EFFECT OF BENZLYAMINOPURINE ON *IN VIVO* MULTIPLICATION AND GENETIC STABILITY OF PLANTAIN (*Musa* spp. AAB) CV. 'ITOKE SEGE'

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ABSTRACT

In vivo macropropagation either alone or in combination with benzlyamimopurine (BAP) is an alternative simple technique for banana multiplication but has not been applied to recalcitrant plantain such as cv. "Itoke Sege". This study was conducted to determine the effect of BAP concentration on *in vivo* multiplication and genetic stability among *in vivo* derived regenerants of plantain cv. 'Itoke sege'. An experiment was laid out in RCBD with four treatments each replicated three times. The treatments consisted of four BAP concentrations (1.5, 3.0, 6.0 mg/l and untreated control). Data were collected on number of days to first shoot emergence, number of shoots per corm, shoot size and morphological and genetic stability of *in vivo* derived shoots. Morphological stability was assessed using banana morphotaxonomic descriptors while genetic instability was assessed based on analysis of 2C nuclear DNA content of *in vivo* derived suckers. Results showed that BAP concentration at 1.5 mg/l significantly ($P \leq 0.05$) enhanced first the shoot emergence at 15.78 days followed by BAP at 3.0, 6.0 mg/l and untreated control with 25.18, 28.39 and 36.43 days, respectively. Moreover, BAP concentration at 1.5 mg/l significantly ($P \leq 0.05$) produced the largest number of suckers of 17.11 suckers per corm followed by untreated control and BAP concentration at 3.0 and 6.0 mg/l and with 15.23, 13.08 and 12.96 suckers per corm, respectively. Similarly, BAP at 1.5 mg /l and untreated control significantly ($P \leq 0.05$) showed the lowest frequencies of off-types with 10.89 and 10.23 % compared to BAP at 3.0 and 6.0 mg/l with 12.08 and 12.86 % of off-types, respectively. However, ploidy analysis revealed that the off-type and normal banana suckers had significantly ($P \leq 0.05$) equal 2C nuclear content and ploidy level. The findings of this research provide evidence for the use of *in vivo* macropropagation coupled with BAP at 1.5 mg L^{-1} as an alternative technology for rapid production of planting materials of recalcitrant plantain varieties.

DECLARATION

I, Grace Victory Kindimba, do hereby declare to the Senate of Sokoine University of Agriculture that this work presented here is my original work done within the period of registration and that it has neither been submitted nor concurrently being submitted for degree award in any university.

_____ _____

Grace Victory Kindimba **Date**

(MSc. Candidate)

The above declaration is confirmed

_____ _____

Prof. T. J. Msogoya **Date**

(Supervisor)

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DEDICATION

I dedicate this work to my father Mr. Victory Kindimba and my mother Valeria Mayemba for laying a foundation for my education, to my husband Tarcisius Mtaki, my children Kersten, and Karen who have waited so patiently to enable me complete this work.

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AA	acuminata-acuminata (intra-specific triploid banana)
AAA	acuminata-acuminata- acuminata (inter-specific triploid banana)
AAAA	acuminata-acuminata- acuminata - acuminata (intra-specific tetraploid banana)
AAB	acuminata-acuminata-balbisiana (intra and inter-specific triploid banana group)
AB	acuminata-balbisiana(inter-specific diploid banana)
ABB	acuminata-balbisiana-balbisiana (inter and intra-specific triploid banana group)
ABBB	acuminata-balbisiana-balbisiana- balbisiana (inter and intra-specific tetraploid banana)
ANOVA	Analysis of Variance
^{0}C	degree celcius
cm	centimetre (s)
CV	Coefficient of variation
cv.	cultivar
DNA	deoxyribonucleic acid
eds.	editors
et al.,	and others
FAO	Food and Agriculture Organization (United Nations)
FAOSTAT	Statistics (corporate statistical database)
Fig.	Figure
GA	Gibberellic Acid
IITA	International Institute of Tropical Agriculture
IPGRI	International Plant Genetic Resources Institute

kg	Kilograms(s)
l	Litre (s)
mg	milligram (s)
mg/l	milligram (s) per litre
No.	Number
sp.	Specie
spp.	Species

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Bananas (*Musa spp*.) are giant perennial herbs which originated from intra- and interspecific crosses of *Musa acuminata* and *Musa balbisiana* (Simmonds, 1995). Bananas are staple food for rural and urban consumers in the humid tropics of East Africa (Rugalema and Okting'ati, 1994). The crop is also an important source of income particularly in locations where small holders produce them in home garden (Rugalema and Okting'ati, 1994).

The major constraints of banana production are germplasm of poor quality, pests, poor soil fertility and agronomic practices, and poor access to healthy and affordable banana seedlings (Mwangi and Muthoni, 2008). To establish new farms, farmers have conventionally relied on suckers that are harvested from their existing farms (Mwangi, 2008). In conventional field propagation, there are two 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). Conversely; plants derived from sword suckers can progress to inflorescence emergence eight months after development of the first normal leaf, under optimal environmental conditions. Conversely 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). The production of suckers in the field is inadequate to meet demand, especially for large scale plantains. This is due to extremely strong apical dominance and very low multiplication rate of the cultivar with about 3-5

suckers per year per stool depending on agro-climatic conditions and management practices (Joab, 2004).

Conventional - derived suckers also contain disease pathogens (Vuylsteke *et al*., 1993) such as banana weevil (*Cosmopolites sordidus*) and parasitic nematodes which spread between farms (Mwangi, 2008). Moreover, conventional propagation of plantain is limited by poor suckering ability caused by the strong hormone-mediated apical dominance (Vuylsteke *et al*., 1990). Banana stool only produces about 5-10 suckers per year of 15- 20 cm tall suckers (Macias, 2001).

In response to constraints of conventional propagation banana can be propagated aseptically in the laboratory through *in vitro* micro-propagation. The production of banana planting material through this technology has higher rates of multiplication of uniform and pest-free planting material within a short period of time (Vuylsteke *et al.*, 1993). Production of plants in test tubes also facilitates safe movement and easy handling of germplasm between laboratories within and across countries. However, tissue culture plants are relatively expensive and not readily accessed by resource poor farmers (Njukwe *et al.*, 2005). The occurrence of somaclonal variation is among the factors that also hinder the application of *in vitro* propagation technology (Vuylsteke *et al*., 1990).

International Institute for Tropical Agriculture (IITA) has developed an alternative technique for the production of healthy planting material known as *in vivo* macropropagation (Njukwe *et al*., 2005). The technique involves desheathing the corm to expose axillary buds and to remove the apical meristem of suckers to suppress apical dominance and stimulate sprouting of axillary buds under *in vivo* conditions (Kwa, 2003). Macropropagation can rapidly multiply plantlets to distribute a new variety or replace

plants in disease-affected fields and give relatively healthy plants when source suckers are from healthy mother plants. The method results in the production of nine to fifteen uniform shoots per plant (Singh *et al*., 2011). Due to the low cost involved in production, the seedlings are priced at 40- 50% less than the suckers produced through tissue culture technology (Njeri *et al*., 2010). Macropropagation can be further enhanced through pouring cytokinin into the decorticated cavity of corms or through soaking the decorticated corms into cytokinin solution (Singh *et al*., 2011). This helps to disrupt the apical meristem and allows the growth of the axillary buds (Arinaitwe *et al*., 2000). This method leads to the production of 45-50 shoots within 3-4 months per corm. The suckers are separated from the mother corm and are subsequently rooted in sterile soil medium under intermittent misting. *In vivo* macropropagation works very well with plantain varieties possibly due to their low apical dominance caused by low levels of endogenous auxins (Kwa, 2003).

1.2 Problem statement and justification

Plantain cv.'Itoke sege' is one of the most important local cultivar potentially used for roasting in Tanzania. This cultivar has a strong apical dominance and this retards lateral shoot growth (Swennen and De Langhe, 1985). The suckering ability of plantains is very low ranging from 5 to 10 suckers per year (Vuylsteke *et al*., 1990). Therefore, the demand for planting materials for this cultivar is too high to be met by the conventional propagation methods. An attempt to *in vitro* propagate this cultivar at Tissue Culture Laboratory of SUA was unsuccessful largely due to blackening of explants caused by oxidation of polyphenols. These phenolic compounds are actively responsible for browning reactions of tissues prior and after *in vitro* incubation due to the oxidation of phenolic compounds by polyphenolic oxidase enzyme present in the tissue when excised (Ko *et al*., 2008). The blackening was reported to result in high culture mortality rate (Ko

et al., 2008). Benzylaminopurine (BAP) is a synthetic cytokinin (Salisbury and Ross, 1985), which is used to induce multiple shoots production (Vuylsteke, 1989). *In vivo* multiplication of suckers can be increased through application of BAP which induces sprouting of axillary buds and adventitious shoots (Singh, *et al*., 2011**;** Langford *et al*., 2012).

Exogenous application of 4.95 mg/L or more of BAP in the growth media triggers chromosomes numerical aberrations (Pesehke and Phillips, (1992). This leads to somaclonal variations associated with different mechanisms including, gene duplication, chromosomal rearrangements, deletions and chromosome numerical changes (Leela *at al*., 2003). The effect of BAP concentration on genetic stability of *in vivo* multiplication derived suckers of 'Itoke sege' is hardly known.

1.3 Objective

1.3.1 Overall objective

The overall objective of this study was to improve the production of planting materials of plantain cv. 'Itoke Sege' through *in vivo* macropropagation coupled with application of BAP.

1.3.2 Specific objectives

i. To determine the effect of benzlyamimopurine concentration on *in vivo* multiplication of plantain cv. 'itoke sege'

ii. To determine the morphological and genetic stability among *in vivo* derived regenerants of plantain cv. 'Itoke sege'.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bananas types, Origin and Diversification

The majority of plantain and banana in the family *Musaceae* originated from two wild species: *Musa acuminata* and *M. balbisiana* that generated the cultivated varieties. The ploidy and genomic composition of the different clones represent *M. acuminata* and *M. balbisiana* as A and B, respectively (Karamura, 1998). Banana includes diverse types such as dessert, cooking, roasting and beer bananas (Karamura, 1998). Dessert bananas are those bananas consumed raw at ripeness and are usually distinguished by the sweet flavour of the fresh ripe fruit, these include exotic bananas like Cavendish, Gros Michel and Pisang awak. Plantains have unpalatable fruits when ripe and are therefore consumed when cooked (Swennen and Vuylsteke, 1987). East Africa Highland bananas (AAA-EAHB) have fruits with lower starchy content than plantains and are therefore cooked when their fruits are green to prepare a meal known as “matoke” (Karamura, 1998). Beer bananas are bananas whose pulp is bitter and astringent and can neither be eaten raw nor cooked (Karamura, 1998).

Banana comprises of a range ploidy levels namely, diploids, triploids and tetraploids. They are categorised into genomic groups on the basis of their ploidy levels and the genomes which they contain (Simmonds and Shepherd, 1955). The resulting genomic groups are classified as AA, AB, AAA, AAB, ABB, AABB, AAAB, and ABBB with the letters A and B representing the contributions of *M. acuminata* and *M. balbisiana*, respectively (Simmonds and Shepherd, 1955; Karamura, 1998).

2.2 Importance of Banana

2.2.1 Major uses of plantains

In Tanzania, bananas and plantains are staple food to more than 5 million out of the 34 million population (Maerere *et al*., 2007). Crop production records show that banana ranks third after maize and cassava in Tanzania (MOAC, 2001). Bananas are principally consumed for their starch, which accounts for over 90 % of the dry matter and provides about 370 kJ per 100 g of raw fruit (Samson, 1986). They are also good sources of vitamin C and B6, and minerals, especially potassium and magnesium. Plantains are consumed after roasting or frying their mature fruits (Swennen and Vuylsteke, 1987; Samsom, 1986). The importance of plantain as a food crop in Tanzania varies from place to place with the highest consumption being in Kagera, Kilimanjaro, Arusha and Mbeya. Mbeya region is the most famous for production and consumption of plantain in the country. About 30 % of the people in rural Tanzania principally derive their carbohydrates from bananas with annual *per capita* consumption in the range of 28-500 kg (Walker *et al*., 1984). Roasted or fried bananas consumed in urban areas are either plantain or Mshale.

2.2.2 Economic and food importance of plantains

According to the FAOstat (2011) Tanzania is the second largest banana producer in the Eastern and central Africa, Uganda being the first. It is estimated that 480,000 ha are under banana production which produces about 3.5 million metric tons per year (FAO, 2011). Banana cultivation in Tanzania occupies 22 % of cultivated land and its production represents 40 % of food production and 15 % of GDP (Ndungo, 1997). It is estimated that 80 % of bananas produced are cooking bananas, 10 % beer bananas, 8 % dessert bananas and 2 % plantains (Kalyebara *et al*., 2007). The major banana producing areas in the country are found in the high altitude zones (Kagera, Kilimanjaro, Arusha and Mbeya regions), as well as in medium and low altitude areas in Morogoro, Kigoma, Rukwa,

Tanga, Coast, Mara, Mwanza and Shinyanga Regions and Zanzibar and Pemba islands (Maerere *et al*., 2007).

'Itoke sege' is an important plantain cultivar in food security because it is widely used for cooking and frying due to its good flavour. The cultivar is largely marketed in Kyela district, Malawi and even in other cities such as Iringa, Morogoro and Dar es Salaam (Maruo, 2007). In 2011 to 2012 the price per bunch of 'Itoke sege' was 7 000 – 9 000 TShs at farm gate price but when transported to cities like Dare es salaam, Iringa and Morogoro the price rises to 13 000 – 15 000 TShs (Zuhura Msigwa, Agricultural Officer, Person communication).

2.3 Banana Plant Morphology

Banana is a perennial herbaceous, monocarpic plant consisting of a corm and shoot axis (Swennen and Ortiz, 1997). The shoot system bears stem-like structure (pseudosterm), leaves and at maturity an inflorencence and fruits (Samson 1986; Swennen and Ortiz, 1997). The pseudostem grows to a height of 2 to 8 m depending on the variety and the conditions. It consists of large overlapping leaf bases which are tightly rolled around each other forming a cylindrical structure of almost 48 cm in diameter (Blomme and Ortiz, 2000). About 9-15 functional foliage leaves are formed in a healthy plant before the emergences of the inflorescences (Blomme and Ortiz, 2000).

The development of the buds from rhizomes or corm is marked by three distinct phases namely peepers, sword and water suckers. These phases of sucker development appear to be under the control of the hormonal system of the mother plant. The apparent dormancy of peepers seems to be induced by apical dominance. In plantain apical dominance is genetically controlled by a major recessive *ad* gene. Most bananas have the alternative

dominant allele, *Ad*, which improves the suckering behavior of the plantain–banana hybrids. The *Ad* allele has incomplete penetrance, genetic specificity, and variable expressivity, which affect the height of the tallest sucker at both flowering and harvest of the mother plant (Ortiz and Vuylsteke, 1994). Cultivars with high apical dominance show inhibited sucker development while those with low apical dominance have one or two well-developed suckers with regulated suckering behaviour or many developing suckers without regulated suckering behaviour (Swennen, *et al*., 1985).

2.4 Plantain Diversification

Plantain as defined by Simmonds (1966) forms a sub group of AAB genomic group. Plantains are diversified by accumulate somatic mutations. A large number of somatic mutations affect pseudosterm colour, dwarfism, fruit colour shape and apex and inflorescence morphology (Tezenas du Montcel *et al*., 1983). An inflorescence is the most striking character among plantains cultivars, which categorize them into four groups according to inflorescence degeneration (Swennen and Vuylsteke 1987). These include French plantains, French horn plantains, False horn plantains and Horn plantains. French plantains have a complete inflorescence at maturity, relatively small and numerous fruits, many biseriate neutral flowers and a large and persistent male bud consisting of bracts and male flowers. These are locally known as ‘Mzuzu’ type including ‘Itoke Sege’. The French horn plantains, however, produce a greater number of fruits and have many more neutral flowers. The Horn plantains have few but big fruits and the male axis is absent (Tezenas du Montcel, 1983; Swennen and Vuylsteke 1987), and these include the locally popular variety namely ‘Mkono wa tembo’. Inflorescence morphologies of different plantain categories are presented in the (Fig. 1).

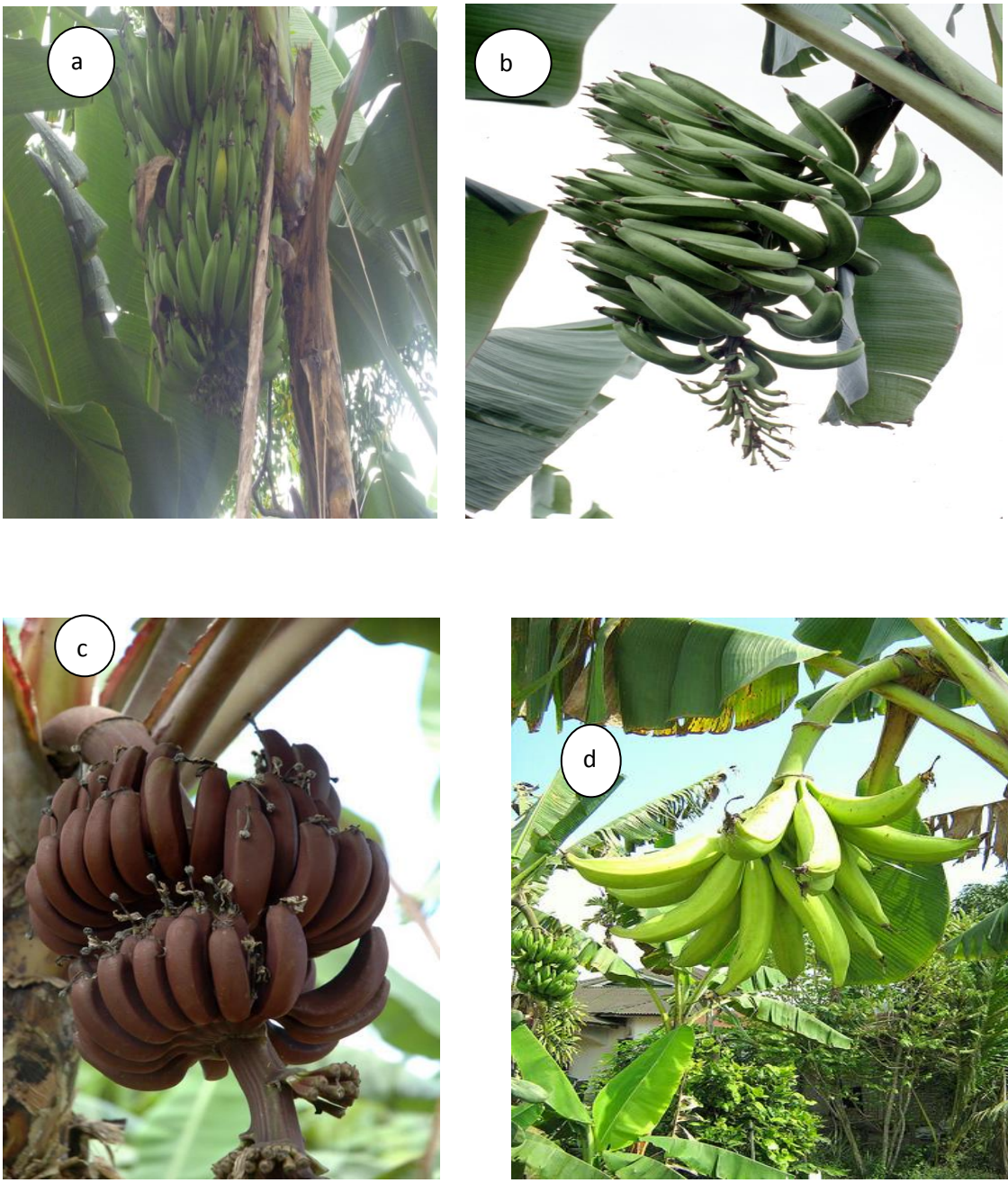

Figure 1: **Morphology of plantain categories:** [**(a) French plantain (b) French horn plantain (c) False horn plantain (d) Horn plantains**]

Pseudosterm height has also been used as criteria for grouping plantain, this include giant, medium and small plant size (De Langhe, 1964). Plant size depends on the number of foliage leaves produced until flowering. Giant plantains have more than 38 leaves, while small plantains produce less than 32 leaves. Giant cultivars are taller and flower much

later than medium ones and they produce more foliage resulting in heavier bunches with more hands and fruits.' Itoke Sege' is an example of the giant plantains.

2.5 Characteristics of 'Itoke Sege'

'Itoke' is a general term for plantain in the "Nyakyusa" language in Mbeya region. The word 'Itooke' (plural 'Matooke') is synonymous to 'Tooke,' which refers to East African highland bananas (AAA) from the north-western Tanzania (Maruo, 2007). 'Itoke sege' is a giant French plantain which grows well in the Southern Highlands especially in Rugwe District in Mbeya Region. The cultivar has a big light green pseudo-stem, persistent bracts on the rachis and large male bud (Rossel, 1998). Fruits are long slender and pointed at both ends. This cultivar takes 15-17 months to flower depending on altitude (Joab, 2004). Being plantain, 'Itoke sege' shows an extremely strong apical dominance and very low multiplication rate with about 3-5 suckers per year per stool depending on agro-climatic conditions and management practices (Joab, 2004). According to Joab (2004) "Itoke Sege" has high yield contributed by big bunch with 25.75 kg, large number of hands of 10 per bunch and number of fruits of 15 per hand.

2.6 Constraints to Banana Production

Despite all the recounted importance of banana, its yield has remained at 9.3 tonnes per hectare over the years which is far below the on-station demonstrated yields of over 30 tonnes per hectare (Mbwana, 1992). The reasons for the low yields have been documented by various authors including Walker *et al*, 1984; Sikora *et al*., 1990 and Bosch *et al.*, 1995. In last 25 years, the soils have been badly degraded due to continuous cropping with no attempts to conserve or replenish the nutrients. Other problems include infestations with the banana weevil borer (*Cosmopolites sordidus*), nematodes such *Radopholus similis* in hot lowlands and *Pratylenchus goodeyi* in highlands; infection with a range of diseases spearheaded by fungal diseases namely black sigatoka, caused by *Mycosphaerella*

fijiensis (Morelet) and Fusarium wilt caused by *Fusarium oxysporum f.sp. cubense*. Banana planting material also is a major problem as farmers have traditionally relied on suckers that are harvested from their existing farms (Mwangi, 2008). These suckers are not only inadequate but are also associated with higher risks of pest and disease spread between farms.

2.7 Banana Propagation

2.7.1 Seeds propagation

Seed propagation is common in wild banana species which are diploid and undergo normal meiosis, fertilization and seed set (Singh, *et al*., 2011). The extent of seed set, germinability and dormancy depends on the species. In *Ensete* sp, seed propagation is the only by means of perpetuation since sucker production is absent (Singh, *et al*., 2011).

2.7.2 Conventional propagation of plantain

All cultivated plantains are triploid, except a few parthenocarpic AA and AB diploids. Therefore, sucker propagation is the only natural means of their perpetuation. A sucker is a lateral shoot that develops from the rhizome and usually emerges close to the parent plant. Swords suckers emerge from the middle part of the corm and are characterised by narrow leaves and strong and conical pseudostems (IPGRI, 1996). Conversely, water suckers have a weak connection to the mother plant, and have broad leaves and weak and cylindrical pseudostems. The central meristem is quiescent during the vegetative growth of banana plant. Thus, banana leaves are produced from the intercalary meristem, and the massive leaf structure is built by the leaf base (IPGRI, 1996) (Fig.1). When these suckers are not available, rhizomes or split rhizomes can also be used as planting materials. Rhizomes well-trimmed around the growing buds each with a centrally placed germinating eye are planted in the nursery for sprouting or directly sown in the main field (Robinson,

et al., 2010). Although immediate planting is preferred, sometimes rhizomes are sun dried for 2-3 days after paring, trimmed of all roots and treated with nematicide before planting.

Figure 2: Types of banana suckers: Sword sucker (left) and water sucker (right) (Singh, *et al*., 2011)

Natural regeneration of cultivated banana through suckers is very slow due to hormone-mediated apical dominance of the mother plant (Macias, 2001). Each banana plant produces only 5-20 suckers during its life time of 12-14 months. Different growth stages of banana are influenced by growth hormones produced by the mother plant (Swennen *et al*., 1984).

2.7.3 Macropropagation of banana

Macro-propagation consists of generating suckers from clean planting material by removing the apical dominance. The technique is achieved by three methods, in the field conditions (*in situ* and *ex situ*) and *in vivo* shed condition. *In situ* multiplication is an induction of sucker multiplication on banana mother plants in the field. It involves several techniques, namely complete decapitation and decortication of the mother plants.

Decapitation involves cutting transversely 2 cm above the collar region of banana plant of about 4-6 months. Pseudostem of the mother plant is cut down and cross cuts incisions are made on the growing meristem so as to stimulate the production of lateral buds. Conversely, in decortication the pseudostem of the mother plant is cut transversely 2 cm above the collar region. Then the apical meristem is removed by making a cavity of 2 cm diameter and 4 cm depth on a growing meristerm. The techniques activate the lateral buds giving rise to more side shoots. Suckers of choice varieties can be maintained in a nursery either in sawdust bed or in a big, bottomless concrete pot (Singh, *et al*., 2011).

In *ex situ* mass multiplication, corms are collected from the field trimmed of all roots and the outer surface and the growing points are excised out with a sterilized sharp knife. These corms are surface sterilized by dipping in 0.3 % bavistin for 15 minutes or by immersion in boiling water for 15 - 20 seconds in water at 50 °C to kill weevil eggs and nematodes (FAO, 2010). The corms are planted directly or put into a multiplication chamber, usually comprising rice husk or sawdust. The initiation medium should essentially provide anchorage, moisture supply and proper aeration to the roots (Singh, *et al*., 2011). These methods result in the production of 9 -15 uniform shoots per plant in a short span of time, and are therefore highly suitable for small and marginal farmers whose requirements of planting material are relatively small.

In vivo macropropagation involves paring, disinfection, desheathing and excision of growing points of detached corms to expose axillary buds followed by removal of apical meristem. Corms can be induced chemically by pouring or soaking them in BAP or GA3. The corms are planted by covering them with sawdust and incubating them in a macropropagation chamber (Singh *et al*., 2011). Shoots are then scarified to allow the growth of secondary buds and shoots from secondary buds are transferred to the nursery

for rooting. Finally, after acclimatization for two month, suckers are ready to be transferred to the field (Njukwe *et al*., 2005). Chemical induction helps to break apical dominances thus promotes sprouting of lateral buds. This could be done on the first generation suckers and continue up to the third generation suckers. This method leads to production of 45-50 shoots per corm in a short span month (Singh *et al*., 2011).

2.7.4 *In vitro* propagation

In vitro propagation in banana is done through shoot tips isolated from suckers (Talengera *et al*., 1994). The technique leads to mass production of axenic planting materials for large scale monoclonal banana plantings. In addition, tissue culture derived suckers produce fruit earlier in the plant crop generation than conventional suckers (Msogoya,*et al*., 2006 and 2008). Although the *in vitro* technique can produce millions of suckers from a single apical meristem, the resulting suckers are prone to infection by the banana streak virus, which is now known to be integrated in the banana genome (Hughes *et al*., 1997). The occurrence of somaclonal variation is among the factors that also hinder the application of *in vitro* propagation technology (Msogoya *et al*., 2008). Moreover, *in vitro* techniques require expensive equipment and expertise which are not available to most banana farmers.

2.8 Banana Genetic Instability

2.8.1 Natural induced genetic instability

The plantain subgroups manifest a wide and unique morphological variation despite botanical homogeneity. African plantain cultivars make - up of supposedly homogenous groups that are derived from a very limited number of botanically different clonal source which greatly diversified by accumulated somatic mutations to give a complex reticulate

pattern of variability (De Langhe 1964). A large number of somatic mutations affect pseudostem colour and dwarfism, fruits colour, shape and apex and inflorescence morphology (Vuysteke, 1994). The frequency of this variation is however, considerably amplified *in vitro*.

2.8.2 *In vitro* induced genetic instability of banana

Genetic variation resulting from *in vitro* micropropagation is known as somaclonal variation. Rates of somaclonal variation in banana plants derived from shoot-tip culture vary from 0 to 70 % according to genotype (Israeli *et al*., 1995). Genetic instability is a risk associated with the application of *in vitro* culture techniques, germplasm handling and storage. Somaclonal variations have been associated with different mechanisms including, gene duplication, chromosomal rearrangements, deletions and chromosome numerical changes. The primary event that possibly triggers chromosomes numerical aberrations during tissue process is exogenously applied growth regulators. Pesehke and Phillips (1992) reported that 4.95mg/L or more of 6- benzylaminopurine in the growth media induce chromosome numerical aberrations in banana. Moreover, maintaining banana tissue culture regime over five subcultures considerably increases the frequency of chromosomes numerical errors (Zhenxum and Hongxian, 1997).

2.9 Detection of Instability

2.9.1 Morphological traits

Different methods are available to detect and monitor morphological traits of tissue culture-derived mutants. Most common quantitative traits include plant height, diameter, leaf length and breath. The ratio of plant height to diameter is used to differentiate dwarf from giant banana plants (Uma, *et al*., 2002). De Kler, *et al*. (1990) proposed to assess the degree of variation in a somaclonal population by determining the value of the

standard deviation for a quantitative trait. The standard deviations being small or large indicate that plants are either true to type (uniform) or not true to type, respectively.

2.9.2 Genetic instability

Genetic instability related to tissue culture suggests that the changes observed at the genetic level are most likely due to a stress-response mechanism (Phillips *et al.,* 1994). The relevant mechanism may be described as a loss of cellular control. The most commonly observed plant tissue culture-imposed changes include chromosome rearrangements, loss or doubling DNA, DNA methylation, and mutations (*Leela, et al*., 2003). These changes affect the stability of regenerated plants that may lead to changes either in the phenotype or the genotype, resulting in regenerated plants that are different from the original clone (Leela, *et al*., 2003). The detection of genetic instability associated with DNA reduction and multiplication is done through measurements of nuclear DNA content by flow cytometry (Dolezel *et al*., 1997). The method is being increasingly used for large-scale ploidy screening of callus cultures and cell suspension cultures which are prone to somatic variation (Dolezel *et al*., 1994).

Flow cytometry is a convenient too for estimation of nuclear DNA content and ploidy level constitutions in plant (Dolezel, l99l). Basically, the Flow cytometry involves in DNA specific fluorochromes and the relative fluorescence intensity emitted by stained nuclei. The DNA content is linearly related to digitized fluorescence signal. Its validity depends on fluorochromes, fluorescence absorption, and standard reference and a linear amplification system. The measurement is based on fluorescence intensity of nuclei ratio between a known nuclear genome and nuclei from sample. The nuclei ratio is calculated to determine the nuclear DNA (Dolezel, 1997), using the following formula:

$DNA_b = DNA_p \times {}^{Gb}/G_p$

Where: DNA_p : 2C nuclear DNA contents (pg) of standard

DNAb : 2C nuclear DNA contents (pg) of samples

G_p : Fluorescence intensity peak of standard

G_b: Fluorescence intensity peak of samples

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Effect of BAP Concentration on *In Vivo* Multiplication of Plantain cv. 'Itoke Sege'

3.1.1 Preparation of propagators and growth media

Propagators used for planting corms were made from soft timbers with a dimension of 200 cm long, 100 cm width and 75 cm deep. Water moistened sawdust was sterilized by heating it at 100 ^{0}C for one hour and thirty minutes. Four propagators were filled three quarter-full with the sterilized sawdust and left for 24 hours to cool before planting the corms.

3.1.2 Preparation of planting material

Suckers of plantain cv. 'Itoke Sege' of 3 - 4 months old were collected from Rungwe District in Mbeya Region. A careful selection of sword suckers was done from healthy mother plants grown by small-scale farmers. Sword suckers were pared to remove roots and packed into plastic bags for transport to Morogoro. The suckers were sterilized in boiling water at 100 ^{o} C for 15 seconds (Fig. 2). Using sterilized sharp knife, the outer leaf sheaths were then removed one by one so as to expose the axillary buds. Cross - cuts were made on the exposed axillary buds and apical meristem to encourage sprouting of axillary buds and to kill apical dominance, respectively.

Figure 3: Surface sterilization of corms in boiling water

3.1.3 Experimental design

The experiment was established on 17 February, 2012, in a randomized complete block design with four treatments replicated four times. The treatments involved four BAP concentrations, namely, 1.5, 3.0, 6.0 mg/l and untreated control. Corms were then soaked overnight (12 hours) in each BAP concentration. Each treatment had 12 corms replicated four times.

3.1.4 Planting and management practices

The corms were removed from each BAP solution and planted into propagators at spacing of 15 cm x15 cm and covered with sawdust. The propagators were irrigated immediately and thereafter, they were irrigated once to twice per week depending on the weather conditions and humidity of the sawdust. Shoots from sprouted corms were treated with booster (poly- feed starter (N-P-K)) at dose of 0.5 g per litre per month after planting. Three months later, the propagules were detached from corms for transplanting plastic bags (Fig. 3).

Figure 4: Propagules ready for detachment from corms

3.1.5 Data collection and analysis

Data collected included number of days to first shoot emergence, number of shoots emerged per corm, number of roots per corm, shoots height and diameter, and number of leaves per shoot. Days to first shoot emergence was collected by counting the days from when corms were planted to the day when the tallest shoots appeared above the sawdust surface. Number of shoots and roots were obtained by counting the shoots and roots, respectively. Shoot height was measured using vernier calliper from the root collar to the tip of cigar leaf while shoot diameter was measured 3.0 cm from root collar using vernier calliper. The data collected were analysed using COSTAT6.4 (Cohort Software, Minneapolis, USA, 2006). The Bartlett's test for normality was performed prior to Analysis of Variance (ANOVA). Mean separation was done based on the least significant difference (LSD) test at a probability of 5 % ($P \leq 0.05$).

3.2 Determination of Morphological and Genetic Stability among *In Vivo* Regenerants of Plantain Cv. 'Itoke Sege'

3.2.1 Morphological instability

3.2.1.1 Description of planting materials

Morphological characterization was carried out using all *in vivo* derived propagules of plantain cv. 'Itoke sege' in section 3.1.3 above. Detached propagules were planted in black

polyethylene bags with a capacity of 4 litres filled with growth media composed of forest soil, farm yard manure and sawdust at a ratio of 4:2:1: by volume. Planted propagules were placed under a plastic tunnel (Fig. 4) and supplied with poly- feed starter (N-P-K) at dose of 0.5 g per litre per month after planting. Irrigation was conducted once per week for three month.

Figure 5: Propagules planted in polybags under the plastic tannel

3.2.1.2 Experimental design

The experiment was established in randomized complete block design with four treatments replicated three times. The treatment consisted of suckers detached from BAP at four concentrations (1.5, 3.0, 6.0 mg/ l and untreated control).

3.2.1.3 Data collection and analysis

Hundred *in vivo* derived suckers of 'Itoke sege' at three months old from each treatment were used for the characterization. The morphological characterisation was carried out based on qualitative and quantitative traits. The qualitative characterization included leaf shape, leaf colour, leaf habit, pseudostem colour, leaf midrib and blotches on leaf and was done using descriptors for banana (IPGRI, 1996) as shown in Table 1.

Table 1: **Qualitative descriptors used for characterization of Plantain cv. 'Itoke Sege'**

Character	Description
Leaf shape	1.Narrow, 2.wide
Leaf colour	1.Green 2.Dark green, 3.Light green
Leaf habit	1. Erect, 2.Intermediate, 3. Drooping
Pseudostem colour	1.Black, 2.Green, 3.Green blackish, 4.Yellow-green
Leaf midrib colour	1. Green, 2. Red
Leaf blotches	1.No blotches , 2.Dull brown blotches, 3.Dark brown blotches

Quantitative trait characterisation was carried out based on plant stature measured by pseudostem height and girth, leaf length and breath as in section 3.1.5 above. Variation in plant stature was determined based on ratio of pseudostem height to girth and leaf length to breath. Suckers with stem height to diameter ratio between 12.0cm and 22.00 were considered as normal plants while those with ratio below 12.0 cm and above 22.0 cm were considered as dwarf and giant plants, respectively. Data collected were analysed as in section 3.1.5 section above.

3.2.2 Karyotypic instability

3.2.2.1 Material and methods

Ten samples of cigar leaves each for normal suckers, dwarf and giant plants were randomly selected from sucker population obtained in section 3.2.1.3.

3.2.2.2 Experimental design

The experiment was conducted as a complete randomized design with three replications and three treatments. The treatments were normal suckers, dwarf suckers and giant suckers. The cigar leaf samples were collected, wrapped in tissue paper, put in plastic bags and packed in styrofoam box containing ice blocks for transportation to National Agriculture Research laboratories, Kawanda, in Uganda.

3.2.2.3 Nuclear DNA Sample preparation

The fresh midrib tissue of approximately 100 mg from a cigar leaf was chopped with a sharp razor blade in a disposable petri dish containing 0.5 ml of cold OTTO I buffer (0.1 M citric acid monohydrate and 0.5 % Tween 20) to form a homogenate. Additional 0.5 ml of cold OTTO I was added to the homogenate and mixed thoroughly. The homogenate was filtered through a nylon filter of 50 μm pore size into a polystyrene tube. Samples were incubated for 1-5 minutes with occasional shaking. To each sample, 2 ml of OTTO II (0.4 M anhydrous disodium hydrogen phosphate), 2 mg /500ml of 4, 6' – diamidino-2-phenylindole and 1 μl/ml of β-mercaptoethanol were added before analysis.

3.2.2.4 Analysis of nuclear DNA content

The instrument used for analysis of nuclear DNA content was Partec GmbH ploidy analyzer (Otto-Hahn-Str. 32, D-48161 Münster). It was first calibrated by running samples of known ploidy. A diploid banana cv. 'Calcutta 4' was used as a standard or control and its peak was set at channel 50. The tetraploid standard or control used was banana cv. '660k-1' and its peak was set at channel 100. East Africa highland banana cv. 'Enyeru' was used as a triploid control and its peak was set at channel 75. Histogram of fluorescence intensity of nuclear DNA of standards and banana cv. 'Itoke sege' are as shown below (Fig. 5).

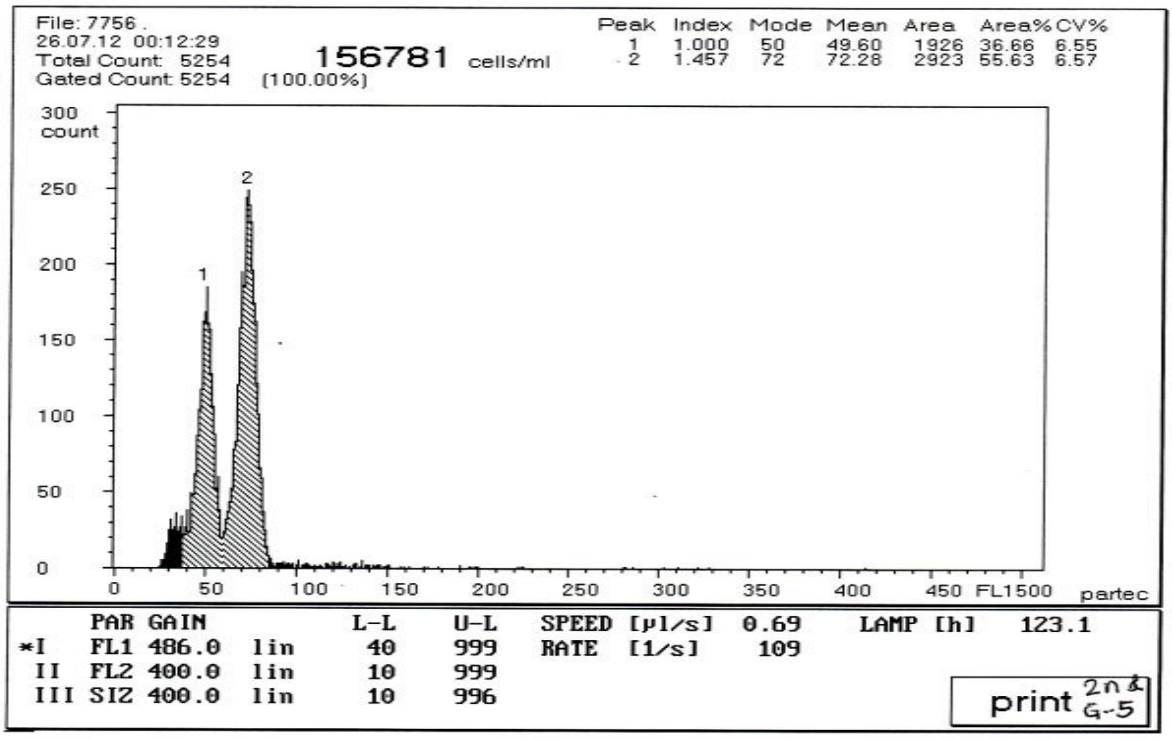

Figure 6: Histograms of relative nuclear DNA content of 'Itoke sege' and 'Calcutta 4': Peak 1 stands for 'Calcutta 4'and peak 2 stands for 'Itoke sege'

After obtaining the histograms of all samples of normal suckers, dwarf suckers and giant cigar leaf, the nuclear DNA amount of these samples in three replicates was calculated as the ratio of the fluorescence intensity of the nuclei of the test samples and the calibration standard multiplied by the nuclear content of the standard (Yokoya *et al*., 2000).

$DNA_b = DNA_p \times {}^{Gb}/G_p$

Where: DNA_p : 2C nuclear DNA contents (pg) of standard

DNAb : 2C nuclear DNA contents (pg) of samples

G_p : Fluorescence intensity peak of standard

G_b: Fluorescence intensity peak of samples

3.2.2.5 Data analysis

The nuclear DNA contents from normal suckers, dwarf suckers and giant suckers calculated in section (3.2.2.4) were analysed using COSTAT6.4 (Cohort Software, Minneapolis, USA, 2006). The Bartlett's test for normality was performed prior to the Analysis of Variance (ANOVA). Mean separation was done based on the least significant difference (LSD) test at probability of 5 %. The 2C nuclear DNA content was further used to estimate the ploidy level and number of chromosomes of plantain cv. 'Itoke sege' where nuclear DNA of 1.8 to 2.5 pg /cell corresponds to 3n ploidy or 33 chromosomes per cell.

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Benzylaminopurine Concentrations on *In Vivo* Multiplication of Plantain cv. 'Itoge Sege'

Results indicate that BAP concentration had a very high significant ($P \leq 0.05$) influence on number of days from banana corm sowing to first shoot emergence in the first cycle (Table 2). Banana corms treated with BAP at 1.5 mg/l produced the first shoot earlier at 15.78 days followed by corms treated with BAP at 3.0, 6.0 mg/l and untreated control with 25.18, 28.39 and 36.43 days, respectively. Moreover, BAP treatment significantly ($P \leq 0.05$) influenced the number of shoots per corm in the first cycle. Banana corms dipped in BAP at 1.5 mg/l produced the largest number of shoots per corm of 9.66 followed by corms dipped in BAP at 3.0, 6.0 mg/l and untreated control, with number of shoots per corm of 6.78, 6.03, and 6.04, respectively (Table 2). Corms treated with BAP at 1.5, 3.0, 6.0 mg/l significantly ($P \leq 0.05$) produced bigger suckers with length of 27.01, 27.27 and 26.73 cm followed by untreated control with shoot length of 22.69 cm, respectively. Similarly, corms treated with BAP at 6.0 mg/l and untreated control had significant ($P \leq 0.05$) larger diameters of 2.41cm and 3.41 followed by corms treated with BAP at 3.0 and 1.5 mg/l with sucker diameters of 2.20 and 2.02 cm, respectively. Suckers from corms treated with BAP at 3.0 mg/l and untreated control had a significant ($P \leq 0.05$) larger number of leaves of 4.56 and 4.77 per sucker followed by corms treated with BAP at 1.5 and 6.0 mg/l with number of leaves of 4.04 and 3.85 per sucker, respectively.

Table 2: Effect of BAP concentrations on *in vivo* regeneration and sucker size of 'Itoke Sege' in the first cycle

BAP conc. (mg/l)	No. of days to first sucker emergence	No. of suckers per corm	Sucker length (cm)	Sucker diameter (cm)	No. of roots per sucker	No. of leaves per sucker
0.0	36.43[a]	6.04[b]	22.69[b]	1.83[c]	15.33[a]	4.77[a]
1.5	15.78[d]	9.66[a]	27.01[a]	2.02[bc]	14.52[b]	4.04[b]
3.0	25.18[c]	6.78[b]	27.27[a]	2.20[ab]	12.74[b]	4.56[a]
6.0	28.39[b]	6.03[b]	26.73[a]	2.41[a]	13.65[ab]	3.85[b]
LSD(0.05)	0.75	4.25	2.87	0.30	4.95	0.73
F-test	***	**	**	**	*	***
CV %	3.13	33.94	12.19	16.0	4.95	10.48

Means followed by the same letter (s) within the column are not significantly different at a probability of 5 % based on LSD test.

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

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

Benzlyaminopurine concentration had a significant ($P \leq 0.05$) influence on number of suckers per corm, sucker length, number of roots per sucker and number of leaves per sucker in the second cycle of *in vivo* multiplication (Table 3). The untreated control produced the highest number of shoots per corm of 9.40 followed by corms treated in BAP at 1.5, 3.0 and 6.0 mg/l with number of suckers per corm of 6.22, 6.80 and 6.69, respectively. However, the control produced the biggest suckers with length of 20.63 cm followed by corms treated with BAP at 1.5, 3.0 and 6.0 mg/l with the sucker length of 13.0, 15.01 and 13.04 cm, respectively. Moreover, corms treated with BAP at 6.0 and 1.5 mg/l produced the largest number of roots per sucker of 23.72 and 23.40 followed by untreated control and BAP concentration at 3.0 mg/l with the number roots per sucker of 19.68 and 17.86, respectively. Corms treated with BAP at 6.0 and 3.0 mg/l also

significantly ($P \leq 0.05$) produced suckers with largest number of leaves of 4.24 and 3.89 per sucker, followed by BAP at concentration 1.5 mg/l and untreated control with 3.69 and 3.51 leaves per sucker, respectively.

Table 3: Effect of BAP on regeneration and sucker size of 'Itoke Sege' in the second cycle of *in vivo* multiplication

BAP conc. (mg/l)	No. of suckers per corm	Sucker length (cm)	Sucker diameter (cm)	No. of roots per sucker	No. of leaves per sucker
0.0	9.40[a]	20.63[a]	1.58	19.68[b]	3.51[c]
1.5	6.22[b]	13.00[c]	1.52	23.40[a]	3.69[bc]
3.0	6.80[b]	15.01[b]	1.52	17.86[b]	3.89[b]
6.0	6.69[b]	13.04[b]	1.34	23.72[a]	4.24[a]
LSD(0.05)	1.75	2.75	0.30	4.29	0.22
F-test	**	***	Ns	*	***
CV %	25.09	18.29	16.0	21.09	6.13

Means followed by the same letter (s) within the column are not significantly different at a probability of 5 % based on LSD test.

ns = not significant, *= significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

Benzlyaminopurine concentration had a significant ($P \leq 0.05$) influence on number of suckers per corm, sucker length, number of roots per sucker and number of leaves per sucker in the combined cycle (Table 4). Banana corms treated with BAP at 1.5 mg/l and untreated control produced the highest number of shoots per corm of 17.11 and 15.23 followed by corms treated with BAP at 3.0 and 6.0 mg/l with number of shoots per corm of 13.08 and 12.96, respectively. Corms treated with BAP at 3.0, untreated control and 6.0 mg/l also significantly ($P \leq 0.05$) produced suckers with largest number of leaves of 4.30, 4.08 and 3.99 per sucker, respectively followed by suckers from corms treated with BAP

at 1.5 mg/l with 3.92 leaves per sucker. Moreover, corms treated with BAP at 1.5 and 3.0 mg/l produced sucker length of 22.40 and 21.58 cm followed by corms treated with BAP at 6.0 and untreated control with sucker length of 19.80 and 17.68 cm, respectively. Corms treated with BAP at 1.5 and 3.0 mg/l also produced sucker diameter of 2.24 and 1.99 cm followed by corms treated with BAP at 6.0 and untreated control with sucker diameter of 1.81 and 1.68 cm, respectively.

Table 4: Effect of BAP concentrations on *in vivo* regeneration and sucker size of 'Itoke Sege' in the combined cycles

BAP conc. (mg/l)	No. of days to first sucker emergence	No. of suckers per corm	Sucker length (cm)	Sucker diameter (cm)	No. of roots per sucker	No. of leaves per sucker
0.0	36.85^{a}	15.23^{ab}	17.68^{c}	1.68^{d}	10.96	4.08^{b}
1.5	15.95^{d}	17.11^{a}	22.40^{a}	2.24^{a}	11.45	3.92^{b}
3.0	25.14^{c}	13.08^{b}	21.58^{a}	1.99^{b}	11.74	4.30^{a}
6.0	28.43^{b}	12.96^{b}	19.80^{b}	1.81^{c}	10.80	3.99^{b}
LSD (0.05)	0.42	2.35	1.33	0.11	1.06	0.17
F-test	***	**	***	***	ns	***
CV %	1.94	19.58	7.96	7.46	11.51	5.10

Means followed by the same letter (s) within the column are not significantly different at a probability of 5 % based LSD test.

ns = not significant, *= significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

4.2 Morphological and Genetic Characteristics of *In Vivo* Regenerants of Plantain Cv. 'Itoke Sege'

4.2.1 Morphological characteristics

All *in vivo* derived regenerants from the four BAP concentrations were normal based on leaf shape, leaf colour leaf habit, pseudostem colour and leaf blotches (Table 5).

Table 5: Qualitative characteristics of plantain cv. 'Itoke Sege'

Character	Description	Inference
Leaf shape	Narrow	Normal
Leaf colour	Dark green	Normal
Leaf habit	Intermediate	Normal
Pseudostem colour	Light Green	Normal
Leaf midrib colour	Green	Normal
Leaf blotches	Dark brown blotches	Normal

Benzylaminopurine treatment had an influence on occurance of off-type suckers based on ratio of sucker height to diameter. The untreated control had the lowest incidences of off-types of 5.5 % followed by BAP at 1.5, 3.0 and 6.0 mg/l with incidences of off-type of 15.51, 16.28 and 17.85 %, respectively (Table 6).

Table 6: Effect of BAP concentration on instability of *in vivo* derived regenerants of plantain cv. 'Itoke Sege'

BAP conc. (mg/l)	Normal plants (%)	Incidence off-type (%)	Difference (%)
0.0	94.50	5.50	89.00
1.5	84.49	15.51	68.98
3.0	83.72	16.28	67.44
6.0	82.15	17.85	64.30

The incidences of dwarf off-types suckers of 12.86 % was significantly ($P \leq 0.05$) the highest for suckers arising from corms treated with BAP at 6.0 mg/l followed by suckers arising from corms treated with 3.0, 1.5 and untreated control with incidences of 12.08, 10.89 and 10.23 %, respectively (Table 7). On the other hand, the occurances of giant off-type suckers of 25.80 and 25.69 % were higesht in corms treated with BAP at 3.0 and 6.0

mg/l followed by incidences of 21.76 cm and 16.56 cm for corms treated with BAP at 1.5 mg/l and untreated control, respectively**.**

Table 7: Effect of BAP concentration on incidence of *in vivo* derived off –type regenerants of 'Itoke Sege'

BAP conc. (mg/l)	Dwarf off –type incidence (%)	Giant off-type incidence (%)
0.0	10.23[d]	16.56[c]
1.5	10.89[c]	21.76 [b]
3.0	12.08[b]	25.80[a]
6.0	12.86[a]	25.69 [a]
LSD (0.05)	0.56	1.48
F-test	***	***
CV	5.05	6.84

Means followed by the same letter (s) within the column are not significantly different at a probability of 5 % based on LSD test.

ns = not significant, *= significant ($p \leq 0.05$), ** = highly significant ($p \leq 0.01$), *** = very highly significant ($p \leq 0.001$).

4.2.2 Genetic characterization of off-types suckers

Karyotypic analysis revealed that normal, giant and dwarf suckers had an equal 2C nuclear DNA content, chromosomes number and ploidy level (Table 8).

Table 8: Karyotypic characteristics of *in vivo* derived suckers of plantain cv.'Itoke Sege'

Plant stature	2C nuclear DNA content (pg)	Chromosome number /cell	Ploidy level
Normal suckers	2.17	33	3n
Dwarf suckers	2.18	33	3n
Giant suckers	2.26	33	3n
LSD (0.05)	0.12	-	-
F-test	ns	-	-
CV %	6.23	-	-

CHAPTER FIVE

5.0 DISCUSSION

5.1 Effect of Benzylaminopurine on *In Vivo* Multiplication of Plantain cv. 'Itoke Sege'

BAP treatment at 1.5 mg/l reduced the number of days from the corm sowing to first shoot emergence as compared with other treatments in the combined cycle of *in vivo* multiplication. The enhanced sucker emergence in corms treated with BAP at 1.5 mg/l was probably due to the suppressed activity of apical meristem (Osei, 2006). Benzylaminopurine is a cytokinin based plant growth regulator which stimulates the growth of lateral meristems.

The number of shoots per corm in the combined cycles was the highest in corms treated with BAP at 1.5 mg/l compared with BAP at 0.0, 3.0 and 6.0 mg/l. The highest *in vitro* multiplication of 7 - 8 shoots per explant has also been reported when MS basal medium was supplemented with BAP at 2.0 mg/l (Kalimutha *et al*., 2007). Furthermore, Muhammad (2007) reported that increasing cytokinin concentration above 3 mg/l resulted into decreased *in vitro* proliferation rate in plantain cultivars. On contrary, Sreeramanan *et al*. (2008) found high shoot induction in BAP at 4.5 mg/l in plantain cv. 'Oniaba' and 'Apantu' (Buah *et al*., 2010). The findings from this study together with those reported earlier suggest that the plantain response to BAP concentration could be cultivar specific.

The significant increase in shoot height and diameter from corms treated with BAP at 1.5 mg/l corresponds well with the enhanced shoot emergence. This is also supported by Swennen (1985) who reported that an injection of BAP in plantain enhanced bud formation as well as the speed of shoot development in plantain. A few number of suckers

per corm results in increased growth due to reduction in competition among the suckers for resources such as nutrients, water and light (Bakelana, 2000).

5.2 Effect of Benzylaminopurine on Morphological and Genetic Stability of *in Vivo* Regenerants of Plantain cv. 'Itoke Sege'

The incidence of off-type was the lowest in untreated control corms compared to suckers treated with BAP. The incidence of off-type banana in untreated control in this study agrees with Vuylsteke (2001) who also reported that giant French plantains showed natural instability under conventional regeneration methods. However, the incidences of off-types increased with increasing concentration of BAP. The increasing incidences of off-type plants with higher BAP concentration in this study is in agreement with Siamak *et al*. (2010) who reported alterations in plant morphology in plantain cv. 'Nangka' and Bluggoe cv. 'Baka Baling' (ABB) under *in vitro* propagation at BAP concentration of 5 mg/l. High concentration of BAP at 4.99 and 9.96 mg/l induced genetic variability in banana (Trujillo and Garcia, 1996).

The 2C nuclear DNA content values of 2.1 - 2.3 pg/cell reported in this study are within the 2C nuclear DNA contents values of normal triploid plantains (Lysak *et al*., 1999). The values of 2C nuclear DNA content imply that the *in vivo* derived regenerants of plantain cv. 'Itoke Sege' are triploidy (3n) with 33 chromosomes per cell. The discrepancies between morphological and genetic characteristics of the *in vivo* derived regenerants suggests that dwarfism and gigantism in plantain cv. 'Itoke Sege' are possibly not under the control of number of chromosomes or ploidy level. Dwarfism in French plantain is reported to be under the control of major recessive gene which shortens the plant internodes (Vuylsteke, 2001). On the other hand, most of variations caused by tissue culture were found to be due to disturbance in endogenous gibberellins metabolism

(Sandoval *et al*., 1995). High levels of gibberellins increase shoot elongation (i.e. gigantism) while its lower level reduces internode elongation (i.e. dwarfism) in most plant species (Hooley, 1994; Olszewski *et al*., 2002). Alternatively, dwarfism and gigantism in plantain cv. 'Itoke Sege' could be under the control of chromosome or DNA structural alterations. Chromosome rearrangements have been found among tissue culture regenerants (Hang and Bregitzer, 1993). Translocations characterised by inversions, insertions and deletions are the most frequent chromosomal abnormality in tissue culture derived regenerants.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In vivo macropropagation coupled with BAP concentration at 1.5mg/l enhances shoot sprouting and increases sucker productivity in plantain cv. 'Itoke sege'. The *in vivo* derived suckers are karyotypically normal though some suckers exhibit morphological abnormality based on plant height. This discrepancy suggests that the observed morphological variations in the regenerants are possibly under the epigenetic control or chromosomal structural alterations.

6.2 Recommendations

The findings of this research provide evidence for the use of *in vivo* macropropagation coupled with BAP at 1.5 mg L^{-1} as an alternative simple and cheap technology for rapid and mass production of plantain planting materials for recalcitrant plantain varieties such as 'Itoke sege'.

Further studies are required to determine the:

(i) Mechanisms underlying dwarfism and giantism on *in vivo* derived suckers of plantain cv. 'Itoke sege'.

(ii) Effect of BAP concentrations on *in vivo* regeneration of other plantain varieties.

(iii) Effect of other cytokine-based growth regulators on *in vivo* multiplication of plantain cv. 'Itoke sege'.

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APPENDICES

Appendix 1: ANOVA tables of all variables in first cycle

Appendix 1.1 ANOVA table for number of days to first shoot formation

Source of variation	d.f.	s.s.	m.s.	F -value	p
REP	3	35.85	11.95	17.34	0.00
Treatment	3	2188.85	729.61	1058.84	0.00
Error	33	22.73	0.68		
Total	39	2247.45			

CV= 3.13%

Appendix 1.2 ANOVA table for number of shoots per corm

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	38.33	12.77	2.18	0.10
Treatment	3	89.81	29.93	5.12	0.00
Error	33	192.85	5.84		
Total	39	320.99			

CV= 33.94%

Appendix 1.3 ANOVA table for sucker length

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	191.61	63.87	6.38	0.00
Treatment	3	141.31	47.10	4.71	0.00
Error	33	329.86	9.99		
Total	39	662.78			

CV = 12.19%

Appendix 1.4 ANOVA table for suckers diameter

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	2.03	0.67	5.88	0.00
Treatment	3	1.82	0.60	5.28	0.00
Error	33	3.80	0.11		
Total	39	7.66			

CV = 16.00%

Appendix 1.5 ANOVA table for number of roots per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	139.56	46.52	1.57	0.21
Treatment	3	258.14	86.04	2.90	0. 04
Error	33	977.76	29.62		
Total	39	1375.47			

CV= 34.98%

Appendix 1.6 ANOVA table for number of leaves per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	1.49	0.49	6.98	0.00
Treatment	3	5.48	1.82	25.61	0.00
Error	33	2.35	0.07		
Total	39	9.33			

CV= 6.19%

Appendix 2: ANOVA tables of all variables in second cycle

Appendix 2.1 ANOVA table for number of shoots per corm

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	38.33	12.77	2.18	0.10
Treatment	3	89.81	29.93	5.12	0.00
Error	41	192.85	5.84		
Total	47	320.99			

CV= 33.94%

Appendix 2.2 ANOVA table for sucker length

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	29.36	9.78	0.67	0. 57
Treatment	3	130.06	43.35	2.98	0. 04
Error	41	595.81	14.53		
Total	47	755.23			

CV=24.95%

Appendix 2.3 ANOVA table for suckers diameter

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	0.40	0.13	6.38	0. 19
Treatment	3	0.63	0.21	2.53	0. 07
Error	41	3.44	0.08		
Total	47	4.48			

CV =19.66%

Appendix 2.4 ANOVA table for number of roots per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	523.73	174.57	8.32	0.00
Treatment	3	298.35	99.45	4.74	0. 00
Error	41	859.28	20.95		
Total	47	1681.37			

CV= 22.95%

Appendix 2.5 ANOVA table for number of leaves per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	4.59	1.53	22.71	0.00
Treatment	3	3.26	1.08	16.16	0. 00
Error	41	2.76	0.06		
Total	47	10.62			

CV= 6.79%

Appendix 3: ANOVA Tables of all variables in combined cycles

Appendix 3.1 ANOVA table for number of days to first shoot formation

Source of variation	d.f.	s.s.	m.s.	F -value	p
REP	3	37.44	12.48	46.79	0.00
Treatment	3	2688.88	896.29	3359.71	0.00
Error	41	10.93	0.26		
Total	47	2737.27			

CV= 1.94%

Appendix 3.2 ANOVA table for number of shoots per corm

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	568.30	189.43	26.92	0.00
Treatment	3	51.54	17.18	2.44	0.07
Error	41	288.44	7.03		
Total	47	908.29			

CV= 21.53%

Appendix 3.3 ANOVA table for sucker length

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	288.11	96.03	11.49	0.00
Treatment	3	185.76	61.92	7.41	0.00
Error	41	342.52	8.35		
Total	47	816.40			

CV = 13.24%

Appendix 3.4 ANOVA table for suckers diameter

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	2.09	0.69	9.60	0.00
Treatment	3	1.83	0.61	8.37	0.00
Error	41	2.98	0.07		
Total	47	6.91			

CV =14.40%

Appendix 3.5 ANOVA table for number of roots per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	2823.20	941.06	16.92	0.00
Treatment	3	1055.33	351.77	6.32	0. 00
Error	41	2279.03	55.58		
Total	47	6157.57			

CV= 25.03%

Appendix 3.6 ANOVA table for number of leaves per sucker

Source of variation	d.f.	s.s.	m.s.	F	p
REP	3	3.61	1.20	26.18	0.00
Treatment	3	0.53	0.17	3.87	0. 01
Error	41	1.88	0.04		
Total	47	6.03			

CV= 5.23%

Appendix 4: ANOVA tables of dwarf off-types, giant off-types and nuclear DNA content of banana suckers

Appendix 4.1 ANOVA table for incidence of dwarf off-type plants

Source of variation	d.f.	s.s.	m.s.	F	p
REP	2	4.67	2.33	21.15	0.00
Treatment	3	7.31	2.43	22.05	0.00
Error	19	2.10	0.11		
Total	24	13.92			

CV= 2.61%

Appendix 4.2 ANOVA table for incidence giant off-type plants

Source of variation	d.f.	s.s.	m.s.	F	p
REP	2	1.83	0.91	0.50	0.60
Treatment	3	65.27	21.75	12.05	0.00
Error	19	34.30	1.80		
Total	24	101.54			

CV= 5.01%

Appendix 4.3 ANOVA table for Nuclear DNA content of banana suckers

Source of variation	d.f.	s.s.	m.s.	F	p
REP	2	0.22	0.11	6.02	0.00
Treatment	2	0.04	0.02	1.22	0.30
Error	28	0.52	0.01		
Total	32	0.80			

CV= 6.23%