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In vitro Protocol Developed for Mass Propagation of Matoke and Nijiru Cultivars Cooking Banana *(Musa Sapientum L.)*

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Abstract

Edible bananas *(Musa spp.)* are the major staple food for rural and urban consumers in the tropical and subtropical countries and an important source of rural income. Banana is the most produced fruit in Ethiopia for food and commercial purposes. However, there are constraints in the production of banana. Among the banana production constraints, low propagation, low quality and quantity of yield. The study was aimed to develop a simple, comprehensive and efficiently repetitive protocol for micropropagation of banana *(Musa sapientum L.)*. For this experiment the banana cultivars used were Matoke and Nijiru. Those two cultivars were taken from the field and grew under green house for three month and taken to the laboratory for initiation. After two month of the culture initiation the developed culture shoot proceed to the shoot multiplication and rooting media.

Keywords: MS medium; Shoot multiplication; Secondary hardening; Proliferated shoots

Introduction

Bananas *(Musa Sapientum L.)* are the most important food crop in the world, with a production approximating 102 million tons per year. It is the world's most widely known and distributed fruit, eaten either raw or cooked, and may be processed into starch, chips, beer, vinegar or dried products. Banana are rich in energy, and vitamin C and A. Banana fruits have a very high content of potassium and a wide K/Na ratio, imparting a protective effect of K against excessive Na intake in diets.

Edible bananas *(Musa spp.)* are the major staple food for rural and urban consumers in the tropical and subtropical countries and an important source of rural income. The genus *Musa* (family Musaceae) originates in Asia. Cultivated banana is derived from two diploid species of genus *Musa. M.* acuminates and M. balbiciana parent genomes [1]. Banana is a good source of carbohydrates, proteins, vitamins and minerals. Many pest and diseases constrain banana production which resulted in serious consequences for environment through the application of pesticides. Thus, major constraints in the banana production system are the non-availability of disease-free, true-to-type planting material, low fertility due to triploidy, slow propagation and long time span from one generation to the next generation. Classical breeding is difficult because of its high degree of sterility and polyploidy of the edible varieties [2].

Bananas belong to group of crops which are normally propagated through vegetative parts of the plant because almost all cultivated banana cultivars are triploid, seedless, or seed sterile. The materials used for conventional propagation include corms, large and small suckers, and sword suckers. Mass propagation of selected genotypes, Somaclonal variation techniques, genetic engineering and other biotechnological applications can be utilized for banana crop improvement which is based on reliable plant regeneration protocols. Tissue culture also plays a vital role in the distribution of germplasm, conservation, safe exchange of internal planting material and rapid propagation of newly selected hybrid cultivars. Several researchers have reported the regeneration of *Musa spp. via* micropropagation [3]. But, propagation percentage and repeatability of the method are matters of concern which ultimately need a comprehensive, repeatable and applied method for a wide range of genotypes to facilitate disease free production of banana crop on commercial scale.

For *in vitro* micropropagation of banana, bacterial contamination is a big problem. Although initially surface sterilization works, latter on microbial contamination at the base of the explant appears within 7 to 15 days after inoculation. Huge number of explants is destroyed in the culture due to endogenous bacteria. The present study suggests a rapid banana multiplication protocol from shoot meristem by using a medium with optimized concentration of auxins cytokinins. Many reports are available on *in vitro* propagation with complicated protocols but less shoot proliferation percentage which eventually yield less number of regenerated plants per culture.

Ethiopian farmers had long time experience of planting and producing this fruit. But they didn't obtain plant materials that free from contaminants. So, to providing farmers with desired planting materials is important through optimizing *in vitro* protocol for selected cultivars [4]. Bananas are asexually propagated by either separating the daughter suckers from the mother plant or tissue culture. Tissue culture, unlike the sucker-

derived planting material has various advantages. These include producing uniform planting materials clean, disease-free materials and many plants in a small space. It offers mass propagation and clean planting material. They are cheaper to transport than conventional suckers and the coupling with virus indexing allows for safe movement, and exchange and conservation of germplasm.

In addition, bananas produced using tissue culture technology are reported to be more vigorous, higher yielding and produce better quality fruits than those produced by conventional means. Inspite of these advantages, tissue culture technology remains unknown orunder exploited in Africa. So, to exploit the merits of this technology, it is inevitable to do protocol optimization work. For this plant, growth regulators are crucial for *in vitro* regeneration of crop plants in artificial medium. Cytokines helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokines and auxins depends on the variety of banana and culture condition. Plantains-cooking banana which might play vital role in food security of Ethiopia. Thus developments of a protocol for shoot tip culture of cooking banana are primarily important to increase production and productivity for banana product to ensure food security of the country.

Statement of the problem

Bananas play an important role in the food security of developing countries, especially in sub-Saharan Africa. They are cultivated in over 120 countries covering about 10 million ha, with an annual production of 130 million tons. In Africa, banana and cooking banana provide more than 25% of the food energy requirements for more than 100 million people, of 20 million are from East Africa alone. Banana is the most produced fruit in Ethiopia for food and commercial purposes. However, there are constraints in the production of banana. Among the banana production constraints, low propagation, low quality and quantity of yield. There is a need for biotechnological applications to improve the production of disease free, develop micropropagation protocols and quality plantlets. Therefore, to optimize development of a reliable and efficient regeneration and transformation protocol of farmer-preferred cultivars using micro propagation and meristematic tissues as explants is a prerequisite for mass propagation of banana.

Significance of the study

Bananas are one of the most studied plants in the field of plant biotechnology. The huge economic importance and the inherent problems associated with conventional propagation of bananas makes the species one of the highly prioritized research crops globally. In addition, an increase in demand by the world growing population implies the need for more planting materials of high quality. Generally, the importance of this study is to develop an optimized micropropagation protocol which could be used in different labs for the rapid micropropagation and dissemination of elite banana cultivars. The work will have a significant impact in addressing food security issues by increasing production and productivity of the banana.

Objectives

General objective

To develop rapid micropropagation protocols of Matoke and Nijiru cultivars cooking banana *(Musa Sapientum L.)*

Specific objectives

- To develop protocols for initiation of Matoke and Nijiru cooking banana cultivars,
- To develop protocols for shoot multiplication of Matoke and Nijiru cooking banana cultivars
- To develop protocols for rooting and rapid mass propagation using shoot culture for quantity and quality improvement of bananas and growth under green house

Literature review

Banana: The genus *Musa L*. consists of four sections: Callimusa, Australimusa, Rhodochlamys and Eumusa. Cultivated bananas belong to the section Eumusa and were formed by interspecific hybridization between *M. acuminate Colla* and *M. balbisiana Colla*, which contributed the A and B genomestohybridprogeny, respectively. Somaticmutations gave rise to several clonal forms of important cultivars with morphological differentiated characteristics. Characterization of banana cultivars is based on 15 morphological characteristics that were proposed. The banana plant is a giant perennial herb that consists of a rhizome and a pseudostem. The rhizome is found underground with a shallow root system and several vegetative buds, from which the suckers develop. The erect pseudostem is composed of several tightly packed leaf bases that are the initial outgrowths of a centrally located apical meristem. At the latter stages, the apical growing point differentiates into an inflorescence meristem. The inflorescence consists of two parts: the male bud and female flowers. The banana fruit develops from the female flowers [5].

Most of the commercially grown banana cultivars are sterile and parthenocarpic. Banana plant reproduces by forming suckers from a vegetative bud on the rhizome. A banana mother plant can produce five to ten suckers per year. The suckers are removed from the mother plant and used as starting material in a new field. Planting material can also be generated in the laboratory by means of *in vitro* propagation. For they a *in vitro* propagation, the shoot tips are isolated from the banana suckers under sterile conditions. Unlimited numbers of shoot tips can be obtained by subdividing the shoot clusters. Shoottip culture of banana is an easy technique to accomplish a 10-fold increase in multiplication rate after every subsequent culture. In addition, *in vitro* plants are an essential source of disease-free plant material apart from viruses.

Tissue culture of banana: Micropropagation of bananas remain as alternative of natural regeneration with tremendous potential for production of quality planting material [6]. Thus, the farmers several antibiotics which are successful in controlling bacterial contaminants in banana tissue culture.

are benefited by tissue-cultured plants because of ease of multiplication of their variety of choice and also suggested that micropropagation is user-friendly technique which does not require much expertise and is suitable for adoption by small and marginal farmers. The success of micro propagation depends on method, variety and price of initiation media. Further, evaluated a micropropagation protocol for local banana in Kenya as an alternative to reduce the unit cost of tissue culture micro propagation. Reported that the planting materials of banana obtained through conventional methods (suckers) do not meet the increasing demand for planting and they are of poor quality.

Micropropagation as the practice of growing plants like banana from meristematic tissue or somatic cells of superior plants *in vitro*. Cultures are established on a separate initiation medium, which has a lower concentration of cytokinin than the multiplication medium, to which cultures are subsequently transferred. Cultured banana explants on MS medium supplemented with 0.5 mg/l BA, Kn and NAA and found that addition of 10% coconut water to the medium resulted in increased number of differentiated shoots per culture. It was also found that acclimatization and transplantation performances of plants was superior for those rooted in liquid medium compared to those rooted in solid medium. The effect of carbon sources (sucrose, glucose, fructose and mannitol) on *in vitro* propagation of banana and found the highest frequency of shoot proliferation on the medium containing sucrose compared to all other carbon sources. It was also reported that MS medium supplemented with sucrose and glucose combination at the concentration of 30 g/l showed the optimum shoot proliferation. Further, optimized medium for *in vitro* proliferation of banana and found that 40 g/l sugar not only facilitated proliferation of buds but also controlled proportion of buds with leaf sheaths and increased the available bud index. The effect of nutrient medium constituents on growth and development of banana plantlets produced *in vitro* was the best evaluated [7]. He found that the medium with sucrose at 30 g supplemented with 0.4 mg/l BAP was the most optimum for banana shoot tip culture as expressed by better growth vigour, plantlet height, fresh weight as well as stronger shoot and root system. Investigated the effect of different carbohydrate source, pH and supporting media on *in vitro* rooting of banana plantlets using MS medium with 0.1 mg/l IBA and activated charcoal. Sucrose in the medium remarkably influences the rooting of plantlets. In the absence of sucrose, culture could not survive after 3 weeks of incubation. In the sucrose containing media, 30 g/l gave the best result. Out of different pH levels tested, minimum time for root initiation with longest length of root was obtained at pH 5.5.

Explain choice and sterilization: Reported that *in vitro* can been cultures of both pineapple and plantain are much growth shoot can be initiated from any growing point of the plant. Thus, dormant buds and shoots that are unsuitable for conventional propagation rates of multiplication *in vitro* are much higher than those achievable in conventional propagation systems. cultured shoot tips, eye bud or floral apex explants of banana cv. Red banana on semi-solid MS medium supplemented with various growth regulators [8]. That banana shoot tips derived from different suckers can be used to study multiplication rate in banana. Were reported that micropropagating banana through shoot tip is the main method used for fast propagation of banana plants. Reported that the protoplast culture and somatic hybridization are feasible technique of micropropagation and to support the genetic improvement of banana. Used male inflorescence tip explants and shoot tip of banana for studying micropropagation in banana. Studied the effect of three different sizes of explants (5, 10 and 20 mm) on the establishment of banana in micropropagation. Three cultivars were used for the study. They found that larger explants (20 mm) responded well with regard to survival of explants, days to swelling and greening of explants, emergence of leaf and days to multiple bud initiation under *in vitro* condition as compared to smaller explants. Explants should be free from surface contaminants to accomplish growth and development under aseptic conditions and this is achieved by surface sterilization. The sterilization treatment should be so selected that it kills the microbes without affecting the plant tissues adversely. Contamination in tissue culture may be caused by endogenous bacteria that escape initial disinfection or by microorganisms introduced during tissue culture manipulations. There are several antibiotics which are successful in controlling bacterial contaminants in banana tissue culture.

Evaluated two methods of banana explant disinfection and the use of 3 indicator culture media for banana micropropagation. Shoot tips of the cultivar Pioneira were disinfected by immersion in 80% alcohol for 2 min and in sodium hypochlorite (2% active chlorine) with 4 drops of Tween-20 for 10 min, with shaking. The contamination observed was only of bacterial nature. Lima and Moraes (2006) evaluated the bacterial contamination control methods in banana explants cv. Caipira using NaOCl, rifampicin antibiotic and their combinations. The best treatment for explants was the immersion in 1% (v/v) NaOCl for 10 minutes, followed by immersion in 300 mg/l rifampicin for 20 minutes. After contamination, the best treatment was the immersion in 1% NaOCl for 10 minutes, followed by immersion in 300 mg/l rifampicin for 24 hours in the dark.

The disinfectants widely used are sodium hypochlorite (which dates back to the mid-18th century), calcium hypochlorite, ethanol (or isopropyl alcohol), mercuric chloride, hydrogen peroxide, silver nitrate and bromine water to enhance effectiveness in sterilization procedure, a surfactant like Tween-20 is frequently added to the sterilizing solution (and in some laboratories a mild vacuum is applied during the procedure). In general, the sterilizing solutions containing the explants are continuously stirred during the sterilization period [9]. The effects of different disinfectant on explants. Mercuric chloride (0.1%) and 0.2% sodium hypochlorite solutions were used to sterilize the sucker buds of Longxuan banana which were used as explants. Sodium hypochlorite showed better effects than mercuric-chloride on the disinfection of explants, the sterilization rate was 90.47% and the explants grew well without any intoxication. The sterilization of explants by firstly treating with savlon for 15 minutes followed by sterilizing with a mixture of 2% NaOCl+1 gm/l Captan or DithaneM-45 and rifampicin (0.1 %) for 45 minutes with Tween-20. Thereafter,

quick dipping of explants (15 sec) in 70 % ethanol. On laminar air flow cabinet explants treated with NaOCl (0.5-1%) for 15 minutes followed by treatment with 0.1% HgCl2 for 7 minutes proved better than the other treatments. Studied the aseptic culture establishment using antibiotics with reference to their efficiency and phytotoxicity in difficult-to-establish native Ney Poovan banana (Musa *AB)*. They standardize antibiotic supplement for obtaining aseptic cultures in native banana variety Elakki Bale. Of the different antibiotics and combinations tried, chloramphenicol was found to give 100 % aseptic cultures followed by rifampicin+chloramphenicol, rifampicin and chloramphenicol+streptocycline combination. Rifampicin was found to cause least phytotoxicity. The plantlets grew normally in subsequent cycles and 96.3 % plantlets could survive up on transfer to ex vitro conditions.

Surface sterilization of the explants primarily by rinsing in tap water for 30 minutes followed by gently rinsing with 70% ethanol for 60 seconds and with 5% sodium hypochlorite solution for 10 minutes. Further, sterilization procedures were carried out in laminar air flow chamber by using 0.1% HgCl2 for 5 minutes. Condition of culture room light, temperature and humidity conditions provided inside the culture room play a significant r ole in success of an *in vitro* technique. The light intensity, quality and duration are the major factors affecting the growth of *in vitro* culture. Found a 16 h photoperiod satisfactory for a wide range of plant species. Relative humidity of the culture room is adjusted to 55 ± 5 %. Banana shoot tip cultures are incubated at an optimal growth temperature of 28 ± 2ºC in a light cycle of 12-16h with a photosynthetic photon flux (PPF) of about 60 µE/m2/s.

Effect of different type and concentration of growth harmone on shoot initation: *In vitro* culture of banana shoot tips result meristematic ball like structure in initiation media containing different concentrations of BAP and IAA. The cultured shoot tip turned brown in color from the initial creamy white in a few days after inoculation. Four weeks later, the external leaf primordial of explants turned green and globular hard coat mass grew from which adventitious plantlets were developed as reported by using BAP [10]. Also reported BAP as the most commonly preferred cytokinin used in banana tissue culture. Furthermore, observed the color change of culture meristems to brown in 4 to 5 days and a development of a green hard ball like structure after 30 to 35 days of inoculation.

Effect of different type and concentration of growth harmone on shoot multiplication: According to reported the multiple shoots from sliced shoot tips of banana and plantain. Auxins and cytokinins have been reported as the most frequently used PGRs for banana micropropagation. Adenine-based cytokinin particularly BAP is the most commonly preferred cytokinin to affect shoot multiplication rate in several*Musa spp.* Differences in rate of multiplication of different *Musa* g enotypes under *in vitro* conditions have also been reported. It has been reported that multiple shoots could be produced from sliced shoot tips of The superfluous corm tissue, roots, and leaf sheathers were are trimmed and removed from the pseudo-stem by sharp knife and finally by blade. Explants were washed thoroughly in running tap banana and plantain. Auxins and cytokinins have been reported as the most frequently used PGRs for banana micropropagation. Adenine-based cytokinin particularly BAP is the most commonly preferred cytokinin to affect shoot multiplication rate in several *Musa spp.*

Differences in rate of multiplication of different Musa genotypes under *in vitro* conditions have also been reported. Yellow friable calluses of banana *(Musa spp)* Gros ichel, cultured on Murashige and Skoog (MS) solid medium supplemented with 2, 4 dichlorophenoxy acetic acid and Coconut Water (CW). Small spherical, compact calluses were formed. Friable calluses were transferred to half-MS liquid media supplemented with 1.5 mg/l 2, 4-D in combination with 5% CW or without 2, 4- D and CW. No shoots were produced from these media. Embryogenic calluses were induced followed by subculture the spherical, compact calluses to half-MS solid medium in the presence of thidiazuron. These embryogenic calluses gave rise to shoots on MS germination medium containing 2.0 mg/l α-naphthalene acetic acid (NAA) and 1.0 mg/l 6-Benzyladenine (BA).

Cultured the shoot tips of banana cv. Basrai on Murashige and Skoog basal medium supplemented with 5.0 mg/l BAP and he recorded on the average, 124 plants produced from each shoot tip after five sub culturing. Studied the effect of different concentrations of BAP and NAA on plant regeneration and shoot multiplication of banana cv. BARI banana-I. Highest shoot proliferation, longest shoot production, maximum no. of leaves and longest leaves were found at concentration of 7.5 mg/l BAP +0.5 mg/l NAA. Added different hormonal combinations in MS medium to induce the adventitious buds in banana cv. Longxuan and to allow its multiplication. The best induction and multiplication medium for adventitious buds.

The effects of five topolins on shoot regeneration of micropropagated 'Williams' bananas and compared to benzyladenine. The highest number of shoots (7.3 ±1.0) was obtained at 30µM mT. Unlike other CK treatments requiring higher concentrations, optimum mean shoot number per explant was attained at the lowest concentration in MemT and MemTTHP (10µM) treatments. In terms of abnormality index, mTR regenerated plantlets were of the best quality across all the CKs tested. studied the effect of coconut water and ascorbic acid on shoot regeneration in banana variety Dwarf Cavendish. They inoculated the shoot tips on MS medium containing BAP (Benzyl Amino Purine) (5.0 mg/l) supplemented with coconut water in various concentration (0, 50, 100, 150 and 200 ml/l) and with various concentrations (0, 25, 50, 75 and 100 mg/l) of ascorbic acid, respectively [11].

As the concentration of coconut water and ascorbic acid was increased up to 100 mg/l and 50 mg/l respectively greater frequency of explant in shoot regeneration, no. of shoots regenerated per explant and shoot length was observed studied the different concentrations of N6 benzylaminopurine and Indole Acetic Acid (IAA) for their effect on shoot multiplication and plant regeneration of the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. Maximum shoot was produced on medium supplemented with BAP at 5 mg/l (Pisang Nangka), 6 mg/l and 7 mg/l with 0.2 mg/l. Further, took different concentration of BAP (0-10 mg/l), Kinetin

(0-10mg/l), NAA (0.3-0.5 mg/l) and different combination of BAP (0-10 mg/l) and NAA (0.3-0.5 mg/l). Highest frequency of shoot regeneration (52.25%), number of shoots regenerated per explants (3.25) and shoot length (4.69 cm) was found at BAP concentration of 5 mg/l, Kinetin concentration of 5 mg/l and combination of 7.5 mg/l BAP+0.3 mg/l NAA. The addition of 5 mg/l BAP was found better than Kinetin for shoot development from shoot tip or male inflorescence tip explants investigated the best plant growth regulators for shoot proliferation and multiplication for cultivar Agnishwar. Among different types and concentration of cytokinins viz. 6-Benzylaminopurine (BAP), Kinetin (KIN), N6 (2-isopentyl) adenine (2iP) tested for multiplication of shoot; maximum multiplication (95%) was obtained in MS medium containing 4.0 mg/1 BAP. The highest average number of shoots for each explant (5.9) was found in MS medium fortified with 4.0 mg/l BAP while maximum elongation of shoot (4.9 cm) was observed in MS medium containing 5.0 mg/l BAP. Ramachandran and Amutha (2013) carried out research work on Cavendish Dwarf variety of banana. Murashig and Skoog's basal medium supplemented with 4 mg/l BAP and 0.2 mg/l NAA was found to be most suitable combination for shooting. Further, for multiplication combination of 5mg/BAP l and 0.3mg/l NAA was found best while found MS medium supplemented with 4.00 mg/l BAP and 2 mg/l IAA best for explant establishment and shoot multiplication of banana cv. Grand Naine did mass propagation of banana. Grand Naine through direct through the main for the organogenesis by using PGRs Benzyl Adenine Purine and Kinetin. Benzyl Adenine Purine (BAP) in five different concentrations were used for shoot proliferation and differentiation and shoot multiplication rate. The study revealed that medium supplemented with 4.0 mg/l BAP produced greater number of shoots and longer shoot (3.0 ± 0.012cm) when compared with other treatments studied the effect of diverse concentration of 6-benzylamino purine (6-BAP) on shoots induction of Grandnaine plantlets *(Musa spp)* cultured shoot tips of *Musa spp.* on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP, KIN and IAA both in individual and in combined form and the best results were obtained from MS medium supplemented with BAP+IAA at the concentration of 3.0 mg/l and 0.5mg/l respectively. Jamir and studied the effect of various levels of cytokinin and auxin for an *in vitro* regeneration of banana cultivars Grand Naine and Jahaji. They tested various concentration of BAP (0-6.5 mg/l). By using 4.5 mg/l BAP was found as the best concentration in induction of highest no. of buds (an average of 7.05 and 7.2) with highest mean length of 0.65 cm and 0.7 cm of shoots. But, the shoot elongation was maximum at lower concentration of BAP (1.5mg/l). Research works were conducted by with BAP, TDZ and coconut water at various concentrations and recorded high frequency of shoot initiation (93.33%) at 5 mg/l BAP. The synergetic effect of BAP (4 to 6 mg/l), TDZ (0.1 to 1.2 mg/l) and coconut water (0.1 to 0.9 ml/l) induced multiple shoot buds and this was optimum at the concentration of 5 mg/l BAP, 0.5 mg/l TDZ and 0.5 ml/l coconut water with 15.90 ± 1.66 frequency of shoots per propagated did the micropropagation of banana cv. Malbhog on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of Indole Acetic Acid (IAA) and Benzyl Amino Purine (BAP). This combination resulted in differentiation of adventitious shoots. The maximum differentiation of shoots (92.05 %) was observed on MS medium with 0.57 µm IAA+17.74 µM BAP. The number of shoots per culture was 16.75. The subculture of differentiated shoots on the same medium resulted in further differentiation (91.97 %) of more than 15 shoots per culture.

Study of different concentration of growth harmones on rooting:

Half strength MS medium supplemented with different levels a of IBA (0, 0.5, 1.0 and 1.50 mg/l) and IAA (0, 0.5 and 1.0 mg/l) for root initiation in banana cv.BARI-1. The highest number of roots (6.50) and longest length of root (5.88 cm) was obtained on 0.5 mg/l IAA+0.5 mg/l IBA found that half strength MS medium fortified with 1 mg/l NAA was suitable for root regeneration from scalps in Malaysian banana cultivars PisangMas, PisangNangka, PisangBerangan and PisangAwak. They found maximum of 7 roots from cv. PisangNangka and PisangBerangan and mean root length was also maximal (4.5 cm) in the latter two cultivars inoculated multiplicities shoots of banana cv. Grand Naine (G-9) on rooting media incorporated with either IBA or NAA and Charcoal (2 g/l) for root induction. IBA (2 mg/l) and Charcoal (2 g/l) produced maximum number of roots (8.5) with a lot of root hairs. investigated the best plant growth regulator for induction of roots in banana cv. Agnishwar. Among different types and concentration of auxin viz. Indole-3-Butyric Acid (IBA) and α-naphthalene acetic acid (NAA), IBA at 1.0 mg/l was found most suitable for rooting of shoot (96% rooting) transferred cultured plants of banana cv. Cavendish Dwarf into media containing activated charcoal and hormone (NAA-1.5 mg/l) obtained rooting on MS (half strength) medium fortified with 1.00 mg/l IBA and 200 mg/l activated charcoal.

Conducted rooting experiments involving the use of IAA, IBA, NAA, smoke–water (SW) and karrikinolide (KAR1). Significantly increased number and length of roots were obtained in SW and KAR1 compared to the control. Overall, when compared to BA, the use of topolin demonstrated higher mean shoot number per explant (MemT and MemTTHP) at lowest cytokinin concentrations and the ease of rooting during the shoot proliferation phase (MemTTHP) assessed the development of roots in regenerated shoots of Musa spp. cv. Grand Naine by treating the shoot with five levels of kinetin (0,0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) supplemented in MS medium. Root development was not observed in the medium devoid of hormone. Whereas, increased number of roots (4.0), length of roots (6.0 cm) and length of shoots (6.5 cm) were observed in medium with 1.0 mg/l kinetin found that best root formation (96%) and root number / explant (11.80) of *in vitro* developed in shoots of *Musa spp.* could be achieved on half strength MS has medium supplemented with IBA at 1.0 mg/l. Studied the effect of IBA and NAA on rooting of banana cultivars Grand Naine and Jahaji. For rooting, NAA and IBA were used individually at the concentration of 0, 0.1, 0.2 and 1 mg/l. Cent percent rooting and also highest no. of functional roots (6.33 and 5.2) with moderate root length (2-4 cm) were achieved on medium with 1 mg/l NAA. found that MS medium supplemented with 1.0 mg/l IBA induced 5.33 ± 1.21 numbers of roots with a mean root length of infection. Greenhouse potting mixture used for growing out banana plantlets include 2 parts of a commercial growing media.

7.50 ± 1.87 cm in banana undertook to study on the effect of various concentrations of growth regulators IAA (0.0, 0.5 and 1.0 mg/l) and IBA (0.0, 0.5, 1.0 and 1.5 mg/l) and their interaction on rooting in banana (Musa paradisiaca). Grand Naine. The short duration were recorded at 0.5 mg/l IAA–8.9 days and 1.5 mg/l IBA-+9.4 days and interaction of both 0.5 mg/l IAA+1.5 mg/l IBA has given almost a week root induction (7.33) days. The highest numbers of roots were produced by treatment of 0.5 mg/l IAA which was 6.2 and 7.8 at 15 and 30 DAI, respectively and 1.5 mg/l IBA produced 5.1 and 7.1 roots at 15 and 30 DAI respectively and in 0.5 mg/l IAA+1.5 mg/l IBA combination gave 7.0 and 8.0 numbers of roots at 15 and 30 DAI respectively. In length of roots, it was observed that 0.5 mg/l IAA produced longest root size 5.7 and 6.7 cm at 15 and 30 DAI, respectively. The interaction, 0.5 mg/l IAA+0.5 mg/l IBA produced 6.33 and 7.33 cm length in 15 and 30 DAI respectively. Studied the effect of IBA on rooting of banana cv. Malbhog. They observed that the *in vitro* developed shoots showed 100% rooting on MS medium supplemented with 4.92 µm IBA.

Acclimatization: Acclimatization is necessar y in the c ase of plantlets because *in vitro* produced plant material is not adopted to natural environmental conditions. They are very poorly adopted to resist the low humidity, higher light levels and more variable temperature prevailing outside. Thus, light, temperature and relative humidity are the three major factors to be controlled during acclimatization to natural environment. Physical, chemical and biological properties of potting mixture are also important factors in establishment of *in vitro* raised the plantlets. Thorough washing of plantlets to remove the traces of agar and nutrient medium, dipping in 0.05% sarbendazim and sterilizing the potting mixture eliminate problem of fungal infection. Greenhouse potting mixture used for growing out banana plantlets include 2 parts of a commercial growing media mixture part perlite, and 3 parts vermiculite (medium to coarse grade).

Plants are generally allowed to acclimatize in the greenhouse for approximately 2 months and to reach a height of about 20 cm (8 inches) before they are transplanted to the field hardened rooted plantlets of banana variety Grand Naine (G9) in portrays containing different potting mixtures viz.soil, sand and cocopeat (1:1:1), soil sand and farmyard manure (1:1:1) and mixture of cocopeat and sand (2:1) of which, the mixture of cocopeat and sand (2:1) showed maximum (96%) survival of plantlets. studied the effect of fertigation and Indole Butryic Acid (IBA) application in nutritive solution on growth of plantlets has been excised during acclimatization process in greenhouse. The experimental unit consisted of 1 transplanted plant and daily application of 10 ml of Steiner's nutritive solution at 10, 25, 50, 75 and 100% without and with 1mg/l of auxin. After 11 weeks of acclimatization, they found higher plants with respect to plant fresh weight, dry weight, height and leaf width which corresponded to treatment from 75% to 100% of Steiner's for as solution. The IBA application had no significant effects on the growth of the M. cavendischii plants. There was no significant interaction between fertigation and IBA applications.

Hardened and acclimatized *in vitro* rooted plantlets by using different treatments. Plants transplanted at the age of 4 weeks after root initiation gave maximum survival during transplanting. These plants were hardened in glass beaker and polythene bags singly or in cluster. The maximum survival during hardening was observed by covering the plantlets with glass beaker individually and kept in culture room. Out of various potting mixture tried, the potting mixture containing soil: sand and FYM gave maximum height and survival of plantlets. The results also showed that out of different potting mixtures tried for hardening soil, sand and plantlets in coconut coir pith to be 84.44% during primary hardening.

Materials and Methods

Preparation of stock plants

This study was conducted at Ethiopian institute of agricultural research in the Tissue Culture Laboratory of MARC, Ethiopia. For this experiment the banana cultivars used were Matoke and Nijiru. These cultivars were 4 months old young shoot grown in green house.

The explants were obtained from healthy looking field grown suckers of these cultivars from Melkassa Agricultural Research Center (MARC) banana propagation nursery. The pseudo stems at lower parts of the suckers containing meristem were used as explants. The shoot tips (meristem and a two leaves primordial) were the starting materials.

Disinfection

The superfluous corm tissue, roots, and leaf sheathes were trimmed and removed from the pseudo-stem by sharp knife and finally by blade. Explants were washed thoroughly in running tap water for 15-20 minutes with detergent solution to remove adherent soils. The leaf sheaths near the bases were again removed from the pseudo-stem leaving the young leaves around the meristem until the shoot tip became about 2 cm in length. For disinfection and sterilization of the mother plant70% alcohol for 30 second and 5% sodium hypochlorite solution for 10 minutes were used. Then, the explants were washed three times for at least 2 minute in double distilled water. Explants were excised from young suckers (0.5 to 0.75 m) of the two banana varieties

Shoot tip culture establishment

The culture medium used for this study was that of basal medium containing 30 g/l sucrose and gelled with 8 g/l agar. The pH of the medium was adjusted to 5.7-5.8 using NaOH or HCl before autoclaving. Plant growth regulators (PGR) were also added to the medium prior to sterilization. The medium was autoclaved at 1.2 KPa and 121ºC for 20 min, and then cooled at room temperature before use. Surface sterilized explants were placed on MS medium containing concentration of 3mg/l BAP for shoot tip initiation. The initiated cultures were incubated for 8 weeks aseptically at 25±2ºC under 16h cool white, fluorescent lights. Evaluation of multiplication rate was done using the same MS medium supplemented with a combination of BAP and IAA

was used for shoot proliferation. After 8 weeks of initiation stage contamination-free shoots were decapitated at 7 to 10 mm height and split longitudinally into two or more parts depending on vigor and thickness. Then, they were transferred to multiplication medium. However, smaller shoots were not split; rather they were put in two or three in a culture vessel. The multiplication rate was studied by sub culturing the shoots on MS media every 4 weeks for 4cycles.

Media

Medium was selected as the basal medium throughout the experiment as it was the most favourable medium particularly for callusing and plant regeneration. MS medium is a widely accepted medium for the tissue culture of banana. For further studies an array of media was generated using MS basal medium supplemented with different combinations and concentrations of phytohormones. Besides MS basal medium, half MS medium were used.

Culture initiation

For culture initiation, after trimming all the outer whorls of the spindle shoot tips measuring about (2-4mm) were excised and placed on MS media having 3ml/l BAP. The cultures were then kept in the growth room at a temperature of 27±2oC and photoperiod of 16h light at an intensity of 2500lux.

Shoot multiplication

After 6-8 weeks of culture in the initiation media the prepared shoots were transferred to multiplication media. The medium at this stage of culture was MS basal medium containing with 30g/l sucrose, 100 mg/l myo-Inositol, 1mg/l of thiamine, and solidified with 7g/l agar. The media were fortified with BAP (3.0, 4.0 mg/l) in combination with IAA. These experiments were carried out by complete randomized design with eight treatments by five replications for both cultivars **(Table 1)**.

BAP	IAA
3	0
3	0.2
3	0.4
3	0.6
4	0
4	0.2
4	0.4
4	0.6

Table 1: Treatment of shoot multiplication.

Rooting and elongation

For rooting, shoots plantlets were transferred to MS medium supplemented with different hormone concentration levels of IAA, IBA and NAA. The experiments were conducted by complete randomized block design with 13 treatments in five replications for both cultivars **(Table2).**

IBA	NAA	IAA
0	0	0
0	0	0.5
0	0	1
0	0	1.5
0	0	2
0.5	0	0
0.16	0	0
0.74	0	0
2.32	0	0
0	0.53	0
0	1.06	0
0	0.59	0
0	2.12	0

Table2: Treatments of rooting.

Acclimatization and hardening of plantlet

Elongated and rooted plantlets (about 6 cm with 3 to 4 leaves) were taken out from culture vessels and roots were carefully washed thoroughly with running tap water to remove the agar. Plantlets were disinfected with Ridomyl (2.0 g/l) for 10 minutes to prevent fungal infections before transplanting. Individual plantlets were then transferred into small polyethylene bags filled with various sterile potting mixtures (sand to soils or sugarcane filter cake) in a. The potting mix was fumigated with 5% formalin ten days prior to planting (kept covered for 3 days and then aerated without cover for remaining 7 days). Plants were kept inside chambers covered with transparent plastic for a week in the greenhouse to maintain high humidity for acclimatization and hardening. The humidity was gradually reduced and plantlets were kept outside the chamber. Then, plants were later transferred to bigger polyethylene bags that were filled with forest soil, sand and manure in the ratio of The hardened plants were eventually transferred to the field and successfully established for further evaluation.

Data collected and analysis

Data on the following categories were recorded, that is completely dead, alive but not growing, or growing slowly, or growing very well and also data were record the sources of infection, as fungal or bacterial contamination. At the subsequent culture stages of shoot multiplication, as well as elongation and/or rooting data related to plantlet growth and development were recorded, as deemed necessary. These include, information on shoot and or root development and growth, including number and length of shoots, number of leaves and length of roots. Data collected from each experiment

were subjected to statistical analyses using the SAS statistical software and ANOVA were constructed.

Results and Discussion

Shoot initiation

In this study, *In vitro* culture of banana shoot tips resulted in hard meristematic ball like structure in initiation media containing 3 ml BAP. The cultured shoot tip turned brown in color from the initial creamy white in a few days after inoculation. Four weeks later, the external leaf primordia of explants turned green and globular hard coat mass grew from which adventitious plantlets were developed **(Figure 1).**

Concentration G.Rs (mg/l)		Dwarf cavendish		Gaint cavendish		Poyo	
BAP	IAA	Survival (%)	Sprout (%)	Survival (%)	Sprout (%)	Survival (%)	Sprout (%)
2.0	0.0	100	90	90	70	100	90
2.0	0.65	100	80	90	70	100	70
3.0	0.0	100	100	100	90	90	60
3.0	0.65	100	70	90	80	100	90

Figure 1: Shoot initiation.

The data collected depend on the percentage of survival of the cultivars. As the result indicated, the shoot tip initiation response survival of 75% and 68% were obtained from explants cultured on MS medium supplemented with 3.0 mg/l BAP alone for the two banana cultivars Matoke and Nijiru respectively. According to the report of using BAP for culture imitation of banana 80% of survival were obtained. BAP as the most commonly preferred cytokinin used in banana tissue culture. Furthermore, observed the color. Change of culture meristems to be brown in 4 to5 days and a development of a green hard ball like structure after 30 to 35 days of culture.

Effect of different concentrations of BAP and IAA on shoot proliferation of Matoke and Nijiru cultivars in MS medium

In this study, the data collected depend on the number of shoot and shoot multiplication rate of the cooking banana cultivars. It was observed that the mean shoot number and multiplication rate of both cultivars were listed among the explants of the different genotypes indicated. The results of this study were for the mean of all parameters for both cultivars were significance different among the treatments. After 2 months of culture initiation, sliced shoots were transferred to the various multiplication medium after which shoots with at least one leaf were emerged **(Figure 2)**.

Figure 2: Shoot multiplication of Matoke and Nijiru cultivars.

Dissected shoot tips from healthy suckers of banana *(Musa sp.)* varieties and cultured on MS medium supplemented with 6 mg/l BAP, 2 mg/l IAA and 200 mg/l adenine sulfate. According to the report shoot initials developed after 3 weeks and were proliferated on induction medium with the BAP concentration reduced to 4 mg/l that multiple shoots could be produced from sliced shoot tips of banana and plantain **(Table 3)**.

Treatment	Concentrations of BAP and IAA	Shoot number	Multiplication factor
1	3.0 ml*0.0 ml	9.0^{bc}	8.6^{b}
2	3.0 ml*0.2 ml	7.0^{d}	8.0^{b}
3	3.0 ml*0.4 ml	8.4^{cd}	9.0^{b}
4	3.0 ml*0.6 ml	14.2^{a}	14.4^{a}
5	4.0 ml*0.0 ml	9.6^{bc}	7.2^{b}
6	4.0 ml*0.2 ml	10.0^{ab}	9.2^{b}
7	4.0 ml*0.4 ml	9.8^{bc}	9.0^{b}
8	4.0 ml*0.6 ml	10.2^{ab}	8.6^{b}
CV (%)		14	17
LSD @ 5%		1.8	2

Table 3: Effect of different concentrations of BAP and IAA on shoot multiplication of matoke cultivar in MS medium. Means expressed in different letters showed statistically significance difference among the treatments, LSD=Least Significant Difference, CV=Coefficient Of Variation

As the result indicated the highest number of shoot and shoot multiplication rate were obtained on MS medium supplemented with a combination of BAP and IAA at concentrations of 3 ml BAP +0.6 ml IAA mg/l for Matoke cultivar and Nijiru cultivar was most productive and produce maximum number of shoots at concentration of BAP and IAA; 3ml BAP (12.2) followed by 3ml BAP+0.2 ml IAA (11.6). The poor response of shoot proferation were observed both in shoot number and multiplication rate for Matoke cultivar were at PGR concentration 3 mlBAP+0.2 ml IAA(7) and 4 ml BAP+0IAA (7.2) respectively. On the other hand, the poor response of shoot initiation for Nijiru cultivar was observed in shoot For multiplication rate of Nijiru cultivar 3 ml BAP*0.6 ml IAA (7.4) and 4 ml BAP*0.6 ml IAA (7.6) combinations of the plant growth regulators were shown poor response. The mean number of shoots and multiplication rate of all treatments obtained after four subcultures of both cultivars were stated. The result showed that there were significance differences among treatments on both cultivars in all parameters. In this

experiment, subculturing of the shoots for multiplication was carried out for 4 cycles. It was observed that the cultures showed higher rate of multiplication for the first four subcultures. According to the report of the best results clearly showed that 5.0 BAP+1.0 IAA+10% CW media found to be most productive for shoot proliferation.

However, after the 4th cycle the multiplication rate was declined. Sub culturing induced multiple axillary shoots more than a three-fold three weeks after the first subculture. Further transfer in the same medium resulted in about 2 to 4 fold increase in proliferation at every subculture cycle. The meristematic shoot tip explants were cultured on MS medium with eight different combinations of BAP and IAA. Among the various treatments, the effective results were obtained from these eight combinations given in **(Table 4)**.

Treatment	Concentrations of BAP and IAA	Shoot number	Multiplication factor
1	3.0ml*0.0ml	12.2[a]	15.0[a]
2	3.0ml*0.2ml	11.6[a]	12.4[b]
3	3.0ml*0.4ml	11.4[a]	11.8[b]
4	3.0ml*0.6ml	8.4[b]	7.6[c]
5	4.0ml*0.0ml	7.4[b]	7.8[c]
6	4.0ml*0.2ml	8.0[b]	9.4[c]
7	4.0ml*0.4ml	8.8[b]	9.4[c]
8	4.0ml*0.6ml	8.2[b]	7.6[c]
CV (%)		17.4	17.7
LSD @ 5 %		2.1	2.3

Table 4 : Effect of different concentrations of BAP and IAA on shoot multiplication of Nijiru cultivar in MS medium.Means expressed in different letters manuscript showed statistically significance difference among treatments, LSD=Least significant difference, CV=Coefficient of variation. After 23 weeks, explants changed colour to green and produced shoots.

The mean number of shoots and multiplication rate of all treatments obtained after four subcultures were stated in. When these antibiotic treated explants were cultured in MS, they produced healthy shoots. After four sub-culturing, the clump formation occurs and the capability of culture to further divide gradually becomes low. The proliferating axillary buds were well defined, pale green and 3.0-4.0 cm long with bulbous base and thin pointed tips. After 3-4 weeks, three-fold increase in multiplication was seen. At every sub culture in the same media resulted in three to four fold increases in multiplicate axillary buds Proliferating shoots continue to produce axillary buds on the same fresh media while elongated shoots were transferred to root induction media for root induction. According among thirteen different combinations with various concentrations of BAP and IAA to analyze the shoot initiation and shoot multiplication and resultantly MS medium of 5.0 mg/l BAP and 1.0 mg/l IAA+10% coconut water showed good results both for shoot initiation and multiplication. Auxins and cytokinins have been reported as the most frequently used plant growth regulators for banana micropropagation. Adenine based cytokinin particularly BAP is the most commonly preferred cytokinin to affect shoot multiplication rate in several *Musa spp.* Differences in rate of multiplication of different *Musa* genotypes under *in vitro* conditions have also been reported. In the present investigation, shoot meristems were cultured on agar (semisolid) similar reports were also made.

Rooting and Elongation

Effect of different concentrations of IBA , NAA IAA rooting of Ma toke and Nijiru cultivars : In this study the data collected depend on four parameters. Those parameters were: Shoot number, shoot length, root number and root length of the plantain. The results of this study were for the mean of all parameters for both cultivars showed significant difference among the all treatments. Three different auxins (IAA, NAA, and IBA) were used in three different combinations in MS supplemented medium for root induction. The best progresses were obtained at PGR concentration of NAA 0.59 on all parameters shoot number (3.8), shoot length (18.7 cm), root of a number (17) and root length (21.2 cm) for Matoke cultivar. But, the poor progresses were obtained at different PGR concentration for Matoke cultivar on those parameters. That were: the poor shoot number was obtained at hormone concentration 0.5IBA ml/l (0.6), control and 0.16 ml/l IBA (1): for shoot length 0.7 cm at concentration of 0.5 IBA and 1.8 cm at control treatment: for number roots 0.2 at concentration of 0.5IBA ml/l, 1.4 at concentration of 0.74IBA ml/l and 1.8 on control treatment: and for root length at PGR concentration of 0.5IBA ml/l 0.3 cm and at 0.16IBA ml/l 0.8 cm **(Table 5)**. Shoots were rooted on MS medium containing 2 mg/l BAP, 2 mg/l IAA and 0.1% activated charcoal

Treatment	concentrations of IBA, NAA and IAA (ml/l)	SHPP	SHL (cm)	RPP	RL (cm)
1	IBA 0* NAA 0,*IAA 0	1.0[ef]	1.8[fg]	1.8[ef]	5.2[f]
2	IBA 0*NAA 0*IAA 0.5	1.8[cde]	2.78[f]	3.6[e]	6.9[e]
3	IBA 0* NAA 0*IAA 1	1.0[ef]	6.1[e]	7.2[d]	4.4[f]
4	IBA 0*NAA 0* IAA 1.5	2.6[bc]	6.7[de]	10.6[c]	2.7[g]
5	IBA 0*NAA 0*IAA 2	1.6[de]	5.9[e]	7.0[d]	7.3[e]
6	IBA 0.5*NAA 0*IAA 0	0.6[f]	0.7[g]	0.2[f]	0.3[h]

7	IBA 0.16*NAA 0*IAA 0	1.0^{ef}	7.8^{dc}	14^{b}	0.8^{h}
8	IBA 0.74*NAA 0* IAA 0	3.0^{ab}	5.8^{e}	1.4^{f}	6.6^{e}
9	IBA 2.32*NAA 0* IAA 0	1.6^{de}	8.8^{c}	14.0b	9.3^{d}
10	IBA 0* NAA 0.53* IAA 0	1.8^{de}	12.78^{b}	17.0a	13.6^{c}
11	IBA 0* NAA 1.06* IAA 0	1.2^{ef}	13.54^{b}	14.6^{b}	18.8^{b}
12	IBA 0*NAA 0.59* IAA 0	3.8^{a}	18.7a	17.0^{a}	21.2^{a}
13	IBA 0* NAA 2.12*IAA 0	2.4^{bcd}	13.2^{b}	10.0^{c}	14.4^{c}
CV (%)		18.9	11.8	18.9	11.5
LSD @ 5%		0.8	1.2	2	1.3

Table 5: Effect of different concentrations of IBA, NAA and IAA on rooting of matoke cultivar. Means expressed in different letters manuscript showed statistically significant different among treatments, LSD=Least significant difference, CV=Coefficient of variation The best number of shoot for Nijiru cultivar was at PGR concentration 0.74 ml/l IBA (4) and 2.32 ml IBA (3.6). In other case, the lowest number of shoot that has been observed at hormone concentration 2.12 ml/l NAA (0.8) followed by 0.5 ml/l IAA (1) and 0.53 ml/l NAA (1) for Nijiru cultivar. As the result indicated the best shoot length was observed at hormone concentration 0.74 ml/l IB A (12.7 cm) and an lowest one were at hormone concentration 0.59 ml/l NAA (1.2 cm) and 2.12 ml/l NAA (1.06 cm) for Nijirucultivar.

According to this study the best progress of root number was 14.4obtained at hormone concentration of 0.74 ml/l IBA and the poor root number was 2.8 obtained at hormone concentration of 2.12 ml/l NAA for Nijiru cultivar and the good progress of root length was 13.6 cm obtained at hormone concentration of 0.7 ml/l IBA and the lowest plant root length were 1.88 cm and 2.7 cm obtained at hormone concentration of 2.12 ml/l NAA and 1 ml/l IAA respectively for Nijiru cultivar. According to the report of the results showed that MS medium supplemented with IAA (2.0 mg/l) gave best results which produced 60% root induction. Four different auxins with varying concentrations were employed check their effect on root induction. Each auxin was applied in four different concentrations. For root induction, the well grown shoots were separated and transferred to MS media containing varying concentrations of auxins [12].

According to the experiment effect of different concentrations of IBA, NAA and IAA on explant of Matoke cultivar rooting in MS medium were studied with thirteen treatments with five replication. The mean number of shoot, shoot length, root number and root length for Matoke and Nijiru cultivars across the treatment were listed in **(Table 6)**.

Treatment	Concentrations of IBA,NAA and IAA(ml/l)	SHPP	SHL(cm)	RPP	RL(cm)
1	IBA 0* NAA 0,*IAA 0	1.6^{cd}	3.8^{e}	5.6^{e}	4.6^{e}
2	IBA 0*NAA 0*IAA 0.5	1.0^{cd}	3.5^{e}	5.2^{e}	4.4^{ef}
3	IBA 0* NAA 0*IAA 1	1.2^{cd}	1.86^{fgh}	4.0^{fghi}	2.7^{fgh}
4	IBA 0*NAA 0* IAA 1.5	1.0^{cd}	2.6^{ef}	5.0^{egh}	3.7^{efg}
5	IBA 0*NAA 0*IAA 2	1.8^{bc}	7.7^{c}	9.6^{c}	8.7^{c}
6	IBA 0.5*NAA 0*IAA 0	2.6^{b}	8.74^{bc}	10.8^{bc}	9.76^{bc}
7	IBA 0.16*NAA 0*IAA 0	1.8bc	5.1^{c}	7.2^{d}	6.1^{d}
8	IBA 0.74*NAA 0* IAA 0	4.0^{a}	12.7^{a}	14.4^{a}	13.6^{a}
9	IBA 2.32*NAA 0* IAA 0	3.6^{a}	9.2^{b}	11.0^{b}	10.2^{b}
10	IBA 0* NAA 0.53* IAA 0	1.0^{cd}	2.3^{fg}	4.2^{fgh}	3.3^{fgh}
11	IBA 0* NAA 1.06* IAA 0	1.6^{cd}	1.7^{fgh}	3.8^{ghi}	2.7^{ghi}
12	IBA 0*NAA 0.59* IAA 0	1.6^{cd}	1.2^{gh}	3.4^{hi}	2.2^{hi}
13	IBA 0* NAA 2.12*IAA 0	0.8^{d}	1.1^{h}	2.8^{i}	1.9^{i}
CV (%)		16.8	18.6	14.8	16.2
LSD @ 5%		0.8	1.1	1.2	1.2

Table 6: Effect of different concentrations of IBA, NAA and IAA on rooting of nijiru cultivar. Means expressed in different superscript letters showed statistically significance difference among treatments, LSD=Least Significant Difference, CV=Coefficient Of Variation.

NAA was found to be effective at very low concentrations for root initiation of banana. NAA 1.0 mg/l is suitable for roots in initiation in *Musa sp.* Observed rooting on MS medium containing 1.2 µM NAA during the study of multiplication rate effects of cytokinins on Kibuzi, Bwara and Ndizwemit banana cultivars. Also reported best rooting response in a combination of IBA and NAA. However, and used half strength MS+1.0 mg/l IBA, whereas used auxin free MS for rooting of banana microshoots. On the other hand, obtained rooted banana shoots in half strength MS medium supplemented with 0.2 mg/l IBA for 1.0 mg/l IBA for best rooting response of *in vitro* cultured plants. Rooting can be stimulated when individual shoots are transferred to a basal medium without any PGR. However, auxins induce further root initiation in bananas.

A similar result was reported by that the root formation was occurred 50 days after shoot transfer. However, noted that plantlets needed 2 to 3 months for root formation. Synthetic growth regulating chemicals that have been found most reliable in stimulating adventitious root production are, most frequently used to induce root initiation in banana. During the current study, the root induction and elongation response to IAA were better than all other auxins evaluated. These findings are contrary to those of who reported that NAA was more effective than IAA in banana tissue culture. From all plantlets plant on rooting medium 77% survival contamination free plants were obtained from both cultivars **(Figure 3).**

Figure 3: Matoke and Nijiru cultivars on rooting stage.

Acclimatization of RootedPlants: The well developed healthy as transferred into small plate bed filled with different soil media mix, and kept inside small chambers covered with transparent plastic in the greenhouse for primary hardening for a week. Plantlets were washed by distilled water and disinfected with Ridomyl (1.0 g/l) for 10 minutes to prevent fungal infections before transplanting. Individual plantlets were then transferred into soil filled plate bed .Thorough washing of plantlets to remove the traces of agar and nutrient medium and sterilizing the potting mixture eliminate problem of fungal infection. Among acclimatized plants the plants were acclimatized (85% survived), and the highest vegetative plant growth in terms of plant height, pseudo stem girth and number of photosynthetic culture from shoot meristem was achieved on sem-solid media a leaf per plant was recorded on media containing sugarcane filter A similar result was reported by that the root formation was a days after shoot transfer. However , noted that formation. cake and sand at 3:1 ratio (v/v). The survival plantlets were planted in soil filled polyethylene bag **(Figures 4 and 5.)**

Figure 4: Acclimatization in greenhouse.

Figure 5: The last stage of acclimatization in greenhouse.

Conclusion

This study was aimed to develop a simple, comprehensive and efficiently repetitive protocol for micropropagation of banana *(Musa Sapientum L.)* using shoot meristem. The shoot on their initiation response with survival of 75% and 68% were obtained from explants cultured on MS medium supplemented with 3.0 mg/l BAP alone for the two banana cultivars Matoke and Nijiru, respectively. After growing *in vitro* cultures on different hormonal combinations, MS medium supplemented with BAP and IAA, the highest number of shoot and shoot multiplication rate was 14.2 obtained on MS medium supplemented with a combination of BAP and IAA at concentrations of 3 ml BAP+0.6 ml IAA mg/l for Matoke and Nijiru cultivar were most productive and produced maximum number of shoots at concentration of BAP and IAA, BAP followed by 3ml BAP+0.2 ml IAA. Antibiotic (cefotaxime) was used to check the endogenously born bacterial contamination. Initiation of shoot bud and establishment of culture from shoot meristem was achieved on sem-solid media.

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