height, leaf length, leaf width and a significant ($p \leq 0.05$) effect on collar diameter (Table 8). Shoots grown in the mixed medium were the biggest with plant height of 23.64 cm and collar diameter of 1.92 cm followed by shoots grown in the cocus peat with 18.94 and 1.80 cm, respectively. Shoots grown in rice husks were the shortest with plant height of 14.28cm and collar diameter of1.53 cm. Moreover, leaves of shoots grown in mixed medium were the largest with leaf length of 24.13 cm and leaf width of 13.10 cm followed by leaves of shoots grown in the cocus peat with 20.36 and 10.83 cm, respectively. Leaves of shoots grown again, in rice husks were the smallest with leaf length of 16.46 cm and leaf width of 8.44 cm.

Table 8: Effect of initiation media on shoot growth of different banana cultivars

Media	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mixed media	23.64a	1.92a	2.80a	24.13a	13.10a
Rice husk	14.28c	1.53b	2.71a	16.46c	8.44c
Cocus peat	18.94b	1.80ab	2.88a	20.36b	10.83b
Mean	18.96	1.75	2.80	20.31	10.79
Fpr	**	*	ns	**	**
CV (%)	11.50	10.10	5.30	10.60	10.30
SE±	1.44	0.12	0.14	1.70	0.79

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant, * = significant ($p < 0.05$), ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

4.1.6 Effect of banana cultivars on shoot growth

Banana cultivars had a highly significant ($p \leq 0.001$) effect on shoot growth based on shoot height, shoot collar diameter, number of leaves per shoot, leaf length and leaf width (Table 9). Banana cv. Mzuzu had the tallest shoots with 26.31 cm followed by cv. Bukoba and cv. Mtwike with 16.33 cm and 14.22, respectively. Moreover, cv. Mzuzu had the biggest shoot collar diameter of 2.40 cm compared to cv. Mtwike and cv. Bukoba with 1.46 and 1.40 cm, respectively. Banana cv. Mzuzu had the largest number of 3.28 leaves per shoot followed by cv. Mtwike and cv. Bukoba with 2.63 and 2.48 leaves per shoot, respectively. Furthermore, cv. Mzuzu had the longest shoot leaf of 27.29 cm followed by cv. Bukoba and cv. Mtwike with leaf length of 17.02 and 16.63 cm, respectively. Banana cv. Mzuzu had the largest shoot leaf with width of 14.98 followed by cv. Bukoba and cv. Mtwike with leaf width of 9.14 and 8.26 cm, respectively.

Table 9: Effect of banana cultivar on shoot growth

Cultivar	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mtwike	14.22b	1.46b	2.63b	16.63b	8.26b
Bukoba	16.33b	1.40b	2.48b	17.02b	9.14b
Mzuzu	26.31a	2.40a	3.28a	27.29a	14.98a
Mean	18.96	1.75	2.80	20.31	10.79
Fpr	**	**	**	**	**
CV (%)	11.50	10.10	5.30	10.60	10.30
SE±	1.79	0.14	0.12	1.75	0.91

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns =not significant, * = significant ($p < 0.05$), ** = highly significant ($p \leq 0.01$).

4.1.7 Interaction effect of banana cultivar-initiation media on shoot growth

Banana cultivar-media interaction had highly significant ($p < 0.001$) influence on shoot growth of different banana cultivars (Table 10). Banana cv. Mzuzu produced the biggest suckers on mixed medium with shoot height and collar diameter of 36.27 cm and 2.90 cm, respectively. This was followed by cv. Mtwike with shoot height and collar diameter of 19.67 cm and 1.73 cm, respectively and cv. Bukoba with shoot height and collar diameter of 15.00 cm and 1.13 cm, respectively. Furthermore, cv. Mzuzu had the largest number of 3.83 leaves per shoot, compared to cv. Mtwike and cv. Bukoba with 2.73 and 1.83 leaves per shoot, respectively. Banana cv. Mzuzu had the biggest shoot leaf size with length and width of 36.70 cm and 20.67 cm, respectively followed by cv. Mtwike with 20.97 cm and 10.30 cm, respectively and cv. Bukoba with 14.73 cm and 8.33 cm, respectively.

Table 10: Interaction effect of banana cultivar-initiation media on shoot growth

Cultivar-Media	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mtwike-Mixed media	19.67c	1.73d	2.73d	20.97c	10.30d
Mtwike-Rice husk	11.23i	1.27h	2.53f	14.37i	7.10i
Mtwike–Cocus peat	11.77h	1.37g	2.63e	14.57h	7.37h
Bukoba–Mixed media	15.00f	1.13i	1.83g	14.73g	8.33f
Bukoba–Rice husk	14.70g	1.43f	2.73d	16.07f	8.30g
Bukoba–Cocus peat	19.30d	1.63e	2.87c	20.27d	10.80c
Mzuzu–Mixed media	36.27a	2.90a	3.83a	36.70a	20.67a
Mzuzu–Rice husk	16.37e	1.90c	2.87c	18.93e	9.93e
Mzuzu-Cocus peat	26.30b	2.40b	3.13b	26.23b	14.33b
Mean	18.96	1.75	2.80	20.31	10.79
Fpr	***	**	**	**	**
CV (%)	16.20	15.00	10.70	17.80	15.60
SE±	2.71	0.23	0.23	2.97	1.44

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

4.1.8 Correlation between assessed parameters for shoot growth

The correlation between traits was computed by regressing values of one variable on those of other variables. The correlations among the variables are presented in Table 11. The results showed very highly significant ($p \leq 0.001$) correlation between all assessed variables with shoot diameter and shoot height having r = 0.91, number of leaf per corm and shoot height having r = 0.70 , number of leaf per corm and shoot diameter having r = 84, leaf length and shoot height having r = 0.97, leaf length and shoot diameter having r = 0.92, leaf length and number of leaf per corm having r = 0.71, leaf width and shoot height having r = 0.98, leaf width and shoot diameter having r = 0.92, leaf width and number of leaf per corm having r = 0.72 and leaf width and leaf length having r = 0.99.

Table 11: Correlation Coefficients of different assessed parameters

	Shoot height	Shoot diameter	Number of leaves per corm	Leaf length	Leaf width
Shoot height	1.00				
Shoot diameter	0.91***	1.00			
Number of leaf per corm	0.70***	0.84***	1.00		
Leaf length	0.97***	0.92***	0.71***	1.00	
Leaf width	0.98***	0.92***	0.72***	0.99***	1.00

*** = very highly significant ($p \leq 0.001$)

4.2 Effect of Initiation Media and Banana Cultivar on Sucker Growth in Secondary Nursery

4.2.1 Effect of initiation media on sucker growth in secondary nursery

Initiation growth media had a significant ($p < 0.01$) influence on shoot growth in secondary nursery based on shoot height and leaf size (Table 12). Shoots initiated from the mixed medium and cocus peat were significantly taller with shoot height of 34.64 cm and 30.05

cm, respectively compared to short shoots initiated from rice husks with shoot height of 23.55 cm. Moreover, shoots initiated from the mixed medium had the largest leaves with length and width of 29.71 cm and 18.83 cm, followed by shoots initiated from the cocus peat with 26.61 cm and 15.49 cm, respectively. On the other hand, shoots initiated from the rice husks had the smallest leaves with leaf length and width of 22.22 cm and 12.87 cm, respectively.

Table 12: Effect of initiation media on sucker growth in second nursery

Media source	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mixed media	34.64a	3.85a	4.78a	29.71a	18.83a
Rice husk	23.55c	3.85a	4.33a	22.22c	12.87c
Cocas peat	30.05b	3.24a	4.54a	26.61b	15.49b
Mean	29.42	3.48	4.55	26.18	15.73
Fpr	**	ns	ns	*	*
CV (%)	11.01	12.59	13.61	21.04	18.90
SE±	3.94	0.66	0.57	3.97	4.23

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant, * = significant ($p < 0.05$).

4.2.2 Effect of banana cultivar on sucker growth in secondary nursery

Banana cultivar had a significant ($p \leq 0.01$) effect on shoot growth in the secondary nursery based on height, leaf length and width (Table 13). Banana cv. Mzuzu was the tallest with shoot height of 36.38 cm followed by cv. Bukoba with shoot height of 27.84 cm and cv. Mtwike with shoot height of 24.02 cm. Moreover, cv. Mzuzu had the largest leaves with 31.39 cm long and 18.48 cm width followed by cv. Bukoba and cv. Mtwike with leaves of 24.68 cm and 22.48 cm long and 14.98 cm and 13.73 cm width, respectively.

Table 13: Effect of banana cultivar on sucker growth in secondary nursery

Cultivar	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mtwike	24.02c	3.36a	4.34a	22.48c	13.73c
Bukoba	27.84b	3.10a	4.35a	24.68b	14.98b
Mzuzu	36.38a	3.99a	4.95a	31.39a	18.48a
Mean	29.42	3.48	4.55	26.18	15.73
Fpr	*	ns	ns	**	*
CV (%)	11.01	12.59	13.61	21.04	18.90
SE±	6.43	1.15	0.99	3.05	4.24

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant, * = significant ($p < 0.05$), ** = highly significant ($p \leq 0.01$).

4.2.3 Interaction effect of banana cultivar-initiation media on sucker growth in secondary nursery

Banana cultivar-initiation media interaction had a significantly ($P < 0.05$) influence on shoot growth in the secondary nursery (Table 14). Shoots of cv. Mzuzu were the biggest on mixed medium with shoot height of 46.53 cm and collar diameter of 4.93 cm followed by cv. Bukoba with 29.27 cm and 3.13 cm, respectively and cv. Mtwike with 28.13 cm and 3.50 cm, respectively. Furthermore, shoots of cv. Mzuzu were the biggest on cocus peat with shoot height of 35.20 cm and collar diameter of 3.63 cm followed by cv. Bukoba with 29.93 cm and 3.17 cm, respectively and cv. Mtwike with 20.03 cm and 2.93 cm, respectively. Shoots of cv. Mzuzu were the biggest on rice husks with shoot height of 27.43 cm compared to shoots of cv. Bukoba and cv. Mtwike with 24.33 cm and 18.90 cm, respectively. Conversely, shoots of cv. Mtwike were the biggest on rice husks with shoot collar diameter of 3.67 cm followed by cv. Mzuzu and cv. Bukoba with 3.40 cm and 3.00 cm, respectively.

Table 14: Interaction effect of banana cultivar-initiation media on subsequent sucker growth in secondary nursery

Cultivar-Media	Shoot height (cm)	Shoot diameter (cm)	Number of leaves per shoot	Leaf length (cm)	Leaf width (cm)
Mtwike-Mixed media	28.13c	3.50c	5.10a	24.03a	16.77a
Mtwike-Rice husk	18.90i	3.67b	3.60a	19.07a	10.83a
Mtwike–Cocus peat	20.03h	2.93i	4.33a	24.33a	13.60a
Bukoba–Mixed media	29.27d	3.13g	4.23a	24.27a	16.77a
Bukoba–Rice husk	24.33g	3.00h	4.13a	24.50a	12.27a
Bukoba–Cocus peat	29.93c	3.17f	4.70a	25.25a	15.90a
Mzuzu–Mixed media	46.53a	4.93a	5.00a	40.83a	22.97a
Mzuzu–Rice husk	27.43f	3.40e	5.27a	23.10a	15.50a
Mzuzu-Cocus peat	35.20b	3.63c	4.60a	30.23a	16.97a
Mean	29.42	3.48	4.55	26.18	15.73
Fpr	*	*	ns	ns	ns
CV (%)	10.80	13.20	12.40	18.10	19.50
SE±	2.22	0.36	0.37	2.40	1.80

Means followed by the same letters(s) within the column are not significant at 5% level based on Student-Newman-Keuls. ns = not significant, * = significant ($p < 0.05$).

4.2.4 Correlation between assessed parameters for shoot growth in second nursery

The correlation between traits was computed by regressing parameter values of one variable on those of other variables. The Correlations among the variables are presented in Table 15. The results showed a significant ($P < 0.05$) correlation between number of leaves per corm and shoot diameter ($r = 0.65$), and highly significant ($P \leq 0.01$) correlation between shoot diameter and shoot height ($r = 0.68$), number of leaves per corm and shoot height ($r = 0.41$), leaf length and shoot height ($r = 0.96$), leaf length and shoot diameter ($r = 0.58$), between leaf length and number of leaves per corm ($r = 0.83$), leaf width and shoot height ($r = 0.96$), leaf width and shoot diameter ($r = 0.60$), leaf width and number of leaves per corm ($r = 0.81$) and leaf width and leaf length ($r = 0.99$).

Table 15: Correlation coefficients of different assessed variables

Variables	Shoot height	Shoot diameter	Number of leaves per corm	Leaf length	Leaf width
Shoot height	1.00				
Shoot diameter	0.65**	1.00			
Number of leaf per corm	0.68**	0.41*	1.00		
Leaf length	0.96**	0.58**	0.83**	1.00	
Leaf width	0.96**	0.60**	0.81**	0.99**	1.00

* = significant ($p < 0.05$), ** = highly significant ($p \leq 0.01$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Initiation Media and Banana Cultivars on *in vivo* Multiplication

5.1.1 Effect of initiation media on *in vivo* shoot multiplication

Rice husks medium reduced the number of days to shoot emergence. The enhancement of shoot emergence in corms planted in rice husks was probably due to high temperature in the medium. In this study, the temperature during emergence period in rice husks medium was higher at 29.0 °C while that in cocus peat and mixed medium was 27.4 and 26.1 °C, respectively. Similary, high temperatures of 20 °C promoted seedling germination and seedling emergence in *Pinus halepensis* (Vozzo, 1983). Corms planted in the mixed medium and cocus peat produced larger number of shoots per corm than those planted in rice husks medium. The higher shoot productivity in mixed medium and cocus peat was associated with the high nutrient contents. In this study, mixed medium has higher total nitrogen (0.53 %), extractable phosphorous (254.46 mg/kg soil) and exchangeable potassium (19.11 cmol (+)/Kg. Idnani *et al.* (1971) showed that forest soils had high nutrient availability, while farmyard manure in the mixes improves the chemical fertility and increases the availability of phosphorous and nitrogen (Allison, 1973; Cooke, 1967). Manyama (1999) also found that mixed medium was suitable for raising nursery seedlings.

5.1.2 Effect of banana cultivar on *in vivo* shoot multiplication

Banana cv. Mzuzu produced the largest number of shoots followed by cv. Bukoba and cv. Mtwike. Similarly, Kwa (2003) reported higher shoot productivity with french plantain and low shoot productivity with banana cv. Grande naine. The differential response among the three banana cultivars is possibily due to their difference in genetic make-up. For exemple, plantains have lower level of cytokinin than Cavendish and East African Highland

bananas. The higher shoot productivity of cv. Mzuzu can therefore be associated with the level of cytokinin in the corms.

5.2 Effect of Initiation Media and Banana Cultivars on Shoot Growth

5.2.1 Effect of initiation media on shoot growth

Banana shoots initiated from the mixed medium and cocus peat were bigger than those from the rice husks medium based on shoot height, leaf length and leaf width. The increased size of shoots derived from the mixed medium and cocus peat was possibly attributed to higher nutrient contents. In this study, the mixed medium had the highest nutrient content of nitrogen, extractable phosphorous and exchangeable potassium, followed by cocus peat and rice husks medium, respectively. Baiyeri (2003) also reported that nutrients coupled with good water holding capacity of the growth medium enhance vigour and quality of plantlets.

In the second nursery, mixed medium increased the shoots size than cocus peat and rice husks medium based on shoot height, leaf length and leaf width. The increase in shoot size could be due to the quality of the initiation medium. Baiyeri (2005) found similar trend that plantlets which had better roots system at transplanting survived and grew better in the nursery than those with poor root systems.

5.2.2 Effect of banana cultivar on shoot growth

Banana cv. Mzuzu produced more vigorous shoots than cv. Mtwike and cv. Bukoba based on shoot height, leaf length, leaf width and number of leaves per shoot. The bigger shoot size of cv. Mzuzu was possibly due to its genetic make-up. Plantains have stronger apical

dominance which allows them to grow faster and vigorously while Cavendish cultivars tend to grow slower with many suckers (Simmonds and Shepherd, 1959; Cline, 2000).

In the second nursery, banana cv. Mzuzu increased shoot size more than cv. Mtwike and cv. Bukoba (on shoot height, leaf length, and leaf width). The enhancement in shoot size in plantains is attributed to their genetic make-up (Champion, 1967) that results in well-developed root system and better quality of suckers. Baiyeri (2005) found similar trend that plantlets which had better roots system at transplanting survived and grew better in the nursery than those with poor root systems.

5.2.3 Interaction effect of banana cultivar-initiation media on shoot growth

Initiation media–banana cultivar interaction showed an increase in shoot size. The differential responses of different banana cultivars to initiation media were probably due to differences in their genetic and response to nutrients. Plantain need more N and K nutrients compared with dessert banana (Fox, 1989; Irizarry, 1981). In this study, mixed medium contained higher nutrient contents than other growth media. Toth *et al.* (2002) found that an increase in nitrogen availability results in higher leaf nitrogen content. Previous studies that conducted foliar analysis (Bosch *et al.*, 1996; Gold *et al.*, 1999; Okech *et al.*, 2004; Rufino, 2003; Smithson *et al.*, 2001; Smithson *et al.*, 2004; Ssali *et al.*, 2003) identified K deficiency as a major constraint, often followed by N and Mg. According to the same authors, East African highland bananas (e.g. cv. Bukoba) require less amount of phosphorus. The results are similar with findings of Baiyeri, 2003; Baiyeri *et al.*, 2005) who reported that root system development and shoot growth are highly related with characteristics of the growth medium (Meerow, 2000).

In the second nursery, initiation media–banana cultivar interaction also showed an increase of shoots size (height and collar diameter). The enhancement in shoot size was possibly due to both initiation medium and genetic make-up. Baiyeri and Ndubizu (1994) reported that the quality of seedlings transplanted has influence on their re-establishment in the orchard, and consequently their future productivity.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Mixed medium enhanced banana shoot emergence comparable to rice husks while cocus peat medium delayed shoot emergence. Mixed medium followed by cocus peat increased shoot productivity as well as sucker size during *in vivo* multiplication stage. On the other hand, banana cv. Mzuzu produced the biggest shoots followed by cv. Bukoba and cv. Mtwike, respectively. In the secondary nursery, suckers initiated in mixed medium grew faster than those initiated in cocus peat and rice husks medium with cv. Mzuzu having the bigger shoots than those of cv. Mtwike and cv. Bukoba. Therefore, mixed medium and plantain cv. Mzuzu are the most suitable for *in vivo* multiplication.

6.2 Recommendations

(i) The findings recommend mixed medium and plantains as the most suitable for *in vivo* multiplication technique.

(ii) Further studies are required to:

a) Evaluate the effect of aeration and water holding capacity of the growth media on *in vivo* multiplication of banana cvs. Mtwike, Bukoba and Mzuzu.

b) Establish relationship between endogenous cytokinin levels and *in vivo* propagation of banana cvs. Mtwike, Bukoba and Mzuzu.

c) Evaluate the effect of externally applied cytokinins on *in vivo* multiplication of banana cvs. Mtwike, Bukoba and Mzuzu.

REFERENCES

Allison, F. E. (1978). Soil organic matter and its role in crop production. *Soil Science for Plante Nutrition* 61: 135 – 217.

Arinaitwe, G., Rubaihayo, P. R. and Magambo, M. J. S. (2000). Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. *Horticulture Sciences* 86: 13 – 21.

Auboiron, E. (1997). *La Multiplication sur Souche Décortiquée.* Fiche Technique, CARBAP, Njombé, Cameroon. 28pp.

Baiyeri, K. P. and Aba, S. C. (2004). Response of *Musa* species to macro-propagation. I: Genetic and initiation media effects on number, quality and survival of plantlets at prenursery and early nursery stages. *African Journal of Biotechnology* 4(3): 223 – 228.

Baiyeri, K. P. and Ndubizu, T. O. C. (1994). Variability in growth and field establishment of Falsehorn plantain suckers raised by six cultural methods. *MusAfrica* 4: 1 – 3.

Baiyeri, K. P. (1997). Upgrading inferior plantain propagules using factorial combinations of nitrogen rates and watering intervals. *African Journal for Tropical Agriculture* 2: 17 – 23.

Baiyeri, K. P. (2003). Evaluation of nursery media for seedling emergence and early seedling growth of two tropical tree species. *Moor Journal of Agricultural Research* 4: 60 – 65.

Baiyeri, K. P. and Aba, S. C. (2005). Response of *Musa* species to Macro-Propagation: I: Genetic and initiation media effects on number, quality and survival of plantlets at pre-nursery and early nursery stages. *African Journal of Biotechnology* 4(3): 103 – 287.

Baiyeri, K. P. and Ajayi, A.R. (2000). Status and constraints of *Musa* species production in a sub-humid zone of Nigeria. *Acta Horticulturae* 540: 73 – 77.

Baiyeri, K. P. and Mbah, B. N. (1994). Growth and yield correlation in Falsehorn plantain (*Musa* AAB cv. Agbagba) in a sub-humid zone of Nigeria. *MusAfrica* 5: 3 – 4.

Baiyeri, K. P. and Mbah, B. N. (2006). Effects of soilless and soil based nursery media on seedling emergence, growth and response to water stress of African breadfruit (*Treculia africana decne*). *African Journal of Biotechnology* 5: 1405 – 1410.

Baiyeri, K. P., Egbufor F. C. and Ndubizu, T. O. C. (1994). Evaluation of sucker production techniques in false horn plantain (*Musa* AAB). *Nigerian Journal of Horticulture Science* 2(2): 41 – 43.

Bartlett, G. N., Craze, B., Stone, M. J. and Crouch, R. (1994). *Guidelines for Analytical Laboratory Safety*. Department of Conservation and Land Management, Sydney, Australia.18pp.

Beardsell, D. V. and Nichols, D. G. (1982). Wetting properties of dried-out nursery container media. *Scientia Horticulturae* 17: 49 – 59.

Berg, L. A. and Bustamante, M. (1974). Heat treatment and meristem culture for production of virus free bananas. *Phytopathology* 64 : 320 – 322.

Beugnon, M. and Champion, J. (1966). Etude sur les racines du bananier. *Fruits* 21: 309 – 327.

Blomme, G. and Ortiz, R. (1996). Preliminary assessment of root systems in *Musa. The International Conference on Banana and Plantain for Africa*, 14 – 18 October 1996, Kampala, Uganda. pp. 10 – 16.

Blomme, G. and Ortiz, R. (2000). Preliminary assessment of root systems morphology in *Musa*. In: (Edited by Craenen, K., Ortiz, R., Karamura, E.B. and Vuylsteke, D.), *Proceedings of the International Conference on Banana and Plantain for Africa International Society for Horticultural Science*, Leuven, Belgium. pp. 259 – 266.

Bonta, E., Verdonck, R. and Gregoire, L. (1995). *La Multiplication Rapide du Bananier et du Plantain au Cameroun.* Notes techniques, AGCD, DIRAGRI, Yaoundé, Cameroun. 116pp.

Bosch, C., Lorkeers, A., Ndile, M. R. and Sentozi, E. (1996). *Diagnostic Survey Constraints to Banana Productivity in Bukoba and Muleba Districts, Kagera Region, Tanzania.* Working Paper No. 8. Tanzania/Netherlands Farming Systems Research Project, Lake Zone. Ari Muruku, Bukoba, Tanzania. 119pp.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I. and Lux, A. (2007). Zinc in plants. *New Phytologist* 173: 677 – 702.

Broschat, T. K. (1998). Root and shoot growth patterns in four palm species and their relationships with air and soil temperatures. *Horticulture Science* 33: 995 – 998.

Buchanan, B. B. (1980). Role of light in the regulation of chloroplast enzymes. *Annual. Review of Plant Physiology* 31: 341 – 394.

Cabrera R. M. A., Gou, P., Foucat, L., Renou, J. P. and Daudin, J. D. (2003). Water transfer analysis in pork supported by NMR imaging. *Meat Science* 67: 169 –178.

Champion, P. (1967). *Les Bananiers et leur Culture.* Botanique et génétique des bananiers. IFAC, Paris, France. 214pp.

Chao, W. S., Serpe, M. D., Anderson, J. V., Gesch, R. W. and Horvath, D. P. (2006). Sugars, hormones, and environment affect the dormancy status in underground adventitious buds of leafy spurge (*Euphorbia esula*). *Weed Science* 54: 59 – 68.

CIRAD-GRET (2002). *Mémento de l'agronome.* Ministère français des affaires étrangères. CRBP, Douala, Cameroun. 4pp.

Cline, M. G. and Choonseok, O. H. (2006). A reappraisal of the role of abscisic acid and its interaction with auxin in apical dominance. *Annals of Botany* 98: 891 – 897.

Cline, M. G. (1997). Concepts and terminology of apical dominance. *American Journal of Botany* 84: 1064 – 1069.

Cline, M. G. (2000). Execution of the auxin replacement apical dominance experiment in temperate woody species. *American Journal of Botany* 87: 182 – 190.

Cooke, G. W. (1976). *A review of the Effects of Agriculture on the Chemical Composition and Quality of Surface and Underground Waters.* In: Agriculture and Water Quality. MAFF Technical Bulletin No. 32. London, UK. 57pp.

Daniells, J., Jenny, C., Karamura, D. and Tomekpe, K. (2001). *Musa*loque: a catalogue of *Musa* germplasm. Diversity in the genus *Musa* (Edited by Arnaud, E. and Sharrock, S.), *International Network for the Improvement of Banana and Plantain*, Montpellier, France. 213pp.

Daniells, J. W. (1988). Comparisons of growth and yield of bananas derived from tissue culture and conventional planting materials. *Banana Newsletter* 11: 2 – 12.

De Boodt, M. and Verdonck, O. (1972). The Phyisical Preperties of Substrates in Horticulture. *Acta Horticulture* 26: 37– 44.

De Langhe, E., Swennen, R. and Wilson, G. F. (1983). Aspects hormonaux durejetonnage des bananiers plantains. *Fruits* 38: 318 – 325.

Dewayne, L. I., Richard, W. H. and Thomas, H. Y. (2003). *Growth Media for Container Grown Ornamental Plants.* The Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. 241pp.

Donahue, R. L., Miller, R. W. and Shickluna, J. C. (1990). *Soils an Introduction to Soils and Plant Growth.* 5th Edition. Prentice Hall, India. 667pp.

Dowiya, N. B., Rweyemamu, C. L. and Maerere, A. P. (2009). Banana (*Musa* spp. Colla) cropping systems, production constraints and cultivar preferences in eastern Democratic Republic of Congo. *Journal of Animal and Plant Sciences* 4(2): 341 – 356.

Espino, R. R. C., Jamaluddin, S. H., Silayoi, B. and Nasution, R. E. (1992). *Musa L. Edible Cultivars.* In: (Edited by Verheij, E. W. M. and Coronel, R. E.), Plant Resources of South East Asia No. 2 edible fruits and Nuts. PROSEA, Bogor, Indonesia. 320pp.

Faivre-Dupaigre, B., Liagre, L., Lassica, Y. and Neu, D. (2002). *Les Filières Agricoles et Agro-alimentaires.* In: Memento de l'agronome. CIRAD/GRET, Douala, Cameroun. 154pp.

FAO (2002). *Production Year Book 2000.* Food and Agriculture Organization of the United Nations, Rome, Italy. 184pp.

FAO (2005). *Report of the Workshop on Production and Marketing Constraints on Roots, Tubers and Plantains in Africa*, Zaire. FAO, Rome. 82pp.

FAOSTAT (2007). Core production data. Food and agriculture organization of the United Nations. [faostat.fao.org/] site visited on 20/7/2011.

Faturoti, B., Tenkouano, A., Lemchi, J. and Nnaji, N. (2002). *Rapid Multiplication of Plantain and Banana*. Macropagation techniques. A Pictorial Guide. IITA, Ibadan. 43pp.

Fox, R.L. (1989). *Banana.* In: (Edited by Plucknett, D. L. and Sprague, H.B.), Detecting mineral nutrient deficiencies in tropical and temperate crops. Westview, Boulder, USA. 12pp.

Gold, C. S., Karamura, E. B., Kiggundu, A., Bagamba, F. and Abera, A. M. K. (1999). Geographic shifts in highland cooking banana (*Musa* spp., group AAA-EA) production in Uganda. *International Journal of Sustainable Agriculture and World Ecology* 6: 45 – 59.

Hodel, D. R., Pittenger, D. R. and Downer, A. J. (2005). Palm root growth and implications for transplanting. *Journal of Arboriculture* 31:171–181.

IITA (2011). *Plantain and Banana Improvement Program-Annual Report for 2010.* International Institute of Tropical Agriculture, Onne, Nigeria. 35pp.

Irizarry, H. F. (1981). Nutrient uptake of intensively managed plantain as related to stage of growth at two locations. *Journal of Agriculture* 65: 331 – 345.

Karamura, D., Mgenzi, B., Karamura, E. and Sharrock, S. (2004). Exploiting IK for the management and maintenance of *Musa* biodiversity on farm. *African Crop Science Journal* 12: 71 – 78.

Karamura, D., Mgenzi, B., Karamura, E. and Sharrock, S. (2007). The biology of Banana and Plantains. *African Crop Science Journal* 12: 71 – 78.

Karamura, E. B., Turyagyenda, F. L., Tinzaara, W., Blomme, G., Ssekiwoko, F., Eden-green, S., Molina, A. and Markham, M. (2008). *Xanthomonas wilt of Bananas in East and Central Africa. Diagnostic and Management Guide.* Biodiversity international, Rome, Italy, 59pp.

Kjeldahl, J. Z. (1883). A new method for the determination of nitrogen in organic bodies. *Analytical Chemistry* 22: 366 – 378.

Kutner, M. H., Nachtsheim, C. J. and Neter J. (2004). *Applied Linear Regression Models.* 4th Edition. McGraw-Hill Irwin, New York, USA. 51pp.

Kwa, M. (1997). *Etude des Techniques de Multiplication du Matériel Végétal in Vivo*. In: Anonymous (Ed.), Rapport Technique. Document Interne. CRBP, Njombé, Cameroun. 100pp.

Kwa, M. (2000). *La Technique de Plants Issus des fragments des Tiges.* Fiche technique, CRBP, Douala, Cameroun. 11pp.

Kwa, M. (2002). New horticultural techniques of mass production of bananas the PIF technique. CARBAP technical data sheet. *CARBAP Njombé* 47: 191 – 226.

Kwa, M. (2003). Activation of latent buds and utilization of stem fragments for plants mass propagation in *in vivo* horticulture conditions. *Fruits* 58: 315 – 328.

Kwa, M. (2006). Activation de bourgeons latents et utilisation de fragments de tige du bananier pour la macroprogation de plants en conditions horticoles *in vivo,* CIRAD, EDP sciences. *Fruits* 15 *:* 315 – 327.

Lahav, E. and Turner, D. W. (1989). *Banana Nutrition.* International Potash Institute, bulletin No. 7. Berne, Switzerland. 5pp.

Lassoudière, A. (2007). *Le bananier et sa Culture.* Editions Quae, Versailles, France. 383pp.

Lawlor, D. W. (2002). Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experiment Botany* 53: 773 – 787.

Lemaire, F. A. D., Riviere, L. M. and Charpentiers, D. (1989). *Cultures en Pots et Conteneurs Principles Pgronomiques et Applications*. INRA, Paris, France. 184pp.

Ma, S. S. and Shii, C. L. (1972). *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *Journal of Chinese Society Horticulture Science* 18: 135 – 142.

Madhulatha, P., Anbalagan, M., Jayachandran, S. and Sakthivel, N. (2004). Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). *Plant Cells and Tissus Organs Culture* 76: 189 – 192.

Maerere, A. P., Kusolwa, P. M., Msogoya, T. J. and Nsemwa, T. L. H. (2003). Comparison of effective *in vitro* regeneration and multiplication potential of local and introduced banana. in: *Proceedings of the Second Collaborative Research Workshop on Food Security,* Sokoine University of Agriculture, Morogoro, Tanzania. pp. 4 – 7.

Manyama, A. A. A. (1999). Characterization and evaluation of local materials suitable for confection of substrates for raising seedlings in a nursery. Dissertation for Award of MSc Degree at Sokoine University of Agriculture, Morogoro, Tanzania, 102pp.

Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. Second Edition. Academic Press, London. 889pp.

Meerow, A.W. and Broschat, T. K. (1992). *Transplanting Palms.* University of Florida IFAS, Gainesville. USA. 63pp.

Mgenzi, S. R. B., Piters, S. and Rwezaura, P. A. (1997). *Banana Seasonality in Karagwe District*: A problem of perception. KDRDP/ARDI, Maruku, Tanzania. 86pp.

MOAC, (2000). *Basic Data Agriculture and Livestock Sector.* Ministry of Agriculture and cooperatives, Dar es Salaam, Tanzania. 22pp.

Msogoya, T. J., Maerere, A. P. and Grout, B. (2006). *Field Performance of Micro-Propagated East African Banana (Musa AAA East Africa).* In: The Eastern Zone of Tanzania. 4pp.

Msogoya, T. J. and Grout, B. (2008). *Altered Response to Biotic and Abiotic Stress in Tissue Culture-Induced off-Type Plants of East African Highland Banana (Musa AAA East Africa).* In: Eastern Zone of Tanzania. 5pp.

Murashige, T. (1974). Plant propagation through tissue culture. *Annual Review of Plant Physiology* 25: 135 – 178.

Ndubizu, T. O. C. (1985). *Plantain Production Manual for Nigerian Growers.* Technical Bulletin 1. Imo State College of Agriculture, Umuagwo, Nigeria. 6pp.

Ndungo,V. (1997). *La Recherche sur le Bananier et la Banane Plantain à l'IRAZ. Priorités et Stratégies.* Presented on third BARNESA steering committee meeting, London, UK. 12pp.

Njukwe, E., Tenkouano, A.. Amah, D., Sadik, K., Muchunguzi, P., Nyine, M. and Dubois, T. (2005). Training manual on macro-propagation of banana and plantain. *IITA Uganda* 69: 129 – 318.

Okech, S. H., Asten van, P. J. A., Gold, C. S. and Ssali, H. (2004). Effects of potassium deficiency, drought and weevils on banana yield and economic performance in Mbarara, Uganda. *Uganda Journal of Agricultural Sciences* 9: 511 – 519.

Ortiz, R. and Vuylsteke, D. (1996). Recent advances *in Musa* genetics, breeding and biotechnology. *Plant Breeding* 66: 1355 – 1363.

Pandey, S. N. and Sinha, B. K. (1995). *Plant Physiology*. 3rd Edition, Vikas Publishing House PVT Ltd., New Delhi, India. 26pp

Pearsall, W. H. (1927). Growth studies on the relative size of plant organs. *Annals of Botany* 41: 449 – 556.

Pertuit, J. R., A. J. and Mazur, A. R. (1981). Development of growth media for Poinsenttias. *Horticultural Science* 16: 216 – 218.

Petit, E. (2010). *La culture du Bananier.* IA-IPR de la Science de la vie et de la terre, Académie de la Martinique. 72pp.

Preece, J. E. and Read, P. E. (1993). *The Biology of Horticulture.* An introductory textbook, New York, USA. 269pp.

Raby, G. (1978). *Jardinage Sans Terre.* Introduction à la culture hydroponique. Enticelle Publisher, Paris, France. 182pp.

Rasheed, A. (2003). Plantain production as a business. *Horticulture Magazine* 1(1): 11 – 12.

Riviere, L. M. and Nicolas, H. (1987). Conduite de l'irrigation des cultures hors-sol sur substrat. Constraints liées au choix des substrats. Milieux poreaux et transfests hydriques. *Bulletin* 22: 47 – 69.

Robinson, J. C. and Bower, J. P. (2010). Transpiration characteristics of banana leaves (cultivar 'Williams') in response to progressive depletion of available soil moisture. *Science of Horticulture* 30: 289 – 300.

Robinson, J. C. and Galan S. V. (2010). *Bananas and Plantains.* 2nd Edition. Crop production science in horticulture Series No. 19. CAB International, Wallingford, UK. 749pp.

Robinson, J. C. and Nel, D. J. (1996). Growth and yield responses of banana cv. Williams to drip irrigation under drought and normal rainfall conditions in the subtropics. *Science of Horticulture* 30: 187 – 202.

Rufino, M. (2003). On-farm analysis of nematode infestation and soil fertility as constraints to the productivity of banana-based production systems in Uganda. Dissertation for Award of MSc Degree at Wageningen University, Wageningen, The Netherland. 91pp.

Samson, J. A. (1986). *Tropical Fruits.* Second Edition. Longman Publisher, New York, USA. 104pp.

SAS (2002). *SAS/STAT User's Guide, Version 9.0.* (4th Edition.), SAS Institute Inc., Cary. 68pp.

Sharif, M., Chaudhary, F. M and Latif, A. (1974). Suppression of super-phosphate phosphorus fixation by farmyard manure. I. High phosphorus uptake from super-posphate. *Soil Science of Plante Nutrition* 20: 187 – 190.

Siamak, S., Fatemeh, M. and Mahmood, M. (2009). Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoottips. *African Journal of Biotechnology* 8(21): 547 – 576.

Simmonds, N. W. and Shepherd, K. (1959). The taxonomy and origins of the cultivated bananas. *Journal of the Linnean Society of London* 359: 302 – 312.

Singh, H. P., Uma, S., Selvarajan, R. and Karihaloo, J. L. (2011). *Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific.* National Research Centre for Banana. Indian Council of Agricultural Research, New Delhi, India. 94pp.

Smale, M. (2006). Assessing the impact of crop genetic improvement in sub-Saharan Africa: Research context and highlights. Promising crop biotechnologies for smallholder farmers in East Africa: Bananas and maize. *Genetic Resources Policies Briefs* 19: 23 – 47.

Smale, M., De Groote, H. and Zepeda, F. (2006). *Biosafety and Biodiversity Risks.* In: (Edited by Melinda, S., Edmeades, S. and De Groote, H.), Promising Crop biotechnologies for smallholder farmers in East Africa: Bananas and Maize. Genetic Resources Policies Briefs, Uganda. 19pp.

Smithson, P. C., McIntyre, B. D., Gold, C. S., Ssali, H. and Kashayij, I. N. (2001). Nitrogen and potassium fertilizers vs. nematode and weevil effects on yield and foliar nutrient status of banana in Uganda. *Nutrient Cycling in Agroecosystems* 59: 39 – 50.

Smithson, P. C., McIntyre, B. D., Gold, C. S., Ssali, H., Night, G. and Okech, S. (2004). Potassium and magnesium fertilizers on banana in Uganda: yields, weevil damage, foliar nutrient status and DRIS analysis. *Nutrient Cycling in Agroecosystems* 69: 43 – 49.

Ssali, H., McIntyre, B. D., Gold, C. S., Kashaija, I. N. and Kizito, F. (2003). Effects of mulch and mineral fertilizer on crop, weevil and soil quality parameters in highland banana. *Nutrient Cycling in Agroecosystems* 65: 141 – 150.

Sussex, I. M and Kerk, N. M. (2001). The evolution of plant architecture, Current opinions. *Plant Biology* 4: 33 – 37.

Swennen, R. and Vuylsteke, D. (2001). *Banana* (*Musa* L.). *Crop Production in Tropical Africa.* Directorate -General for International Co-operation, Brussels, Belgium. 552pp.

Taiz, L. and Zeiger, E. (1998). *Plant physiology.* Second Edition. Sinauer Associates, Sunderland, Mass, USA. 372pp.

Taiz, L. and Zeiger, E. (2006). *Plant Physiology.* Sinauer Associates, Inc., Sunderland Masachusetts, USA. 690pp.

Taiz, L. and Zeiger, E. (2003). *Plant Physiology.* (3rd Ed.), Paniona Publishing Corporation, New Delhi, India. 67pp.

Tenkouano, A., Hauser, S., Coyne, D. and Coulibaly, O. (2006). Clean planting material and management practices for sustained production of banana and plantain in Africa. *Horticulture Science News* 46: 14 – 18.

Toth, V. R., Meszkaros, I., Veres, S. and Nagy, J. (2002). Effects of the available nitrogen on the photosynthetic activity and xanthophyll cycle pool of maize in field. *Journal of Plant Physiology* 159: 627 – 634.

UNCST (2007). *The Biology of Bananas and Plantains.* Program for biosafety systems. Uganda National Council for Science and Technology, Kampala, Uganda. 12pp.

Vozzo, J. A. (1985). Mulch and temperature effects on germination of southern pine seeds. In: (Shoulders, E. Ed.), *Proceedings of Third Biennial Southern Silvicultural Research Conference* 7 – 8 November 1984, Atlanta, USA. pp. 42 – 44.

Vuylsteke, D. and De Langhe, E. (1985). Feasibility of *in vitro* propagation of bananas and plantains. *International Institute of Tropical Agriculture* 62: 323 – 328.

Vuylsteke, D. and Talengera, D. (1998). *Postflask Management of Micropropagated Bananas and Plantains.* A manual on how to handle tissue-cultured banana and plantain plants. International Institute of Tropical Agriculture, Ibadan, Nigeria. 15pp.

Wootton, R. D., Gouin, F. R. and Stark, F. C. (1981). Composted, digested sludge as a medium for growing flowering annuals. *Journal of America Society Horticulture Science* 106: 46 – 49.

APPENDICES

Appendix 1: ANOVA tables of all parameters for shoot multiplication

Appendix 1.1 ANOVA Table for Days to shoot emergence

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	12.121	6.06	0.47	
Cultivar	2	81.212	40.606	3.14	0.151
Error (a)	4	51.659	12.915	1.39	
Media	2	82.459	41.229	4.43	0.036
Cultivar*Media	4	46.588	11.647	1.25	0.341
Error (b)	12	111.627	9.302		
Total	26	385.665			

Grand mean = 20.76; CV (a) = 10.10; CV (b) = 14.80; (a) = Cultivar; (b) = Media;
CV = Coefficient of variation.

Appendix 1.2 ANOVA Table for Corm survival

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.175556	0.087778	9.87	
Cultivar	2	0.108889	0.054444	6.12	0.061
Error (a)	4	0.035556	0.008889	1.3	
Media	2	0.008889	0.004444	0.65	0.54
Cultivar*Media	4	0.015556	0.003889	0.57	0.691
Error (b)	12	0.082222	0.006852		
Total	26	0.426667			

Grand mean = 86.30; CV (a) = 6.40; CV (b) = 9.70

Appendix 1.3 ANOVA Table for Number of shoots per corm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.4867	0.2433	1.9	
Cultivar	2	40.4867	20.2433	157.74	<.001
Error (a)	4	0.5133	0.1283	0.26	
Media	2	5.4867	2.7433	5.54	0.02
Cultivar*Media	4	3.7067	0.9267	1.87	0.181
Error (b)	12	5.9467	0.4956		
Total	26	56.6267			

Grand mean = 4.09; CV (a) = 5.10; CV (b) = 17.20

Appendix 2: ANOVA tables of all parameters for shoot growth

Appendix 2.1 ANOVA Table for Shoot height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	77.902	38.951	2.71	
Cultivar	2	750.462	375.231	26.12	0.005
Error (a)	4	57.462	14.366	1.53	
Media	2	394.807	197.403	21.05	<.001
Cultivar*Media	4	372.764	93.191	9.94	<.001
Error (b)	12	112.509	9.376		
Total	26	1765.907			

Grand mean = 18.96; CV (a) = 11.50; CV (b) = 16.20; (a) = Cultivar; (b) = Media;
CV = Coefficient of variation.

Appendix 2.2 ANOVA Table for Shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.97852	0.48926	5.19	
Cultivar	2	5.68519	2.84259	30.16	0.004
Error (a)	4	0.37704	0.09426	1.37	
Media	2	0.71185	0.35593	5.18	0.024
Cultivar*Media	4	1.53037	0.38259	5.57	0.009
Error (b)	12	0.82444	0.0687		
Total	26	10.10741			

Grand mean = 1.75; CV (a) = 10.10; CV (b) = 15.00

Appendix 2.3 ANOVA Table for Number of leaves per corm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	3.1163	1.55815	23.84	
Cultivar	2	3.23852	1.61926	24.77	0.006
Error (a)	4	0.26148	0.06537	0.72	
Media	2	0.12519	0.06259	0.69	0.519
Cultivar*Media	4	3.32593	0.83148	9.22	0.001
Error (b)	12	1.08222	0.09019		
Total	26	11.14963			

Grand mean = 2.80; CV (a) = 5.30; CV (b) = 10.70

Appendix 2.4 ANOVA Table for Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	56.81	28.41	2.06	
Cultivar	2	657.29	328.64	23.83	0.006
Error (a)	4	55.16	13.79	1.06	
Media	2	265.29	132.64	10.19	0.003
Cultivar*Media	4	347.8	86.95	6.68	0.005
Error (b)	12	156.22	13.02		
Total	26	1538.57			

Grand mean = 20.31; CV (a) = 10.60; CV (b) = 17.80

Appendix 2.5 ANOVA Table for Shoot width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	13.692	6.846	1.85	
Cultivar	2	240.019	120.009	32.46	0.003
Error (a)	4	14.788	3.697	1.31	
Media	2	97.556	48.778	17.27	<.001
Cultivar*Media	4	108.37	27.093	9.59	0.001
Error (b)	12	33.893	2.824		
Total	26	508.319			

Grand mean = 10.79; CV (a) = 10.30; CV (b) = 15.60

Appendix 3: ANOVA tables of all parameters for shoot growth in second nursery

Appendix 3.1 ANOVA Table for Shoot height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	63.37	31.69	0.31	
Cultivar	2	36.41	18.21	0.18	0.844
Error (a)	4	412.38	103.09	2.27	
Media	2	38.4	19.2	0.42	0.664
Cultivar*Media	4	360.55	90.14	1.99	0.161
Error (b)	12	544.36	45.36		
Total	26	1455.47			

Grand mean = 29.42; CV (a) = 11.01; CV (b) = 10.80; (a) = Cultivar; (b) = Media;
CV = Coefficient of variation.

Appendix 3.2 ANOVA Table for Shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	1.0341	0.517	0.36	
Cultivar	2	0.803	0.4015	0.28	0.769
Error (a)	4	5.7215	1.4304	1.71	
Media	2	0.5719	0.2859	0.34	0.717
Cultivar*Media	4	6.9304	1.7326	2.07	0.148
Error (b)	12	10.0444	0.837		
Total	26	25.1052			

Grand mean = 3.48; CV (a) = 12.59; CV (b) = 13.20

Appendix 3.3 ANOVA Table for Number of leaves per corm

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	0.643	0.321	0.17	
Cultivar	2	12.181	6.09	3.27	0.144
Error (a)	4	7.446	1.861	0.8	
Media	2	7.879	3.939	1.69	0.226
Cultivar*Media	4	10.157	2.539	1.09	0.405
Error (b)	12	27.964	2.33		
Total	26	66.27			

Grand mean = 4.55; CV (a) = 13.61; CV (b) = 12.40

Appendix 3.4 ANOVA Table for Shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	95.02	47.51	0.39	
Cultivar	2	45.12	22.56	0.18	0.839
Error (a)	4	491.51	122.88	2.24	
Media	2	112.54	56.27	1.03	0.388
Cultivar*Media	4	307.96	76.99	1.4	0.291
Error (b)	12	657.93	54.83		
Total	26	1710.07			

Grand mean = 26.18; CV (a) = 21.04; CV (b) = 18.10

Appendix 3.5 ANOVA Table for Shoot width

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP	2	14.59	7.29	0.22	
Cultivar	2	11.29	5.65	0.17	0.847
Error (a)	4	130.35	32.59	2.29	
Media	2	22.23	11.12	0.78	0.479
Cultivar*Media	4	66.2	16.55	1.16	0.374
Error (b)	12	170.55	14.21		
Total	26	415.22			

Grand mean = 15.73; CV (a) = 18.90; CV (b) = 19.50