# RESPONSE OF BANANA AND PLANTAIN CULTIVARS TO MACROPROPAGATION USING BUD MANIPULATION TECHNIQUE

**BY**

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## CERTIFICATION

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## ABSTRACT

In Nigeria, bananas and plantains are important staple and cash crops. Nevertheless, one of the major constraints to their production is the unavailability of healthy planting materials at planting time. Fortunately, micropropagation using bud manipulation technique could be used to produce large numbers of healthy plantlets in a short time. The response of five Musa cultivars to micropropagation using bud manipulation technique was evaluated in the horticultural Nursery of the Department of Crop Science, University of Benin, Benin City, Nigeria in June, 2017. Three sword suckers (about 5-40cm in height) of each Musa cultivar were used. Two sword suckers (about 5-40 cm in height) of each Musa cultivar were used. The corms of each cultivar were cut through to remove apical dominance, and were treated with neem leaves extract/fungicide, and allowed to air dry for 72 hours under a shade. This was followed by planting the corms of each cultivar in high humidity plastic propagators. The experiment was laid out in randomised complete block design with three replications. The results indicated that there was no significant difference among the Musa cultivars in their response to micropropagation. The mean number of sprouted shoots per corm for plantain was 3.2-4.3. while banana and hybrid had 1.0 and 1.5 respectively. The mean total number of plantlets produce among plantain cultivar varied from 8.7-14.0, while banana and hybrid banana 3.0. The response of banana and plantain bud manipulation technique depends on the variety, and since the technology is simple and affordable there is hope of high farmer adoption through workshop training and collaborative research with farmers.

# CHAPTER ONE

# INTRODUCTION

Banana and plantain evolved by natural hybridization between *Musa accuminata* (contributing genome A) and Musa balbisiana (contributing genome B) (Heslop-Harrison and Schwarzeder, 2007; Ngomnoel et al., 2014).

Bananas and plantain (Musa spp.) are important staple foods for nearly 400 million people in many developing countries, especially in Africa where bananas and plantains provide more than 25% of food energy requirement for over 70 million people. The pulp of a ripe banana is essentially a super-rich, easily digested food. The cooked banana is nutritionally similar to the potato. Bananas consist of about 70% water, 27% carbohydrate, 1.2% protein and 0.3% fat. In energy terms, each gram provides one calorie. The fruit is considered a good source of vitamins A, B1, B2 and C (Arvanitoyannis et al., 2008a).

The nutritional qualities of bananas and plantains have given the crops a huge market demand and this required large healthy and uniform plantations for the sustainable export of the crop (Roux et al., 2001; Izsy 2001; Ray et al., 2006), which raises the need for large quantities of planting materials. The limitation however is that plantains and bananas have a slow sucker production rate and also produces them at different times, giving non-uniform production. This results in a definite number of planting materials, which are also non-uniform for commercial production.

This has led to the need to use other protocols to ensure the production of large numbers of uniform and healthy suckers for commercial production, tissue culture technique has been one of such techniques for the mass propagation of bananas and plantains in vitro.

Bananas and plantain can be propagated aseptically in the laboratory through tissue culture technique. In-vitro micro-propagation eliminates all sucker-transmitted pests and diseases with the exception of viruses (Bush et al., 2010). The benefits of the tissue culture technique in the mass propagation form of planting materials is however not sustainable to the farmers in developing countries due to the cost of producing these in-vitro plantlets (Gitongaet al., 2010). Apart from the fact that it is expensive, it is also delicate and hence cannot easily be carried out by the farmers on their own (Gitonga et al., 2010).

The International Institute of Tropical Agriculture (IITA) has been investigating alternative means of producing clean planting materials; as a way of coming up with alternatives that are comparable to tissue culture and macro-propagation has been considered as suitable alternative, (Bush and Tachiu-Henson, 2015). Macro-propagation is a relatively easy technique that is carried out in a shed or even in the field (Baiyeri and Aba, 2005; schill et al., 2000). It consists of generating suckers from clean planting material by removing apical dominance. Macro-propagation can be classified into two categories: Field-base techniques, based on complete or partial decapitation and detached corm techniques, practiced in a shed and this method could be an excellent option for producing low cost quality planting material in large quantities and also reduces the cost of producing planting material (Gitoganetal., 2010) and has the potential of producing 50-60 shoots per sucker in 4-5 months. This is a simple method because of the ease of the protocol for plantlet regeneration. Macro- propagation is achieved by two methods and could be adopted either in the field conditions (in situ) or in the nursery (ex-situ). It involves decapitation, decortication and hardening. A more easily adopted technology of propagation is the bud manipulation method, which is less expensive.

**Objective of the Study**

The objective of this study was to evaluate the response of banana and plantain cultivars to macro- propagation using bud manipulation technique.

# CHAPTER TWO

# LITERATURE REVIEW

## Systematics

Banana and plantain belong to the family Musacea. They evolved by natural hybridization between *Musa accuminata* contributing genome A) and *Musa balbisiana* (contributing genome B) Heslop- harrison and Schwarzeder, 2007; Ngomuo et al., 2014), Worldwide, there is no sharp distinction between "bananas" and "plantains", especially in the Americas and Europe, "banana" usually refers to soft, sweet, and dessert bananas, particularly those of the Cavendish group, which are the main exports from banana-growing countries. By contrast, Musa cultivars with firmer, starchier fruit are called "plantains". In other regions, such as Southeast Asia, many more kinds of banana are grown and eaten, so the simple two-fold distinction is not useful and is not made in local language.

The term "banana" is also used as the common name for the plants which produce the fruit. This can extend to other members of the genus Musa like the scarlet banana (*Musa coccinea*), pink banana (*Musa velutina*). It can also refer to members of the genus Ensete, like the snow banana (*Enseteglaucum*) and the economically important false banana (*Enseteventricosum*). The scientific names of most cultivated bananas are Musa acumunata, *Musa balbisaina*, and Musa paradisiaca for the hybrid *Musa acuminate* and *Musa albisiana*, depending on their genomic constitution. The old scientific name Musa sapientum is no longer used. Musa is one of two or three genera in the family Musa cae; it includes bananas and plantains. Around 70 species of *Musa* are known with abroad variety of uses. The genus Musa was first named by Carl Linnaeus in 1753, as a latinization of the Arabic name for the fruit, Mauz.

From the time of Linnaeus until the 1940s, different types of edible bananas and plantains were given Linnaeus binomial names, such as Musa cavendishii, as if they were species. The genus Musa has an extremely complicated origin involving hybridization, mutation, and finally selection by humans. Most edible bananas are seedless (parthenocarpic), hence sterile, so they are propagated vegetatively. The giving of species names to what are actually very complex, largely asexual, hybrids (mostly of two species of wild bananas, Musa acuminate and Musa balbisiana) led to an endless confusion in banana botany. In the 1940s and 1950s, it became clear to botanists that the cultivated bananas and plantains could not usefully be assigned Linnaean binomials, but were better given cultivar names. When the Linnaean binomial system was abandoned for cultivated bananas, an alternate genome- based system for the nomenclature of edible bananas in genus Musa was devised. Thus, the plant previously known by the "species" name *Musa cavendishii* became Musa (AAA Group) "Dwarf Cavendish". The "new" name shows clearly that Dwarf Cavendish is a triploid, with three sets of chromosomes, all derived from *Musa accuminata*, which is designated by the letter "A". When Musa balbisiana is involved, the letter "B" is used to denote its genome. Thus, the cultivar 'Rajapuri" may be called Musa (AAB Group) 'Rajapuri. Rajapuri is also a triploid, expected to have two sets of chromosomes from e from Musa balbisiana. In the genome of edible banana from the genus Musa, combinations such as AA, BB, ABB and even AAAB can be found.

## Origin and Distribution

*Musa* species are native to tropical, Indomalaya and Australia and are likely to have been first domesticated in Papua New Guinea. They are grown in 135 countries, primarily for their fruits and to a lesser extent to make fiber, banana wine and banana beer and as ornamental plants.

In 2016, world production of bananas and plantains was 148 million tonnes, led by India and China with a combined total (only for bananas) of 28% of global production (Table 1). Other major producers were the Philippines, Ecuador, Indonesia, and Brazil, together accounting for 20% of the world total of bananas and plantains.

## Botanical Description

The banana plant is the largest herbaceous flowering plant. The banana is an edible fruit botanically a berry produced by several kinds of large herbaceous flowering plants in the genus Musa In some countries, bananas used for cooking may be called plantains, in contrast to dessert bananas. The fruit is variable in size, colour and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be green, yellow, red, purple or brown when ripe. All the above-ground parts of a banana plant grow from a structure usually called a "corm". Plants are normally tall and fairly sturdy, and are often mistaken for trees, but what appears to be a trunk is actually a "false stem" or pseudostem". Bananas grow in a wide variety of soils, as long as the soil is at least 60cm deep, has good drainage and is not compacted. The leaves of banana plants are composed of a "stalk" (petiole) and a blade (lamina). The base of the petiole widens to form a sheath; the tightly packed sheaths make up the pseudo stem, which all that supports the plant. The edges of the sheath meet when it is first produced, make it tabular. As new growth occurs in the centre of the pseudo stem, the edges are forced apart. Cultivated banana plants vary in height depending on the variety and growing conditions. Most are around 5m (16ft) tall, with a range from "Dwarf Cavendish" plants at around 3m (10ft) to "Gros Michel" at 7m (23ft) or more. Leaves are spirally arranged and may grow 2.7 meters (8.9ft) long and 60cm (2.0ft) wide. They are easily torn by the wind, resulting in the familiar frond look.

When a banana plant is mature, the corm stops producing new leaves and begins to form a flower spike or inflorescence. A stem develops which grows up inside the pseudostem, carrying the immature inflorescence until eventually it emerges at the top. Each pseudo-stem normally produces a single inflorescence, also known as the "banana heart". (More are sometimes produced; an exceptional plant in the Philippines produced five). After fruiting, the pseudostem dies, but offshoots will normally have developed from the base, so that the plant as a whole is perennial. In the plantation system of cultivation, only one of the offshoots will be allowed to develop in order to maintain spacing.

The inflorescence contains many bracts (sometimes incorrectly referred to as petals) between rows of flowers. The female flowers (which can develop into fruit) appear in rows further up the stem (closer to the leaves) from the rows of male flowers. The ovary is inferior, meaning that the tiny petals and other flower parts appear at the tip of the ovary.

The banana fruits develop from the banana heart, in a large hanging cluster, made up of tiers (called "hands"), with up to 20 fruit to a tier. The hanging cluster is known as a bunch, comprising 3-20 tiers, or commercially as a "banana stem", and can weigh 30-50 kilograms (66-110lb). Individual banana fruits (commonly known as a banana or "finger") average 125 grams (0.276 lb), of which approximately 75% is water and 25% dry matter (Table 2). The fruit has been described as a "leathery berry".

There is a protective outer layer (a peel or skin) with numerous long, thin strings (the phloem bundles), which run lengthwise between the skin and the edible inner portion. The inner part of the common yellow dessert variety can be split lengthwise into three sections that correspond to the inner portions of the three carpels by manually deforming the unopened fruit. In cultivated varieties, the seeds are diminished nearly to non-existence; their remnants are tiny black specks in the interior of the fruit. Bananas are naturally slightly radioactive, more so than most other fruits, because of their potassium content and the small amounts of the isotope potassium-40 found in naturally occurring potassium. The banana equivalent dose of radiation is sometimes used in nuclear communication to compare radiation levels and exposures.

**Production and Export**

As reported for 2013, total world exports were 20 million tonnes of bananas and 859,000 tonnes of plantains. Ecuador and the Philippines were the leading exporters with 5.4 and 3.3 million tonnes, respectively, and the Dominican Republic was the leading exporter of plantains with 210,350 tonnes. In the table below, some countries produce statistics distinguishing between bananas and plantain production, but four of the top six producers do not, requiring comparisons using the total for bananas and plantains combined.

**Table 1. 1. Global Production of banana and plantain in millions of tonnes**

| Country | Bananas | Plantains | Total |
|---|---|---|---|
| India | 29.1 | | 29.1 |
| China | 13.1 | | 13.1 |
| Philippines | 5.8 | 3.1 | 8.9 |
| Ecuador | 6.5 | 0.6 | 7.1 |
| Indonesia | 7.0 | | 7.0 |
| Brazil | 6.8 | | 6.8 |
| Colombia | 2.0 | 3.5 | 5.5 |
| Cameroon | 1.2 | 4.3 | 5.5 |
| Uganda | 0.6 | 3.7 | 4.3 |
| Ghana | 0.09 | 4.0 | 4.1 |
| Guatemala | 3.8 | 0.3 | 4.1 |
| **World** | **113.3** | **35.1** | **148.4** |

Source: FAOSTAT of the United Nations

## Banana Propagation and Conservation of Germplasm

Bananas and plantains are usually propagated mainly in the tropics- regions within latitude 320 north (tropics of Capricorn) and latitude 32º south (tropics of cancer). Bananas grow in a wide variety of soils, as long as the soil is at least 60cm deep, has good drainage and is not compacted. The field propagation is optimally a spacing of 1 meter x 1 meter using sword suckers. However, novel technologies include the use of aseptic tissue propagation to generate suckers and the adventuring use of bud manipulation technique to generate suckers (macro-propagation).

Given the narrow range of genetic diversity present in bananas and the many threats via biotic (pests and diseases) and abiotic (such as drought) stress, conservation of the full spectrum of banana genetic resources is ongoing. Banana germplasm is conserved in many national and regional gene banks, and at the world's largest banana collection, the International Musa Germplasm Transit Centre (ITC), managed by Bioversity International and hosted at KU Leuven in Belgium. Musa cultivars are usually seedless, and options for their long-term conservation are constrained by the vegetative nature of the plant's reproductive system. Consequently, they are conserved by three main methods: in vivo (planted in field collections), in vitro (as plantlets in test tubes within a controlled environment), and by cryopreservation (meristems conserved in liquid nitrogen at-196 °C). Genes from wild banana species are conserved as DNA and as cryopreserved pollen and banana seeds from wild species are also conserved, although less commonly, as they are difficult to regenerate. In addition, bananas and their crop wild relatives are conserved in situ (in wild natural habitats where they evolved and continue to do so). Diversity is also conserved in farmers' fields where continuous cultivation, adaptation and improvement of cultivars is often carried out by small-scale farmers growing traditional local cultivars (including home gardens).

**Consumption and Utilization**

Plantains could be eaten raw when ripe, roasted, fried, boiled and milled into flour to produce further more by-products like plantain/banana bread, beer etc. Raw bananas (not including the peel) are 75% water, 23% carbohydrates, 1% protein, and contain negligible fat. A 100-gram serving supplies 89 Calories, 31% of the US recommended Daily Value of vitamin B6, and moderate amounts of vitamin C, manganese and dietary fiber (see table below).

**BANANAS, RAW (DAILY VALUE)**

**Nutritional value per 100 g (3.5 oz)**

| | | |
|---|---|---|
| **Energy** | 371 kJ (89 kcal) | |
| **Carbohydrates** | 22.84 g | |
| Sugars | 12.23 g | |
| Dietary fiber | 2.6 g | |
| **Fat** | 0.33 g | |
| **Protein** | 1.09 g | |
| **Vitamins** | **Quantity** | **%DV†** |
| Thiamine (B1) | 3% | 0.031 mg |
| Riboflavin (B2) | 6% | 0.073 mg |
| Niacin (B3) | 4% | 0.665 mg |
| Pantothenic acid (B5) | 7% | 0.334 mg |
| Vitamin B6 | 31% | 0.4 mg |
| Folate (B9) | 5% | 20 µg |
| Choline | 2% | 9.8 mg |
| Vitamin C | 10% | 8.7 mg |
| **Minerals** | **Quantity** | **%DV†** |
| Iron | 2% | 0.26 mg |
| Magnesium | 8% | 27 mg |
| Manganese | 13% | 0.27 mg |
| Phosphorus | 3% | 22 mg |
| Potassium | 8% | 358 mg |
| Sodium | 0% | 1 mg |
| Zinc | 2% | 0.15 mg |
| **Other constituents** | **Quantity** | |
| Water | 74.91 g | |

- Units
- µg = micrograms • mg = milligrams
- IU = International units

Percentages are roughly approximated using US recommendations for adults.

Source: USDA Nutrient Database

## POTASSIUM

Although bananas are commonly thought to supply exceptional potassium content, [91] their actual potassium content is relatively low per typical food serving at only 8% of the US recommended Daily Value (Table 2). Vegetables with higher potassium content than raw dessert bananas (358 mg per 100 grams) include raw spinach (558 mg per 100 grams), baked potatoes without skin (391 mg per 100 grams), cooked soybeans (539 mg per 100 grams), grilled portabella mushrooms (437 mg per 100 grams) and processed tomato sauces (413-439 mg per 100 grams). Raw plantains contain 499 mg potassium per 100 grams. Dehydrated dessert bananas or banana powder contain 1491 mg potassium per 100 grams.

## Problems of Ripening, Storage and Transportation

### Ripening

Export bananas are picked green, and ripen in special rooms upon arrival in the destination country. These rooms are air-tight and filled with ethylene gas to induce ripening. The vivid yellow color consumers normally associate with supermarket bananas is, in fact, caused by the artificial ripening process. Flavor and texture are also affected by ripening temperature. Bananas are refrigerated to between 13.5 and 15 °C (56.3 and 59.0 °F) during transport. At lower temperatures, ripening permanently stalls, and the bananas turn gray as cell walls break down. The skin of ripe bananas quickly blackens in the 4 °C (39 °F) environment of a domestic refrigerator, although the fruit inside remains unaffected.

Bananas can be ordered by the retailer "ungassed" (ie. not treated with ethylene), and may show up at the supermarket fully green. Guineos verdes (green bananas) that have not been gassed will never fully ripen before becoming rotten. Instead of fresh eating, these bananas can be used for cooking, as seen in Jamaican cuisine. [66]

A 2008 study reported that ripe bananas fluoresce when exposed to ultraviolet light. This property is attributed to the degradation of chlorophyll leading to the accumulation of a fluorescent product in the skin of the fruit. The chlorophyll breakdown product is stabilized by a propionate ester group. Banana-plant leaves also fluoresce in the same way. Green bananas do not fluoresce. The study suggested that this allows animals which can see light in the ultraviolet spectrum (tetrachromats and pentachromats) to more easily detect ripened bananas.

**Storage and Transport**

Bananas must be transported over long distances from the tropics to world markets. To obtain maximum shelf life, harvest comes before the fruit is mature. The fruit requires careful handling, rapid transport to ports, cooling, and refrigerated shipping. The goal is to prevent the bananas from producing their natural ripening agent, ethylene. This technology allows storage and transport for 3-4 weeks at 13 °C (55 °F). On arrival, bananas are held at about 17 °C (63 °F) and treated with a low concentration of ethylene. After a few days, the fruit begins to ripen and is distributed for final sale. Ripe bananas can be held for a few days at home. If bananas are too green, they can be put in a brown paper bag with an apple or tomato overnight to speed up the ripening process.

Carbon dioxide (which bananas produce) and ethylene absorbents extend fruit life even at high temperatures. This effect can be exploited by packing banana in a polyethylene bag and including an ethylene absorbent, e.g., potassium permanganate, on an inert carrier.

The bag is then sealed with a band or string. This treatment has been shown to more than double lifespans up to 3-4 weeks without the need for refrigeration.

# CHPATER THREE

# MATERIALS AND METHODS

The experiment was conducted using the following protocols

**Source of Planting Materials**

The Musa cultivars (three plantain, one Musa hybrid and one banana) were obtained from the Department of Crop Science, University of Benin, Benin City. The five varieties are designated as follows: FT04UG, FTOSBC, FTO9LC, IITAHB, MSOIBA.

**Preparation of Planting Materials**

Cleaning of suckers, Bud Manipulation and treatment with phytochemical and fungicide. The corms of young sword suckers of up to 3-5mm above the corm, each cultivar about 5-45cm in heights were harvested and the shoots were cut back to reach the corm with the buds in the corm being visible. The roots were also removed. The corms with the visible buds were cross-cut at the centre to remove the apical dominance.

It was then further washed thoroughly in running water to wash away any traces of infections. The corms were then treated with Azadichrata indica (ncem) leaves extracts mixed with crushed garlic and fungicide.

The treated corms were left to dry for about 24 hours before sowing into propagators stacked with a bottom layer of 10cm sharp sand and a top layer of saw dust of about 20cm thick.

**Experimental Site, Design, Propagators, Planting and Experimental Layout**

The experiment site was carried out at the Department of Crop Science Horticultural Nursery, University of Benin, Benin City, Edo State Nigeria. The Experiment was laid out in complete block design with three replications. Three corms of each cultivar were planted in separate plastic propagators. The propagators were carried out with transparent polythene in other to create a very humid atmosphere for the banana/ plantain suckers. After sowing, watering was delayed till the following day.

Plastics for propagators materials are not perforated at the bottom and moderate watering is needed subsequently to avoid fungal infestation. The propagation was covered with transparent polythene to provide hematic covering and placed under thatched shade of 50% sunlight penetration. The growth of plantlets was observed 20-40 days after sowing and multiple shoots were cut with sharp knifeand transplanted polythene bags placed under a shade.

**Cultural practices**

**Watering**

Watering was done periodically to ensure the propagating chamber was kept humid but not flooded to avoid fungal infestation.

**Weeding**

Periodic weeding was done to avoid nutrient competition by competing weeds and to prevent alternate host to insect pest.

**Plantlets excision**

Two to three weeks after planting, growth of young plantlets were usually observed are excised from the corm and transplanted into polythene bags filled with compost mostly with roots to ensure casy survival.

**Care of plantlets**

Plantlets are also taken care of under the same shade made for the propagator in the nursery to prevent the attack of insect infestation under warm temperature. The regime of water application was also maintained as required for the young plantlets.

**Data Collection and Statistical Analysis**

The data were subjected to analysis of variance and means were compared using Student Neuman Keul Test.

# CHAPTER FOUR

## RESULTS

The experiment tested for number of shoots per corm, shoot height, and total number of plantlets and number of plantlets per corm are presented in Table 1 as shown below:

**Table 1: Response of** Musa **cultivars to micropropagation using bad manipulation techniques**

| Cultivar | Number of Sprouted shoots per corm | Shoot height (cm) | Total number of plantlets | Number of plantlet per corm |
|---|---|---|---|---|
| FT04UG | 4.3 | 8.7 | 14.0 | 4.7 |
| FT05BC | 4.0 | 11.5 | 8.7 | 2.9 |
| FT09LC | 3.2 | 19.3 | 12.7 | 4.2 |
| IITAHB | 1.5 | 4.5 | 3.0 | 1.0 |
| MS01BA | 1.0 | 0.5 | 3.0 | 1.0 |

However, data for total number of plantlets are presented graphically in a bar chart in Figure 1 below.

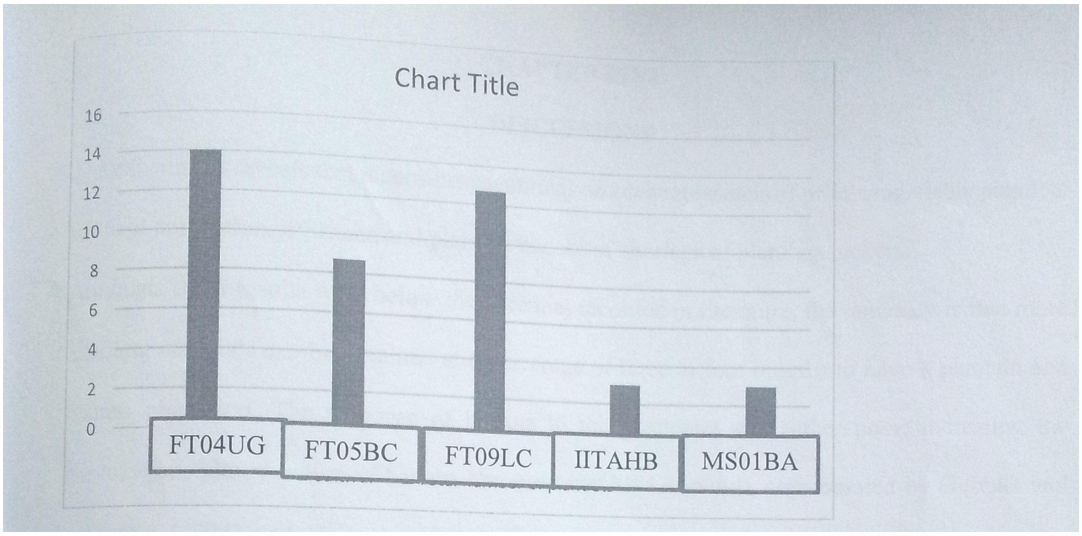


Figure 4.1: Bar Chart the relation between the cultivars and total number of Plantlets

The result showed that FT04UG cultivar (plantain) had an average sprouted shoots of 4.3 short height of 8.7cm 14.0 mean number of plantlets. FT05BC cultivar (plantain) had an average of 4.0 sprouted shoots, 11.5cm short height and 8.7 mean total number of plantlets. FT09LC cultivar (plantain) had an average of sprouted shots, 19.3cm short height and 12.7 mean total number of plantlets. IITAHB cultivar (plantain) had an average of 1.5 sprouted shoots, 4.5cm shoot height and 3.0 mean total number of plantlets. MS01B cultivar (plantain) had an average of 1.0 sprouted short, 0.5cm short height and 3.0 mean total number of plantlets.

The ANOVA showed there were no significant differences among the Musa cultivars.

# CHAPTER FIVE

## DISCUSSIONS

The experiment reveals that macro-propagation is an adequate means of producing viable plantlets for field cultivation of banana and plantain in case of shortage of planting suckers. Although, these results were below the potential recorded in literature, the tendency is that more shooting materials can be obtained at the average of three to four months to have a plantain and banana plantation. The response of banana to this treatment was rather poor; indicating the homozygous ploidy nature of banana chromosome (AAA-triploid), corroborated by Gubbuk and pekmezci (2004) and Baiyeri and Aba (2007). Thus the genotype of a given plant species determines its bud proliferation in vitro-Baiyeri and Aba (2007).

I hereby recommend this practice to potential plantain farmers who which to cultivate larger hectares with the aim of more economic benefit.

# CHAPTER SIX

## CONCLUSION AND RECOMMENDATION

In conclusion to this experiment, I will say there is hope for more suckers production using bud manipulation technique as an alternative to tissue culture for ease of production and cost reduction.

Economically, production economics support this production technique; as output maximization is achieved at input minimization. Giving the nature of tropical Agriculture with huge constraints of production factors, this method with some level of man power will ensure the availability of plantain/ banana produce all through the year.

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