EFFECT OF GROWTH REGULATORS ON *IN VIVO* SHOOT PROLIFERATION AND ROOTING OF SELECTED LOCAL BANANA (*Musa* spp. L.) CULTIVARS

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ABSTRACT

Dipping banana corms in BAP at 1.5 mg /litre for 12 hours enhances banana sucker proliferation. Thidiazuron (TDZ) is a growth regulator with both cytokinin and auxin effect and has a higher residual effect than BAP. The influence of TDZ on shoot proliferation and root formation in banana is hardly known. The objectives of this study were to evaluate the effect of (1) *in vivo* propagation combined with temporary TDZ treatments on sucker multiplication and growth and (2) indole-3-butyric acid on rooting and growth of TDZ *in vivo* derived suckers of banana. The study was conducted at Sokoine University of Agriculture and comprised of two experiments established in a split plot arrangement in a randomized complete block design (RCBD). The main factor for both experiments was banana cultivars (Mtwike, Mzuzu and Bukoba). The sub-factor for experiment one was TDZ concentrations (0.5, 1.0, 2.0 and 3.0 mg/l) while the sub-factor for experiment two was IBA at 0.06 % and control (no IBA). Data were subjected to analysis of variance (ANOVA) and mean separation was done based on Student-Newman-Keuls at $P < 5\%$ using GenStat Statistical Programme 12th Edition. Results showed that TDZ at 2.0 mg/l led to the highest proliferation of 6.3 suckers per corm followed by TDZ at 3.0 and 1.0 mg/l with 4.6 and 4.0 suckers per corm, respectively. Banana cv. Bukoba produced the highest number of 6.4 suckers per corm followed by cv. Mzuzu and cv. Mtwike with 2.9 and 2.3 suckers per corm, respectively. Treatments of TDZ-derived suckers in IBA powder at 0.06 % reduced root formation and growth. Further studies are required to determine alternative cytokine-based growth regulators for enhancing banana shoot productivity under *in vivo* multiplication

DECLARATION

I Juliana Amos Mwakisitu, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation 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.

Juliana Amos Mwakisitu — Date: 29/10/2014

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The above declaration is confirmed:

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DEDICATION

This work is dedicated to my father Amos Mwakisitu and my mother Tusajighwe Mwinama for laying the foundation and inspiration of my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
BK	Cultivar Bukoba
cv	Cultivar
CV	Coefficient of variation
d.f	Degree of freedom
FAO	Food and Agriculture Organization of the United Nations
Fpr	F probability
IAA	Indole Acetic Acid
IBA	Indole-3-Butyric Acid
IITA	International Institute of Tropical Agriculture
LSD	Least Significant Difference
m.s	Means of square
MAFS	Ministry of Agriculture, Food Security and Co-operative
MT	Cultivar Mtwike
MZ	Cultivar Mzuzu
NAA	α-Naphthalene Acetic Acid
NB	Nota bane
ns	Not significant
s.s	Sum of square
spp.	Species
SUA	Sokoine University of Agriculture
TDZ	Thidiazuron
USA	United States of America
v.r	Variation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

In Tanzania, bananas are staple food for more than 5 million among 34 million of population (Maerere, 2007). The main banana cultivars cultivated in Tanzania belong to the East Africa Highland bananas (*Musa* spp. AAA), Cavendish subgroup (*Musa* spp. AAA) plantains (*Musa* spp. AAB), Gros Michel (*Musa* spp. AAA) and Pisang awak (*Musa* spp. AB/ABB). According to MAFS (2001) Kagera region is leading for banana production followed by Mbeya, Kilimanjaro, Arusha, Tanga and Kigoma Regions. The production has been fluctuating from 250 000 to 350 000 hectares per annum for the past ten years.

Banana production has been declining over the years due to the number of factors. including inadequate availability of good quality planting materials (Dowiya *et al.*, 2009). Edible bananas do not produce seeds and therefore are clonally propagated by using traditional propagation methods. The most popular traditional method involves digging and detaching suckers from orchard-grown banana plants. This method is cheap and simple but poses risks of transmitting pests from infected mother plants (Staver *et al.*, 2010) and leads to inadequate supply of planting materials for large plantations mainly due to low suckering ability of most banana cultivars (Rasheed, 2003). The low suckering ability of most banana cultivars is largely due to high hormone-mediated apical dominance of the mother plant (Singh *et al.*, 2011).

In vivo macro-propagation is an alternative technique by which suckers are produced from banana corms under nursery conditions (Singh *et al.*, 2011). In this technique, desuckered maiden suckers of 4–6 months old are decapitated just above the root collar and

dcshealthed to expose axillary buds. Moreover, the decapitated corms are disinfected by hot water treatment and their apical meristems are damaged by decortications to reduce the apical dominance and stimulate sprouting of the axillary buds (Signh *et al.*, 2011). The corms are then planted in sawdust growth media for regeneration (Baiyeri and Aba, 2004). This method is simple, leads to production of high quality planting materials and has the potential of producing 50 - 60 shoots per corm in 4 - 5 months (Baiyeri and Aba, 2004).

1.2 Problem Statement

In vivo macro-propagation works well when maiden suckers are used particularly from plantain cultivars (Baiyeri and Ajayi, 2000). The inability of axillary buds from small banana suckers to sprout under *in vivo* macro-propagation is associated with the high apical dominance caused by high auxin levels. Higher auxin levels have been reported in Cavendish and East African highland banana (Arinaitwe *et al.*, 2000). Auxins works antagonistically with cytokinins and thus application of the later suppresses apical dominance while promoting sprouting of axillary buds (Naseem, and Dandekar, 2012).

Thidiazuron (TDZ) is a cytokinin-based growth regulator that leads to high proliferation rates at very low concentrations in woody plant species (Huetteman and Preece, 1993). According to Huetteman and Preece (1993) Thidiazuron is useful in inducing proliferation in plant species that respond poorly in 6-benzylaminopurine (BAP) supplemented growth medium. Thidiazuron has the ability to increase the biosynthesis of endogenous adenine-based cytokinins in plants. More over it involved in cell membrane modification, increasing energy levels, facilitating the nutrient uptake and nutrient assimilation in plants thus activate the basic survival mechanism in plant tissues especially for asexual reproduced species survival (Guo *et al.*, 2011).

Thidiazuron has been used for stimulation of *in vitro* shoot proliferation in recalcitrant banana genotype and increased proliferation rates in non-recalcitrant banana (Arinaitwe *et al.*, 2000). Moreover, TDZ is used for *in vitro* callus formation in triploid banana (Srangsam and Kanchanapoom, 2003). A recommended thidiazuron concentration for banana *in vitro* propagation ranges from 0.2 to 0.4 mg/l (Crouch *et al.*, 1998) and thidiazuron concentration above or below the recommended level reduces banana shoot proliferation and suppresses shoot elongation. Benzylaminopurine (BAP) has ability of inducing sprouting of axillary buds and adventitious shoots. The effect of temporary treatment of TDZ on *in vivo* sucker proliferation and growth from young corms of major banana varieties is hardly known.

Auxins, particularly naphthalene acetic acid (NAA) and indole- 3 - butyric acid (IBA) are usually used to induce root formation in stem cuttings and *in vitro* derived banana suckers (Rahman *et al.*, 2005). As TDZ has high residual effects (Makara *et al.*, 2010), its use for induction of *in vitro* proliferation in banana might inhibit root formation in suckers. The effect of dipping suckers on indole-3-butyric acid (IBA) powder on root formation of banana suckers derived from TDZ mediated *in vivo* macro propagation is hardly known.

1.3 Objectives

1.3.1 Overall objective

The overall objective of this study was to enhance the production of planting materials of selected major banana cultivars through application of *in vivo* macro-propagation combined with thidiazuron and rooting by using indole-3- butyric acid (IBA) treatments.

1.3.2 Specific objectives

(i) To determine the effect of *in vivo* macropropagation combined with temporary TDZ treatment on bud proliferation of selected banana cultivars.

(ii) To determine the IBA on rooting of banana suckers derived from TDZ mediated *in vivo* propagation.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Importance and Uses of Banana

2.1.1 Importance of banana

Banana is globally ranked the fourth, after rice, wheat and maize in terms of gross value of production in the world. According to FAOSTAT (2011) the crop is appreciated for its nutritive value with 22.2 % carbohydrates, 0.84 % fibre content, 1.1 % protein, 0.2 % fat and 75.7 % water. Banana is thus considered as a staple food in tropical and sub tropical countries (Ammar- Khodja, 2000).Apart from being a major staple food crop for about 400 millions of people, banana also provides income through local and international trade (Singh *et al.*, 2011).Worldwide, banana ranks the second after citrus in terms of values through exporting by earning US $ 5.2 billion annually (FAO, 2007) it thus provides income and employment in many tropical countries (Heslop - Harrison and Schwarzacher, 2007). Currently banana is grown in more than 150 countries worldwide over a harvested area of approximately 10 million hectares with a production of about 100 million tonnes per year (FAOStat, 2007).

In Africa, about 25 million tonnes of banana are produced which are equivalent to 34 % of the annual world production (Swennen and Vuylsteke, 2001). Banana provides more than 25 % of the carbohydrate requirements for over 70 million of African people (IITA, 1998). Eastern and Southern Africa produce over 20 million tonnes of bananas which account for 25.58 % of total world output (Karamura, 1998). Generally, the Great Lakes Region covering parts of Uganda, Rwanda, Burundi, Tanzania, Kenya and Democratic Republic of Congo are the largest producer and consumer of bananas in Africa (Smale, 2006).The *per capita* consumption has been estimated at between 250 and 450kg per year in Uganda, Burundi and Rwanda (Aliguma and Karamura, 2006).

In Tanzania banana crop occupies 22 % of the total cultivated land which represents 40 % of the total food production contributing about 15 % to the country's GDP (Ndungo. 1997). Crop production records showed that banana ranks the third after maize and cassava in Tanzania (Vice President Office. 2001). Tanzania produces about 4 % of the world banana ranking the second in Africa after Uganda and 7th producer in the world (Maerere. 2007). The Lake zone. Northern highlands. Southern highlands. Eastern and Western zones are the major banana producers in Tanzania and the yield is approximately 4 tons/ha (Jackson and Mgenzi. 2003). According to MAFS (2001) the Lake Zone is leading in banana production since 1998/1999 with a production of about 43 to 45 % of the total banana production in the country. Generally. the crop can improve food security and income of the country since it grows under diverse ecological conditions and produces throughout the year (Kasyoka *et al.*, 2010).

2.1.2 Uses of selected banana cultivars.

Banana fruits are nutritious. containing carbohydrates (98%). Potassium. vitamin C and vitamin B_6 (Bezerra *et al.*, 2013). There are different types of bananas which nutritionally differ in sugar contents and starch. Dessert bananas are fruits which are eaten raw after ripening. They are high in sugar content and therefore sweet while they are low in starch content (Robnson. 1996). Examples of dessert banana include Mtwike (Grand naine). Jamaica (Gross Michel) and Kisukari (Silk). Plantains are fruits with high starch and low sugar content. They are consumed as boiled. roasted or fried meal (Pillay *et al.*, 2002). Plantain fruits are also processed in flour for different uses. Examples of plantains include Mzuzu (French plantain) and Mkono wa tembo (True horn plantains). On the other hand. East African Highland banana have fruits with low sugar and starch contents. They are largely consumed as boiled meal popularly known as "Matoke". Example of East African Highland banana includes Bukoba. Uganda, Embwairuma and Enyoya (Karamura, 1998).

2.2 Morphology and Biology of Banana Plant

A banana is a monocotyledonous herbaceous perennial plant that grows to a height of 2 -8 m depending on cultivars and environmental conditions (Swennen and Ortiz, 1997). The aerial shoot is called a pseudostem, which consists of large overlapping leaf bases which are tightly rolled round each other forming a cylindrical structure of about 48 cm in diameter (Sharrock *et al.*, 2001). Normally, banana plant produces 35 to 50 leaves (this means it has about 35- 50 axillary buds) in its growth cycle and when the banana plant has formed an average of 40 leaves (within 8 to 18 months), the terminal bud of the corm develops directly into the inflorescence which emerges at the top in the centre of the leaf cluster (Blomme and Ortiz, 2000).

A banana plant comprises of suckers, which are the lateral shoots produced from the underground stem (corm) which can form roots and become a separate individual plant (mainly used in vegetative propagation) (Simmonds, 1966). The sucker development includes three distinct stages which are peeper (young sucker bearing scale leaves only), sword sucker (bearing narrow sword leaves) and maiden sucker (large but not fruiting yet) (Swennen *et al.*, 1984). Unfortunately, these stages are highly influenced by the growth hormone produced by the mother plant (Swennen *et al.*, 1984), thus suckers of plantains remain small until the time of flowering of the mother plant due to the weak demand sink for assimilation which is caused by apical dominance.

The release of endogenous growth hormone (auxin and cytokinins) normally depends on the genomic composition of the banana cultivar and thus influences differential proliferation ability. For instance, banana with AAA genome composition (example, East African highland bananas, Cavendish banana and Gross Michel) have low natural proliferation ability due to their strong apical dominance (Baiyeri and Aba, 2005). Several

researchers reported that the exogenous application of auxin and cytokinins in banana tissue culture is known to reduce the apical dominance and induce both axillary and adventitious shoot formation (Madhulatha *et al.*, 2004).

A corm is an underground stem consisting of roots and stem which bears nodes, internodes, axillary buds and apical meristem (Fig. 1). Aerial shoots or suckers arise from the lateral buds while leaves and flowers arise from apical meristem. The continuous vegetative growth of suckers perpetuates the corm's life and hence the perennial status of bananas (Blomme and Ortiz, 2000).

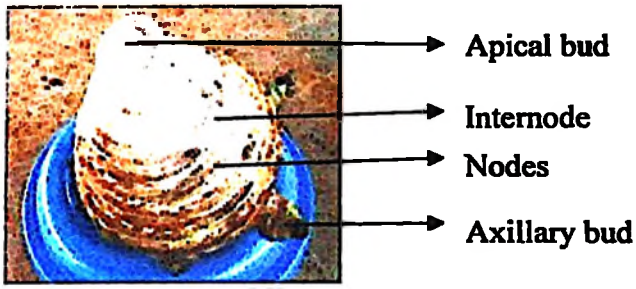


Figure 1: A banana corm (Njukwe, 2005)

The roots are initiated from the corm and range from 50 to 100 cm and rarelysub-horizontal roots reach 300 cm (Blomme, 2000). The roots serve to anchor the plant, store food products, and absorb water and nutrients, conduct water and nutrients to the rest of the plant parts (Walker *et al.*, 2003). Moreover, banana roots are the sources of plant growth regulators that facilitate lateral shoot growth (De Langhe *et al.*, 1983; Stover and Simmonds, 1987).

In banana, the roots emerge from the corm either at adjacent or within the internodes commonly formed either individually or in groups of up to four roots (Belalcázar, 1991). According to Belalcáza *et al.* (1990) banana root system consists primary, secondary and

tertiary roots and root hairs. The secondary roots branch off the primary roots which are consistently thick and are not exceeding 1 cm in diameter. The tertiary roots branch off the secondary roots. The primary, secondary and tertiary roots are short and thick. At the end of the tertiary roots are root hairs with roles of absorbing water and nutrients. Root hairs grow to a few millimeters and their capacity is direct proportional to its range.

Generally, root colour depends on the age and stage of the plant growth may vary from creamy white to yellowish brown though becoming darker at the time of maturity. Young roots are protected with a root cap at the tip to maintain their flexibility. Several researches observed that the functional life of primary roots may reach 5 to 8 months while functional roots may still be recorded at a time of bunch harvest, at 18 months after planting and about 11 months after the root differentiation process has ended (Kwa, 1998).

There are parameters which influence root growth and development in plants such as edaphic (soil temperature), environmental (light intensity), genetic compositions (genotypes) and biological factors (enzymes and growth regulators) as reported by Blomme *et al.* (2006). Several researches were done on the factors affecting root growth and development. For instance Irizarry *et al.* (1981) reported the effect of soil type on root system development and Blomme *et al.* (2006) also reported the root traits of different *Musa* genotypes that are influenced by the agro-ecological environment. Moreover, the manipulation of root growth and development can be achieved by application of different types of exogenous auxin such as indole-3-butyric acid, indole-3- acetic acid and α-naphthalene acetic acid. These auxins reported by several researchers differ in their efficacy on root induction.

2.3 Banana Propagation Methods

2.3.1 Sexual propagation

This is a method of propagation where seeds are used for multiplication purpose. Sexual propagation is common in wild banana species which are diploid and undergo normal meiosis, fertilization and seed set. The extent of seed set, germinability and dormancy depends on the species. In *Ensete* (the genus of the family *Musaceae*) seed propagation is the only means of multiplication since the genus cannot produce suckers.

2.3.2 Asexual propagation

This is the process of using a part of an existing plant (stem, cutting, suckers or other tissues) to produce a new plant under *in situ*, *in vivo* or *in vitro* condition (Arinaitwee*t al.*, 2000; Dayarani *et al.*, 2013; Singh *et al.*, 2011). All edible banana cultivars are seedless, or set sterile seeds, and thus vegetative propagation is the only natural means of their perpetuation (Cronauer and Krikorian, 1984a; Arias, 1992).

2.3.2.1 Traditional propagation of cultivated banana

Banana is traditionally propagated preferably by using sword, water, maiden suckers and peepers (Fig. 2) obtained from field-grown plants (Morton, 1987).

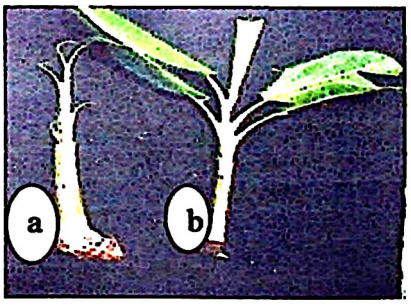


Figure 2: Sword (a) and water sucker (b)
Source: Singh *et al.* (2011)

This method is a common limiting factor for large scale production of bananas and plantains or expansion of existing plantation due to the difficulties in obtaining adequate planting materials (Baiyeri and Ajayi. 2000). The method is very slow on emitting suckers due to hormone-mediated apical dominance of the mother plant (Singh *et al.*, 2011). especially for the plantain, which have low suckering ability than other cultivars. The phenomenon of apical dominance is controlled by the growth hormone (auxins) released by the terminal bud which inhibits the growth of the lateral buds (De Langhe *et al.*, 1983). According to Swennen *et al.*, (1984) the apical dominance is strongly related with suckering ability of the banana plants thus it affects sucker development.

Small scale farmers rely on the natural regeneration of existing banana plants to obtain banana planting materials (Kasyoka, 2013). These suckers are good source of pests and diseases, thus reduce banana productivity and increase the production cost through the need of pest control measures. Moreover, transplanting contaminated planting materials facilitates persistence and spread of pests and diseases to new farms thus shortening the lifespan of plantations (Coyne *et al.*, 2005).

2.3.2.2 *In vitro* propagation

Micro-propagation or *in vitro* multiplication is a modern technique of rapidly multiplying plant materials under aseptic conditions (Signh *et al.*, 2011). Normally the process involves growing explants in an aseptic growth media under controlled environment. Special containers specifically petri - dishes, test tubes or any other suitable containers are used. The growth media consists of macro and micronutrients, vitamins, sugar and plant growth hormones. Depending on the plant species, genetic nature and the contents of the growth media various forms of callus/embryos/shoots/ roots or direct plantlets can be induced (Siamak *et al.*, 2010).

This technique facilitates the production of pest-free and large number of planting materials throughout the year within a short period of time (Msogoya *et al.*, 2006). Smith (1991) reported that tissue culture provides advantages over traditional propagation method such as physiological uniformity of the young plants, easy for rapid dissemination of new planting materials throughout the world, early growing stages and faster growth, early maturity which leads to short harvesting interval compared to conventional materials. Moreover, tissue culture plays a fundamental role in the distribution of germplasm, conservation, safe exchange of planting material and rapid propagation of newly selected hybrid cultivars (Arias, 1992).

Regardless of its advantages, micro-propagation technology is more expensive than the conventional methods of plant propagation due to the fact that it is more sophisticated, skill-intensive and requires high investment (Vuylsteke and Talengera, 1998). Moreover, clonal production of banana planting materials is highly affected by somaclonal variation which is mutation induced by *in vitro* conditions (Msogoya and Grout, 2008). Consequently, the unit cost of the *in vitro* planting material is usually higher and often unaffordable by small-scale farmers (Mwangi and Muthoni, 2008).

2.3.2.3 *In situ* propagation

This is a technology of banana multiplication which involves the whole suckers, large pieces of parent corms or sword suckers. The repression of apical dominance to stimulate lateral buds development and increased suckering ability is achieved through decapitation of a sucker while still in the field (*in-situ*) (Dayarani *et al.*, 2013). Four to seven suckers can be produced in three to four weeks after the removal of apical dominance (Njukwe *et al.*, 2005). The suckers are separated from the mother corm and then rooted in sterile soil medium under irregular misting (Singh *et al.*, 2011). According to Kwa (2006) *in situ*

propagation method is slow due to the fact that it takes six to twelve months to obtain enough planting materials. On the other hand, many buds formed on the mother plant in this case remain unexploited for a long period.

2.3.2.4 False decapitation (decortication)

False decapitation involves a complete destruction of apical meristem by making a small window to view the meristem (Bonte *et al.*, 1995). Therefore, the mother plant continues it photosynthetic and metabolic processes while the energy produced can be used for the production of new suckers (Njukwe *et al.*, 2005). This method results in the production of nine to fifteen banana shoots per plant in a short period (Singh *et al.*, 2011).

2.3.2.5 *In vivo* macro-propagation

Usually suckers contain axillary buds with a number of buds depending on the sucker age. These buds cannot sprout under both *ex situ* and *in situ* conditions for they are strongly inhibited by the apical bud or meristem (Baiyeri and Ajayi, 2000). Macro-propagation is a technique in which these axillary buds are induced to sprout under *in vivo* conditions. Briefly, the technique involves decapitation of the detached maiden (four to six month old) suckers to remain with the corm, disinfection and desheathing of the corm as well as wounding of the exposed axillary buds and apical meristem to stimulate lateral bud sprouting under *in vivo* conditions (Singh *et al.*, 2011).

The *in vivo* macro propagation allows rapid production of large quantities of planting material of highly acceptable quality in nursery conditions. The prepared corms are then planted in sawdust growth media for regeneration. The sawdust used as the initiation media serves to provide plant anchorage, moisture and root aeration (Baiyeri and Aba, 2005). This method is simple and results in production of high quality planting materials

and has the potential of producing 50-60 shoots per corm within 4 -5 months (Baiyeri and Aba. 2004). The method is also cheap and affordable even by small-scale farmers (Kwa. 2003). According to Kwa (2006) the method can spread diseases when infected corms are used without disinfection. Njukwe *et al.* (2005) also reported that macro propagation does not result in mass production of planting materials in comparison with *in vitro* multiplication.

The prepared corms can either be incubated in cytokinin solution to stimulate further the proliferation of axillary buds or can be planted without being treated with cytokinin. Application of 0.4 mg/l BAP in *in vivo* multiplication of ornamental banana (*Musa laterita*) resulted into high number of buds and shoot regeneration (Dayarani *et al.*, 2013). Crouch *et al.* (1998) reported that BAP concentration ranging from 2.0 to /4.9 mg/l is recommended for *in vitro* propagation of *Musa* spp (Crouch *et al.*, 1998). Thidiazuron (TDZ) is a synthetic plant growth regulator which is characterized by cytokinins and auxin effects in plant growth (Guo *et al.*, 2011). Huetteman and Preece. (1993) stated that TDZ has ability of increasing the biosynthesis of endogenous adenine-based cytokinin, which implies that it can persist for a long time in plant tissues (Murch and Saxena. 2001). This makes TDZ an effective cytokinin for stimulation of *in vivo* shoot bud proliferation when banana corms are dipped in a solution for short period. Moreover. TDZ can enhance high proliferation at a very low concentration in woody plant species which exhibit low proliferation on BAP supplemented medium. Due to the ability of increasing the biosynthesis of endogenous cytokinins, thidiazuron becomes effective cytokinin for stimulation of shoot bud proliferation in recalcitrant banana genotypes (Youmbi *et al.*, 2006).

Auxins are growth regulator or similar substances that promote and regulate the growth and development of plants. They are produced in the meristem of shoot tips and move down the plant to cause various growth effects. Auxins are also essential for root induction in plants as reported by Ngomuo *et al.* (2013) and commonly for root induction in stem cuttings and *in vitro* derived banana suckers. There are several types of auxin namely indole -3- aceti cacid (IAA), indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA). Indole-3-butyric acid reported in different plant species is the best for root induction. According to Lindiro *et al.* (2013) indole -3-butyric acid at concentration 2.0 mg/l showed the best results for regenerating roots in pyrethrum *in vitro* derived shoots than α-naphthalene acetic acid (NAA) at concentrations of 2.0 mg/l. Moreover, (Gübbük and Pekmezci, 2004) reported that in *in vitro* shoot and root elongation is enhanced by supplementing 0.19 mg/l IAA in basal medium containing different TDZ concentration.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Planting Materials and Corm Preparation

Three banana cultivars were used in this study namely "Mtwike" (Grande naine - Cavendish), "Mzuzu" (French plantain) and Bukoba (East African Highland banana) (Table 1). These cultivars were collected from Kauzeni village located 12 km West from Sokoine University in Morogoro region.

Table 1: Description of banana cultivars used in in vivo multiplication

Local name	Referrence name	Subgroup	Genomic group	Use
Bukoba	East African Highland	Matoke	AAA	Cooking
Mzuzu	French plantain	Plantain	AAB	Roasting
Mtwike	Grande naine	Cavendish	AAA	Dessert banana

Young and healthy banana suckers of about 4 - 6 months old of each cultivar were collected from different farmers. About 50 suckers for each cultivar were collected but only 45 corms were used for experiment in each cultivar. The induction of axillary bud sprouting was conducted by using Thidiazuron (TDZ). Three propagators each of 3.0 m x 1.0 m x 0.5 m dimension were constructed by using soft timber. The sawdust used as the initiation and growth media was sourced from a saw mill in Morogoro town.

3.2 Methods

3.2.1 Experiment one

Assessment of the effect of TDZ concentrations on in vivo proliferation of different banana cultivars.

(a) Experiment design

The experiment was set as a split plot in a randomized complete block design (RCBD) with three replications. Replications consisted of five corms of each cultivar. Three banana cultivars (Mtwike, Mzuzu and Bukoba) were the main plot factor while the four TDZ concentrations (0.5, 1.0, 2.0 and 3.0 g/l) were the sub- plot factor.

(b) Materials

Propagator, initiation medium (sawdust), banana suckers polythene paper and TDZ hormone.

(c) Methodology

Sawdust as the initiation medium was sterilized by dipping a bag of 70 kilograms of the material in a drum with 40 litre of boiling water at 100 0c for 45 minutes. The sterilized saw dust was then poured in a propagator for cooling (Fig. 3). The suckers were headed back about 30 cm and corm sterilized by dipping them into hot water at 100 0c for 15 seconds before being deshealthed to expose the axillary buds (Fig. 4). The sterilized corms were deshealthed and the exposed buds were wounded by cutting them transversely to stimulate their sprouting (Fig. 5). Moreover, the apical meristem of each corm was destroyed by decortication to overcome the apical dominance. These corms were there after soaked for 12 hours into each TDZ concentration.



Figure 3: Propagator filled with sterilized sawdust



Figure 4: Sterilization of banana corms



Figure 5: Deshelthed corms ready for soacking in TDZ solution

The treated corms were planted into the moistened saw dust in the propagators. The propagators were placed under a plastic tunnel. After the removal of the corms, the remaining 25 litres of TDZ solution for each concentration were poured into 3 m2 of the planted area in the propagator to further stimulate sprouting of the corms. Management such as irrigation was carried out twice per week. After two months, fertilizer application was done by supplying poly-feed starter (contains N-P-K and micro elements) at a concentration of 0.5 g/L of water. The fertilizer solution was supplied all over the medium at a rate of 20 litres per 9 m2.

(d) Data collection (Experiment 1)

The following data were collected daily by counting:

(i) Number of days to first shoot emergence from the date of planting

(ii) Number of sprouted buds per corm

(iii) Number of shoots per bud

(iv) Number of shoots per corm

(v) Total survived shoots per corm

(vi) Shoot size:

a. Shoot height from root collar up to the base of the cigar leaf measured using vernier caliper (0-150mmx0.05/6"x1/28", Japan).

b. Shoot collar diameter measured by vernier caliper (0-150mmx0.05/6"x1/28", Japan) at the root collar.

c. Number of leaves per shoot

3.2.2 Experiment two

Determination of the effect of indole-3-butyric acid (IBA) on rooting and growth of TDZ induced in vivo banana suckers.

(a) Experiment design

The experiment was set as a split plot in a randomized complete block design (RCBD) with three replications. A replication consisted of eight suckers of each cultivar. Three banana cultivars (Mtwike, Mzuzu and Bukoba) were the main plot factor and the rooting hormone IBA at concentration of 0 and 0.6% was the subplot-factor.

(b) Materials

Sprouted banana shoots from experiment 1 above. Black polythene tubes, forest soil, organic manure, rice husks and indole-3- butyric acid (IBA) were sourced locally.

(c) Methodology

The black polythene tubes of 19 cm diameter and 30 cm height were sealed at the bottom using a sealing machine. Four holes of about 3 cm size were made at the bottom of black polythene tubes to facilitate drainage of excess water during irrigation. Forest soil was mixed with farmyard manure and rice husks at a ratio of 1:0.5:0.25 by volume basis, filled in the polythene tubes and then moistened before planting the suckers.

The suckers were detached from their corms and their sized was determined by measuring their height and collar diameter and by counting their number of leaves. Eighteen suckers

were dipped into IBA powder at 0.6% before planting into polythene tubes while the rest 18 suckers were planted without dipping in IBA powder as a control. The sucker leaves were pruned to minimize transpiration prior to transplanting. Managements such as irrigation and weeding were carried out to facilitate plant growth.

(d) Data collection

After two months from sprouting 12 suckers for each treatment were selected and removed from the polythene tubes for each cultivar. The sucker roots were washed with tape water to remove soil remains. The following data were collected:

(i) Sucker collar diameter measured by using vernier caliper (0-150mmx0.05/6''x1/28'', Japan) from root collar up to the base of the cigar leaf.

(ii) Number of explorer roots per sucker

(iii) Sucker height measured by using vernier caliper (0-150mmx0.05/6''x1/28'', Japan) from root collar up to the base of the cigar leaf.

(iv) Number of leaves per sucker

(v) Root fresh weight (g)

(vi) Sucker fresh weight (g)

(vii) Sucker and root dry weights: Fresh roots and shoots were kept in envelopes and dried until constant weight in the oven at 70 0C for seven days (Srinivasan and Vijayalakshmi, 2011).

3.3 Data Analysis

The collected data in both experiment were subjected to Analysis of Variance (ANOVA) using GenStat Statistical Programme 14th Edition (GenStat, 2011). Mean separation was done based on Student-Newman-Keuls (SNK) at $p < 5\%$.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Thidiazuron on *in vivo* Multiplication and Sucker Growth of Selected Local Banana Cultivars

4.1.1 Effect of thidiazuron concentration on *in vivo* multiplication of different banana cultivars

Thidiazuron concentration had a highly significant ($p < 0.01$) effect on the number of sprouted buds per corm, number of shoots per bud and number of shoots per corm (Table 2). Thidiazuron concentration at 0.05 g/litre resulted in sprouting of 1.7 buds per corm followed by TDZ concentration at 1.0, 2.0 and 3.0 mg/l with 2.6, 2.9 and 2.5 sprouted buds per corm, respectively. Similarly, TDZ at 2.0 g/l resulted in production of 2.0 shoots per bud whereas corms treated with TDZ at 0.5, 1.0 and 3.0 mg/l produced 1.2, 1.5 and 1.8 shoots per bud, respectively. The concentration of TDZ at 0.5 mg/l resulted in lower production of shoots per 1.9 corm while corms treated with TDZ at 1.0, 2.0 and 3.0 mg/l produced 4.0, 6.3 and 4.6 shoots per corm, respectively. Conversely, TDZ concentrations had no significant ($P < 0.05$) effect on the number of days to first shoot emergence and shoot survival.

Table 2:: Effect of TDZ concentration on *in vivo* multiplication of different banana cultivars

TDZ conc. (mg/l)	Days to 1st shoot sprouting	No. of sprouted buds/corm	No. of shoots per bud	No. of shoots per corm	Total survived shoots/corm
0.5	29.4	1.7^{a}	1.2^{a}	1.9^{a}	1.5
1.0	25.0	2.6^{b}	1.5^{ab}	4.0^{b}	2.8
2.0	28.8	2.9^{b}	2.0^{b}	6.3^{c}	3.3
3.0	28.5	2.5^{ab}	1.8^{ab}	4.6^{bc}	3.4
LSD (5%)	3.7	0.6	0.6	1.5	1.8
F-test (5%)	ns	**	*	***	ns
S.E	3.8	0.6	0.58	1.5	1.8
CV (%)	13.9	26.4	34.9	35.5	29.4

Means followed by the same letters(s) within the column are not significant different at probability of 5 % level based on Student-Newman-Keul

ns = not significant, * = significant ($p < 0.05$), ** = highly significant ($p < 0.01$) and *** = Very highly significant at ($p < 0.001$)

4.2 Effect of Thidiazuron on Size of Shoots Derived from *in vivo* Banana Multiplication

Thidiazuron concentration had a significant ($p < 0.05$) effect on the sucker diameter and number of leaves per sucker (Table. 3). Corms treated with TDZ at 0.5 mg/l produced bigger suckers with diameter of 2.2 cm followed by corms treated with TDZ at 1.0, 2.0 and 3.0 mg/l with shoot diameter of 1.7, 1.6 abd 1.6 cm, respectively. Moreover, corms treated with TDZ at 0.5 mg/l produced bigger suckers with 3.3 leaves per sucker followed by corms treated with TDZ at 1.0, 2.0 and 3.0 mg/l with 4.5, 4.9 and 4.3 leaves per sucker, respectively.

Table 3: Effect of TDZ concentration on size of shoots derived from *in vivo* multiplication

TDZ conc. (mg/l)	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
0.5	15.2	2.2^{b}	3.3^{a}
1.0	15.5	1.7^{ab}	4.5^{b}
2.0	15.1	1.6^{a}	4.9^{b}
3.0	15.5	1.6^{a}	4.3^{ab}
LSD (5%)	2.9	0.4	0.84
F-test (5%)	ns	*	**
Se	3	0.4	0.86
CV (%)	19.8	22.8	20.2

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 at ($p < 0.01$). ***= Very highly significant at ($p < 0.001$)

4.2.1 Effect of banana cultivars on *in vivo* multiplication

4.2.1.1 Effect of cultivar on shoot in vivo proliferation

Banana cultivars had a significant ($P < 0.05$) effect on the number of sprouted buds per corm, number of shoots per bud and number of shoots per corm (Table. 4). In banana cv. Bukoba, 3.2 buds sprouted per corm while 1.7 and 2.4 buds sprouted per corm in banana cv. Mtwike and Mzuzu, respectively. Banana cv. Bukoba also produced 2.0 shoots per bud while cv. Mtwike and cv. Mzuzu produced 1.4 and 1.6 shoots, respectively. Cultivar Bukoba produced a larger number of shoots of 6.4 per corm compared with banana cv.

Mtwike and Mzuzu with 2.3 and 2.9 shoots per corm, respectively. Conversely, banana cultivars had no a significant ($P < 0.05$) effect on the number of days to first shoot sprouting.

Table 4: Effect of banana cultivar on shoot *in vivo* proliferation

Cultivar	No. of days to 1st shoot sprouting	No. of sprouted buds per corm	No. of shoots per bud	Total no. of shoots per corm	Total survived shoots
Mtwike	25.0	1.7[a]	1.4[a]	2.3[a]	2.1
Mzuzu	27.2	2.4[ab]	1.6[ab]	2.9[a]	2.6
Bukoba	23.2	3.2[b]	2.0[b]	6.4[b]	3.6
LSD (5%)	5.4	0.6	0.46	1.67	1.86
F-test (5%)	ns	**	*	**	ns
S.E	2.4	0.3	0.5	0.7	0.8
CV (%)	8.6	11.5	12.2	17.5	29.4

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

4.2.1.2 Effect of cultivars on size of in vivo multiplication derived suckers

Banana cultivar had no significant ($P<0.05$) effect on shoot height, shoot diameter and number of leaves per sucker (Table 5).

Table 5: Effect of cultivar on size of shoots derived from *in vivo* multiplication

Cultivar	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
Mtwike	14.11	1.71	4.05
Mzuzu	15.98	1.75	4.18
Bukoba	16.04	1.88	4.52
L.S.D (5%)	2.4	0.4	0.7
F-test (5%)	ns	ns	ns
S.E	3.0	0.18	0.34
CV(%)	6.9	10.3	7.9

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 at (($p < 0.05$). **= Highly significant at ($p < 0.01$) and ***= Very highly significant at ($p < 0.001$)

4.2.1.3 Effect of interaction of cultivar and thidiazuron on in vivo multiplication of selected local banana cultivars

Banana cv. Mzuzu - TDZ interaction had a significant ($p< 0.05$) effect on the number of shoots per corm and number of survived shoots (Table 6). Banana cv. Mzuzu produced the largest number of shoots of 10.4 per corm in TZD at 2.0 mg/l. However, cv. Mtwike produced the smallest number of shoots per corm of 1.3 shoots in TDZ at 0.5 mg/l. Similarly, cv. Mzuzu had higher number of survived shoots of 5.1 shoots per corm in TDZ at 2.0 mg/l while cv. Bukoba had 3.5 survived shoots at TDZ of 3.0 mg/l and cv. Mtwike was the lowest in number of survived shoots of 2.5 at TDZ of 3.0 mg/l. Conversely, the interaction of banana cultivars and TDZ concentrations had no significant ($p< 0.05$) effect on the number of days to first shoot sprouting, number of buds per corm, number of shoots per bud, shoot height, shoot diameter and number of leaves per shoot.

Table 6: Interaction effect of banana cultivar and thidiazuron concentrations on *in vivo* proliferation and sucker growth

Cultivar-TDZ conc. Interaction mg/l	No. of days of 1st sucker sprouting	No of buds per corm	No. of suckers per bud	No. of suckers per corm	No. of survived suckers	Sucker height (cm)	Sucker diameter (cm)	No. of leaves per sucker
Bukoba x 0.5	24.9	1.2	1.6	1.9^{cd}	2.6^{ab}	16.2	2.2	3.6
Bukoba x 1.0	25.0	2.5	1.2	3.2^{cd}	1.4^{b}	15.3	1.5	4.6
Bukoba x 2.0	26.6	2.7	1.7	5.2^{bc}	3.4^{ab}	15.1	1.8	5.0
Bukoba x 3.0	24.4	2.8	1.8	5.3^{bc}	3.5^{ab}	14.1	1.6	4.0
Mtwike x 0.5	33.7	1.3	1.0	1.3^{d}	1.4^{b}	11.7	2.0	3.4
Mtwike x 1.0	26.6	1.5	1.0	1.6^{d}	1.7^{b}	15.8	1.9	4.4
Mtwike x 2.0	32.1	1.9	1.5	3.1^{cd}	2.4^{ab}	14.0	1.3	4.6
Mtwike x 3.0	32.3	2.0	2.0	3.2^{cd}	2.5^{ab}	14.2	1.7	4.4
Mzuzu x 0.5	29.4	2.3	1.2	2.7^{cd}	1.4^{b}	16.6	2.1	3.3
Mzuzu x 1.0	23.6	3.6	2.1	7.2^{b}	4.7^{a}	15.3	1.7	4.2
Mzuzu x 2.0	27.1	3.9	2.9	10.5^{a}	5.1^{a}	13.6	1.6	4.2
Mzuzu x 3.0	28.1	2.5	1.9	5.3^{bc}	3.7^{ab}	16.0	1.6	4.3
LSD (5%)	7.1	0.9	0.8	2.5	3.4	5.3	0.7	1.5
F-test (5%)	ns	ns	ns	*	*	ns	ns	ns
S.E	2.4	0.3	0.3	0.8	1.2	1.8	0.2	0.5
CV (%)	14.3	23.6	31.9	35.5	36.2	22.2	23.5	22.8

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 at ($p < 0.01$) and ***= Very highly significant at ($p < 0.001$).

4.2.2 Effect of cultivar and indole -3 - butyric acid (IBA) on rooting and growth of in vivo derived banana suckers

4.2.2.1 Effect of cultivar on rooting of in vivo macropropagation derived banana suckers

Banana cultivars had a very highly significant ($p < 0.001$) effect on the number of roots per sucker, root fresh weight, root dry weight, sucker height, sucker diameter, number of leaves per sucker, sucker fresh weight and sucker dry weight (Table 7). Banana cv. Bukoba produced the tallest shoots with height of 38.7 cm followed by cv. Mtwike and cv. Mzuzu with sucker heights of 30.4 and 28.6 cm, respectively. However, suckers produced by cv. Mtwike were bigger in diameter with 3.5 cm followed by suckers of cv. Bukoba and cv. Mzuzu with diameters of 3.0 and 2.8 cm, respectively. Cultivar Mtwike also produced a larger number of leaves and roots per sucker of 7.2 leaves and 19.7 roots, respectively followed by cv. Bukoba with 6.4 leaves and 13.6 roots per sucker and cv. Mzuzu with the number of leaves and roots per sucker of 5.0 leaves and 10.2 roots, respectively

Cultivar Mtwike and Bukoba produced higher root fresh weight of 61.5 g and 60.9 g per sucker, respectively while cv. Mzuzu produced root fresh weight of 38.6 g per sucker. Cultivar Bukoba and Mtwike also produced higher root dry weight of 5.4 g and 5.3 g per sucker, respectively while cv. Mzuzu produced lower root dry weight of 4.0 g per sucker. Cultivar Mtwike had higher shoot fresh weight of 170.0 g compared with cv. Bukoba and cv. Mzuzu with fresh weight of 146.9 g and 145.9 g, per sucker respectively. Similarly, cv. Mtwike produced higher dry weight of 65.9 g per sucker compared with cv. Mzuzu and Bukoba cultivar with dry weight of 61.3 g and 60.0 g per sucker, respectively.

Table 7: Effect of cultivar on rooting of *in vivo* derived banana suckers

Cultivar	Number of roots per sucker	Root fresh weight (g)	Root dry weight (g)	Sucker height (cm)	Sucker diameter (cm)	Number of leaves per sucker	Sucker fresh weight (g)	Sucker dry weight (g)
Mtwike	19.7[a]	61.5[a]	5.3[a]	30.4[b]	3.5[a]	7.2[a]	170.0[a]	65.9[a]
Mzuzu	10.2[c]	38.6[b]	4.0[b]	28.6[b]	2.8[c]	5.0[c]	145.9[b]	61.3[b]
Bukoba	13.6[b]	60.9[a]	5.4[a]	38.7[a]	3.0[b]	6.4[b]	146.9[b]	60.0[b]
F- test 5%	***	***	***	***	***	***	***	***
LSD 5%	1.2	6.6	0.6	1.8	0.2	0.3	11.9	3.1
CV (%)	25.4	37.5	39.1	17.2	19.8	15.5	23.6	15.5

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

4.2.2.2 Effect of IBA on rooting and growth of in vivo derived banana suckers

Indole -3-butyric acid concentration had a significant ($p < 0.05$) effect on shoot length, shoot diameter, number of roots per shoot and shoot dry weight (Table 8). Indole -3-butyric acid untreated shoots produced taller suckers with height of 33.6 cm while treatment of suckers with IBA at 0.6 % resulted in shorter suckers with height of 31.6 cm. Similarly, treatment of IBA at 0.6 % resulted in smaller number of roots of 13.8 roots per sucker while untreated suckers produced larger number of roots of 15.3 roots per suckers.

Moreover, shoots treated with IBA at 0.6% had lower shoot dry weight of 61.0 g per sucker while untreated resulted in producing suckers with dry weight of 63.8 g per sucker. Suckers treated with IBA at 0.6% resulted in smaller suckers with collar diameter of 3.0 cm while untreated suckers had collar diameter of 3.3 cm. Conversely, IBA at 0.6 % had no significant ($p < 0.05$) effect on the number of leaves per shoot, root fresh weight, root dry weight and sucker fresh weight.

Table 8: Effect of IBA on rooting and growth of *in vivo* derived banana suckers

Conc.IBA (%)	Number of roots per sucker	Root fresh weight (g)	Root dry weight (g)	Sucker height (cm)	Sucker diameter (cm)	Number of leaves per sucker	Sucker fresh weight (g)	Sucker dry weight (g)
0.0	15.3[a]	56.6	4.9	33.6[a]	3.3[a]	6.2	155.3	63.8[a]
0.6	13.8[b]	50.8	5.0	31.6[b]	3.0[b]	6.3	153.2	61.0[b]
F-test (5%)	*	ns	ns	*	**	ns	ns	*
L.S.D (5%)	1.4	6.0	0.5	1.9	0.1	0.3	10.1	2.6
CV (%)	36.9	42.1	41.2	21.7	21.2	21.1	24.6	15.8

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

ns = not significant and * = significant ($p < 0.05$) and ** = highly significant at ($p < 0.01$) and ***= Very highly significant at ($p < 0.001$).

4.2.2.3 Effect of cultivar and IBA interaction on rooting and growth of in vivo derived banana suckers

Indole-3-butyric acid had a very highly significant ($P < 0.001$) effect on rooting of cv. Mzuzu and Bukoba but insignificant effect on rooting of cv. Mtwike (Table 9). For instance, IBA treated suckers of cv. Mzuzu produced 11.1 roots with fresh weight of 47.9 g per sucker while untreated suckers of the same cultivar produced 9.3 roots with fresh weight of 29.3 g per sucker. Conversely, IBA treated suckers of cultivar Bukoba produced 15.6 roots with fresh weight of 76.0 g per suckers whereas IBA treated suckers produced 11.6 roots with fresh weight of 45.9 g per sucker. Furthermore, IBA treated suckers of cv. Mzuzu and cv. Bukoba produced roots with dry weight of 4.9 g and 6.1g per sucker, respectively while the untreated suckers of the same cultivars produced root dry weight of 3.1 g and 4.7 g per sucker, respectively.

Indole-3-butyric acid had very highly significant effect on sucker length and diameter of cv. Mtwike, Mzuzu and Bukoba.Suckers of cv. Mtwike, Mzuzu and Bukoba which were treated with IBA, produced suckers with length of 29.3, 33.3 and 38.1 cm with diameter of 3.3, 3.1 and 3.3 cm per sucker, respectively while the untreated suckers of the same cultivars produced suckers with length of 31.5, 24.0 and 39.3 cm and collar diameter of 3.6 cm, 2.5 cm and 2.8 cm per sucker, respectively.

Moreover, IBA treatment had very highly significant ($p < 0.001$) effect on the number of leaves per sucker of cv. Mtwike but insignificant effect on cv. Mzuzu and Bukoba (Table 9). Indole-3-butyric acid treated suckers of cv. Mtwike produced suckers with 6.8 leaves per sucker while the untreated suckers produced 7.6 leaves per sucker. However, IBA had no significant ($p< 0.05$) effect on sucker fresh weight and dry weight of cv. Mtwike, Mzuzu and Bukoba.

Table 9: Effect of cultivar X IBA interaction on rooting and growth of *in vivo* derived banana suckers

Cultivar X IBA conc.	No. of roots/sucker	Root fresh wt (g)	Root dry wt (g)	Sucker height (cm)	Sucker diameter (cm)	No. of leaves/sucker	Sucker fresh wt (g)	Sucker dry wt (g)
MTK X 1	19.0^{a}	64.4^{b}	5.4^{ab}	29.3b	3.3^{b}	6.8^{b}	167.5	65.5
MTK X2	20.5^{a}	58.5^{b}	5.2^{ab}	31.5^{bc}	3.6^{a}	7.6^{a}	172.4	66.2
MZ X 1	11.17^{c}	47.93^{c}	4.9^{b}	33.3^{c}	3.1^{b}	5.1^{c}	143.1	64.3
MZ X 2	9.33^{d}	29.30^{d}	3.1^{c}	24.0^{a}	2.5^{d}	5.0^{c}	148.7	58.4
BK X 1	15.6^{b}	76.0^{a}	6.1^{a}	38.1^{d}	3.3^{b}	6.5^{b}	155.3	61.6
BK X 2	11.6^{c}	45.9^{c}	4.7^{b}	39.3^{a}	2.8^{c}	6.3^{b}	138.4	58.4
F-test (5%)	***	***	***	***	***	***	ns	ns
LSD	1.6	8	0.8	2.2	0.2	0.4	16.8	4.4
CV (5%)	23.8	32.2	36.9	15	18	15	23.5	15.3

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

ns = not significant and * = significant ($p < 0.05$) and ** = highly significant at ($p < 0.01$) and ***= very highly significant at ($p < 0.001$). 1 = suckers treated with IBA at 0.6 % and 2 = suckers untreated with IBA

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effects of TDZ on Banana *in vivo* Shoot Proliferation and Growth

Thidiazuron concentration at 2.0 mg/l resulted in the highest shoot proliferation of plantain cv. "Mzuzu" followed by TDZ at 3.0 mg/l. This result is in line with the findings by Arinaitwe *et al.* (1999) who reported the highest *in vitro* proliferation of 9.5 shoots per explant when banana cv. Ndiziwemiti (ABB plantain) was treated with TDZ at 2.0 mg/l. Later, Arinaitwe *et al.* (2000) also reported the highest *in vitro* proliferation of 6.4 shoots per explant of banana cv. 'Bwara' (AAA Cavendish) at TDZ concentration of 0.25 mg/l. The differential response to TDZ concentrations of banana cv. Ndiziwemiti and cv. Bwara underscores the need to optimize the TDZ concentration for each banana cultivar. In the present study banana cv. Mzuzu exhibited the highest *in vivo* shoot proliferation at TDZ of 2.0 mg/l followed by cv. Bukoba and cv. Mtwike at TDZ of 2.0 and 3.0 mg/l. The highest *in vivo* shoot proliferation in banana cv. Mzuzu agrees with Kwa (2003) who reported the highest *in vivo* shoot productivity in plantain cultivars than Cavendish banana cultivars. The differential *in vivo* proliferation rates is probably due to the difference among cultivars in endogenous cytokinin levels and uptake rate. This is in line with Arinaitwe *et al.* (2000) who reported that *in vitro* proliferation rate depended on TDZ concentrations and genomic factors. Specifically, Blakesley (1991) reported that cultivars differed in cytokinin uptake, translocation to the meristematic region and degradation. For instance, Youmbi *et al.* (2006) reported increased *in vitro* proliferation rate of 18.7 shoots per explant of banana (AAA Gros Michel) at TDZ concentration of 0.08 mg/l. According to the same authors, banana cv. Fougamou (ABB) required TDZ concentration of 0.2 mg/l to produce the same number of shoots in *in vitro*.

In the present study TDZ at 1.0, 2.0 and 3.0 mg/l resulted in the highest growth of *in vivo* derived banana shoots based on the number of leaves per shoot. The results in shoot growth reported in this study disagree with previous reports in which the number of leaves per *in vitro* derived shoot increased with decreasing TDZ concentration. For instance, Youmbi *et al.* (2006) obtained the high number of leaves of 43 leaves per *in vitro* derived shoot of banana (AAA Gros Michel) at TDZ of 0.1 mg/l while banana cv. Pelpita (ABB), Fougomou (ABB), Dwarf Kalapua (ABB) and Topala (AAB) produced 19.7, 18.0, 6.7 and 2.0 leaves per sucker at the same TDZ concentration, respectively. The difference in the number of leaves between *in vivo* and *in vitro* derived banana shoots is possibly due to the fact that in the later the shoots are permanently incubated in TDZ solution while in the former the corms are temporarily dipped in TDZ and planted in sawdust without nutrients.

5.2 Effect Of IBA on Rooting and Growth of *in vivo* Macropropagation Derived Banana Suckers

Banana cv. Mtwike produced the largest number of roots followed by cv. Bukoba and Mzuzu. Similarly, cv. Mtwike and cv. Bukoba had higher root fresh and dry weight than cv. Mzuzu. The differential rooting and root growth among the three banana cultivars was possibly due to their genetic make-up. Swennen *et al.* (1986) also reported on the variability in root development amongst *Musa spp.* genotypes. According to Sebuwufu *et al.* (2004) roots of East African Highlands banana cv. Bukoba and cv. Kibuzi had the largest root system with the highest root dry weight while plantain cv. Mzuzu had the smallest root system with lower root dry weight. In the present study banana cv. Mtwike and cv. Bukoba also had larger sucker size based on plant height, collar diameter, number of leaves per sucker, sucker fresh and dry weights than cv. Mzuzu. The higher sucker growth in banana cv. Mtwike and Bukoba was possibly due to their larger root system. Sebuwufu *et al.* (2004) also reported significant positive correlation between shoot and

root growth traits in East Africa Highland banana cv. Kibuzi which exhibited the most vigorous shoot growth.

The results of the present study showed that IBA treatment at 0.06 % reduced the number of roots, sucker height and collar diameter of *in vivo* propagation-derived banana suckers. These results contradict with previous studies in which IBA promoted root formation and development in banana cv. Bari (Al-Amin *et al.*, 2009). Specifically, IBA at 0.8 - 1.0 mg/l increased *in vitro* root induction in banana (AAB) cv. Bari-1 (Molla *et al.*, 2004; Sultan *et al.*, 2011). The same authors reported a lower root fresh weight of the same cultivar at IBA ranging from 0.0 to 0.3 mg/l and a smaller number of leaves at IBA ranging from 0.4 to 0.6 mg/l. This descrepancy is probably due to the fact that *in vivo* derived suckers were initiated with TDZ which has both cytokinin and auxin effect. The presence of residual auxin in TDZ derived banana suckers could possibly be enough to enhance root formation.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

The first objective of this study was to determine the effect of temporary TDZ treatment combined with *in vivo* macropropagation on bud proliferation of selected banana cultivars. The results from this study have revealed that *in vivo* shoot multiplication and growth of banana cv. Mzuzu, Bukoba and Mtwike can be increased by dipping for 12 hours banana corms in TDZ at 2.0 and 3.0 mg/l.

The second objective of this study was to determine the effect of IBA at 0.6 % on rooting and growth of banana suckers derived TDZ combined with *in vivo* macro propagation. The results from the present study showed that transplanting TDZ derived banana suckers without dipping them in IBA promotes more root formation and sucker growth of banana cv. Bukoba, cv. Mtwike and cv. Mzuzu. This means that TDZ derived suckers of banana cv. Bukoba, Mtwike and Mzuzu must be transplanted without being dipped in rooting hormone.

6.2 Recommendations

The use of TDZ at 2.0 is recommended for enhancing *in vivo* macropropagation of banana cv. Mzuzu, cv. Bukoba and cv. Mtwike. Transplanting of TDZ derived suckers without dipping them in rooting hormone is further recommended for promoting root formation and sucker growth of banana cv. Mzuzu, cv. Bukoba and cv. Mtwike.

Further studies are required to:

i. Determine the alternative cytokine-based growth regulators for enhanced shoot productivity under *in vivo* multiplication of banana cv. Mtwike and cv. Bukoba.

ii. Determine the alternative auxin-based growth regulators for enhancing rooting and sucker growth of banana cv.Mzuzu. Mtwike and cv. Bukoba.

iii. Establish relationship between banana endogenous auxin levels and induced rooting/shoot proliferation for enhancing *in vivo* macropropagation of different banana cultivars.

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APPENDICES

Appendix 1: ANOVA tables for *in vivo* shoot proliferation variables

ANOVA Table of number of days for first sprouting shoot

Source of variation	d.f	s.s	m.s	v.r	F.pr
Replication	2	93.45	46.73	0.41	0.691
Cultivar	2	1251.74	625.87	5.43	0.072
Error (a)	4	460.82	115.21	1.52	
TDZ conc.	3	527.17	175.72	2.32	0.101
Error (b)	24	1817.29	75.72	1.59	
Cultivar*TDZ conc.	106	5051.85	47.66		
Total	141	8033.27			

ANOVA Table for number of buds per corm

Source of variation	d.f	s.s	m.s	v.r	F.pr
Replication	2	3.499	1.750	1.12	0.411
Cultivar	2	62.760	31.380	20.07	0.008
Error (a)	4	6.255	1.564	0.76	
TDZ conc.	3	37.239	12.413	6.02	0.003
Error (b)	24	49.450	2.060	1.02	
Cultivar*TDZ conc.	107	217.051	2.029		
Total	142	332.280			

ANOVA Table for number of shoots per bud

Source of variation	d.f	s.s	m.s	v.r	F.pr
Replication	2	4.4985	2.2493	2.75	0.177
Cultivar	2	13.8604	6.9302	8.48	0.036
Error (a)	4	3.2678	0.8170	0.49	
TDZ conc.	3	18.5910	6.1970	3.71	0.025
Error (b)	24	40.0854	1.6702	1.93	
Cultivar*TDZ conc.	108	93.5576	0.8663		
Total	143	152.3963			

ANOVA Table for number of shoots per corm

Source of variation	d.f	s.s	m.s	v.r	F.pr
Replication	2	30.41	15.20	1.40	0.347
Cultivar	2	507.08	253.54	23.28	0.006
Error (a)	4	43.57	10.89	0.62	
TDZ conc.	3	431.10	143.70	8.20	<.001
Error (b)	24	420.47	17.52	1.65	
Cultivar*TDZ conc.	108	1145.64	10.61		
Total	143	2149.27			

ANOVA Table for number of survived shoots.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	90.645	45.323	3.35	0.140
Cultivar	2	81.952	40.976	3.02	0.158
Error (a)	4	54.189	13.547	0.76	
TDZ conc	3	98.564	32.855	1.84	0.171
Error (b)	21	374.591	17.838	3.21	
Cultivar*conc.	97	538.907	5.556		
Total	129	1033.231			

Appendix 2: ANOVA table for in *vivo* sucker size for all variables

ANOVA Table for shoot length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr
Replication	2	134.51	67.25	2.98	0.161
Cultivar	2	144.00	72.00	3.19	0.149
Error (a)	4	90.35	22.59	0.49	
TDZ concentration	3	5.89	1.96	0.04	0.988
Error (b)	21	971.07	46.24	1.96	
Cultivar*concentration	97	2290.60	23.61		
Total	129	3089.42			

ANOVA Table for shoot collar diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	0.1704	0.0852	0.13	0.885
Cultivar	2	0.9967	0.4983	0.74	0.534
Error (a)	4	2.7085	0.6771	0.82	
TDZ concentration	3	10.1709	3.3903	4.10	0.020
Error (b)	21	17.3788	0.8276	1.77	
Cultivar*Concentration	97	45.2937	0.4669		
Total	129	64.0358			

ANOVA Table for number of leaves

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	1.034	0.517	0.23	0.804
Cultivar	2	6.985	3.493	1.55	0.317
Error (a)	4	8.988	2.247	0.61	
TDZ Concentration	3	69.537	23.179	6.25	0.003
Error (b)	21	77.855	3.707	3.41	
Cultivar*Concentration	97	105.610	1.089		
Total	129	176.273			

Appendix 3: ANOVA table for sucker rooting variables

ANOVA Table for number of roots.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	77.19	38.60	3.52	0.131
Cultivar	2	342.03	171.01	15.59	0.013
Error (a)	4	43.89	10.97	0.17	
IBA concentration	1	5.56	5.56	0.09	0.777
Error (b)	8	519.44	64.93	2.51	
Cultivar*concentration	54	1394.50	25.82		
Total	71	2382.61			

ANOVA Table for root fresh weight.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	872.4	436.2	0.80	0.511
Cultivar	2	1908.8	954.4	1.75	0.285
Error (a)	4	2187.1	546.8	0.36	
IBA concentration	1	1061.1	1061.1	0.70	0.428
Error (b)	8	12190.8	1523.9	5.0	
Cultivar*concentration	54	16302.8	301.9		
Total	71	34522.9			

ANOVA Table for root dry weight.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	12.521	6.260	1.28	0.372
Cultivar	2	6.543	3.271	0.67	0.561
Error (a)	4	19.547	4.887	0.45	
IBA concentration	1	4.157	4.157	0.38	0.554
Error (b)	8	87.274	10.909	3.54	
Cultivar*conc.	54	166.318	3.080		
Total	71	296.359			

Appendix 4: ANOVA table for sucker growth variables

ANOVA Table for shoot height

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	285.53	142.76	1.12	0.410
Cultivar	2	541.36	270.68	2.13	0.234
Error (a)	4	507.64	126.91	1.05	
IBA concentration	1	48.35	48.35	0.40	0.545
Error (b)	8	968.03	121.00	2.03	
Cultivar*con.	54	3216.75	59.57		
Total	71	5567.65			

ANOVA Table for shoot diameter.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	3.5833	1.7917	2.15	0.232
Cultivar	2	1.3333	0.6667	0.80	0.510
Error (a)	4	3.3333	0.8333	0.56	
IBA concentration	1	1.3889	1.3889	0.94	0.361
Error (b)	8	11.8611	1.4826	3.34	
Cultivar*conc.	54	24.0000	0.4444		
Total	71	45.5000			

ANOVA Table for number of leaves.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	0.194	0.097	0.22	0.815
Cultivar	2	12.194	6.097	13.51	0.017
Error (a)	4	1.806	0.451	0.17	
IBA concentration	1	0.125	0.125	0.05	0.832
Error (b)	8	20.750	2.594	1.81	
Cultivar*conc.	54	77.250	1.431		
Total	71	112.319			

ANOVA Table for shoot fresh weight.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	17426.	8713.	22.57	0.007
Cultivar	2	7288.	3644.	9.44	0.031
Error (a)	4	1544.	386.	0.09	
IBA concentration	1	217.	217.	0.05	0.831
Error (b)	8	35532.	4441.	2.95	
Cultivar*conc.	54	81229.	1504.		
Total	71	143235.			

ANOVA Table for shoot dry weight.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	624.5	312.3	21.25	0.007
Cultivar	2	543.8	271.9	18.51	0.010
Error (a)	4	58.8	14.7	0.02	
IBA concentration	1	43.2	43.2	0.06	0.807
Error (b)	8	5433.5	679.2	5.34	
Cultivar*conc.	54	6866.5	127.2		
Total	71	13570.4			