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# *In vitro* Propagation of Banana (*Musa paradisiaca* L.*)* Plant Using Shoot Tip Explant

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ABSTRACT

Banana is a fruit crop which has high demand in Ethiopia, but its production is constrained by lack of disease free planting material with conventional propagation methods. For shoot initiation, shoot tip explants were cultured on MS medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/L BAP. Similarly, MS medium supplemented with BAP at 1.0, 1.5, 2.0 mg/L in combination with IBA at 0.25 and 0.50 mg/L were used for shoot multiplication. Half- strength MS medium augmented with IBA at 1.0, 2.0, 3.0 and 4.0 mg/l were used for root induction. MS medium without PGRs were used as controls. Finally, hardening of the *in vitro* derived plantlets was carried out in green house both in the primary and secondary acclimatization stages. Results showed that the highest shoot initiation percent (93.40%), highest mean number of shoots per explant (4.67) and lesser day for shoot induction (11.00) were observed in explant cultured on MS + 1.0 mg/L BAP. With shoot multiplication, highest shooting percent (92.60%), maximum number of shoots (7.67) and highest shoot length (5.27 cm) were recorded on MS + 1.5 mg/L BAP + 0.5 mg/L IBA. The highest rooting percent (93.40%), maximum root number per shoot (7.67) and highest root length (11.00 cm) were found on a half strength MS medium + 2.0 mg/L IBA. The survival rate of plantlets were 96.00% in coco peat substrate in primary acclimatization and 97.92% in forest soil, sand and manure substrates mixed at 3:2:1 ratio in secondary acclimatization. Overall, the result showed that the PGRs type, concentrations and combinations used are effective for mass propagation of banana variety studied in this experiment.

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## Introduction

Banana (*Musa paradisiaca*, family Musaceae) is a fruit crop of the tropical and subtropical areas of the world grown in over 120 countries worldwide (Thangavelu and Mustaffa, 2012). It is being cultivated on approximately 10 million hectares with an annual world production estimated at 101.99 million tons (FAOSTAT, 2012). It serves as food source for more than 400 million people throughout the developing countries of the tropics and subtropics (Frison and Sharrock, 1999; Nelson et al., 2006). Banana ranks as the fourth major food crop after rice, wheat and maize and is considered as a poor man's apple in tropical and subtropical areas of the developing countries (Salvador et al., 2007). In terms of nutrition, banana is a thriving source of carbohydrate with calorific value of 67 calories per 100g fruit and is one of the best well-liked and broadly traded fruits across the globe (Kumar et al., 2012). In Africa, banana provides more than a quarter of the carbohydrate requirements for over 70 million people. It is also the main staple food and a key component of food security in the Great Lakes region of Eastern Africa where it makes the highest contribution to household income (Smale and Tushemereirwe, 2007).

Ethiopia is one of the countries which has highly diverse agro-ecology that can grow different fruits where its massive areas are suitable for banana cultivation. Banana production in Ethiopia ranges from smallholding farmers to large commercial plantations under rain fed or with supplementary irrigation conditions (Kahessay et al., 2010). In Ethiopia, banana cultivation occupies the largest area (53,956.16 ha) with production of 4,782,510 quintals compared to other fruit crops. However, the global share of Ethiopia in banana export was only 0.02% which could be related to problems associated with low production and poor quality to meet export standards for international market (CSA, 2014).

Biotic factors such as attacks from nematodes, viruses, and insect pests are the major causes for low banana production in Ethiopia. The insect pests (fruit flies) and diseases caused by *Xanthomonas* wilt and *Fusarium oxysporum*, for example, are reported to be major

challenges for banana production in Ethiopia (Mekonen, 2014). Furthermore, most commercial bananas are propagated vegetatively through suckers, which are collected from an existing field or from a multiplication plot planted only for the production of suckers. Conventional vegetative propagation of banana has its own limitations such as presence of diseased mother plants, low production, poor preservation of original plant genetic material (Ngomuo et al., 2014) and supply is season dependent (Hanumantharaya et al., 2009). In addition, due to the high degree of sterility and polyploidy of the edible varieties, only on average 5 to 10 or less suckers can be produced per year per plant depending upon variety and clone (Vuylsteke, 1998; Singh et al., 2011).

Generally, traditional propagation method is laborious; time consuming and inefficient to supply the increasing demand for healthy banana planting material. Banana production through conventional method also exposes the plant to different bacterial, fungal and viral diseases (Rahman et al., 2004). As a result, banana productivity decreases and the yield becomes very poor. To improve banana productivity and safeguard sustainable banana production, the use of clean and high quality planting material is crucial (Karembu et al., 2010). Thus, in order to overcome problems of conventional propagation and to avoid diseases transmitted by different pathogens, biotechnological methods can be used widely for the reliable propagation, genetic improvement, conservation and distribution of banana germplasms (Arvanitoyannis et al., 2008). *In vitro* propagation is one the applications of biotechnology that offers alternative and large number of plants using only a few explants (Dadjo et al., 2014).

Several researchers have reported the regeneration of *Musa spp*. through *in vitro* micro propagation (e.g., Uzaribara et al., 2015; Noor et al., 2017 and Singh et al., 2017). *In vitro* propagation of bananas offers excellent advantages relatively to traditional propagation methods which include large scale production of disease-free planting materials all year round, physiological uniformity, requires small space to multiply large number of plants and availability of disease-free material (Waman et al., 2014). Moreover, micro-propagated banana plants, in general establish more quickly, grow more vigorously, have a shorter and more uniform production cycle, and produce higher yields as compared to traditionally propagated bananas (Singh et al.,2013). Even though numerous reports are present on banana *in vitro* propagation, variation in propagation protocols may result in different results because of such factors as genotype difference, explant type, culture media composition, plant growth regulators (PGR) and culture environment (Vuylsteke, 1998). Therefore, this study was undertaken to evaluate efficiency of a protocol for of banana cv. Giant Cavendish.

## Materials and Methods

### *Description of the Study Area*

The experiment was conducted at Tigray Biotechnology Center, in the Quality Assurance Research and Development section, Mekelle city, Northern Ethiopia. The center was located 789 Km North of Addis Ababa at an altitude of 1979 meter above sea level, 13º 30' 0" N latitude and 39º 28' 11" E longitude.

### *Stock Solution and Growth Media Preparation*

Stock solutions of the components of MS basal medium (Murashige and Skoog, 1962) were prepared by dissolving the appropriate and recommended amount of macronutrients, micronutrients and organic supplements in double distilled water. In the same way, plant growth regulators (BAP and IBA) stock solution were prepared using the proportion of 1 mg: 1 ml and stored in a refrigerator at 4°C. In preparation of iron stock (1D), $FeSO_4.7H_2O$ and $Na_2$.EDTA were dissolved in hot double distilled water separately. After dissolving, iron sulphate solution was added over $Na_2$.EDTA solution in half liter beaker gently by stirring. EDTA makes Fe accessible at wide range of pH and overcomes its problem of precipitation and dissolvability after media preparation (Murashige and Skoog, 1962). Iron stock solution was protected from light by storing the solution in black bottle covered with aluminum foil.

The Murashige and Skoog (1962) medium used for shoot initiation, shoot multiplication and maintenance and regeneration of roots from multiplied shoots were prepared from its respective stock solutions of the MS basal medium and plant growth regulators. Sucrose (30 g/l) was added to the medium solution before pH adjustment. The stock solutions were mixed properly using Magnetic stirrer. The pH of the medium was adjusted to 5.80 with 0.1 N HCl and 0.1 N NaOH followed by addition of 6 g/l of agar. Sucrose (30 g /l) and agar (6 g/l) were used because they had been the optimal concentrations from previous work with *Musa* species (Buah *et al*., 2010; Ahmed *et al.*, 2014). Then 40 ml of the medium was dispensed in to a sterilized culture bottle and the media was autoclaved at 121ºC, 15 psi for 20 min. After autoclaving the media were allowed for solidification and kept for 72 hours in the media storage room before utilization for inoculation in order to check up the contamination status of the media.

### *Plant Material Preparation and Surface Sterilization*

Young and healthy sword suckers of *Musa paradisiaca* cv. Giant Cavendish were used as experimental plant material in this study. The explant was collected from healthy field grown banana mother plants of Tigray Biotechnology Research Center banana propagation nurseries. The pseudo stems at the lower parts of the suckers containing meristems were used as explants. The sword suckers containing meristems were carefully detached from field grown fruiting banana plants. Then the selected and collected sword suckers were brought into the plant tissue culture laboratory and transplanted in controlled greenhouse until used. Then the corm tissue, roots, leaf sheathes and outer tissues of the suckers were trimmed and removed from the pseudo stem by sharp knife with the young leaves near the shoot tip meristem was left to about 3-4 cm.

After removing the outer sheaths of the suckers and collection of the sword suckers, the suckers were thoroughly washed with tap water for 20 minutes followed by detergent soup solution for 20 minutes to remove adherent soils. Then the explants were washed thoroughly with sterile distilled water four times so that no chemical residues are remaining. The explants were then surface sterilized in a combination of fungicide and bactericide of kocide, baylton and redimol (each 0.25 g/100 ml of distilled water) for 20 minutes followed by soaking in 2%

NaOCl with two drops of Tween 20 (wetting agent) for 10 minutes. Furthermore, the sword sucker tips were treated with 0.1% $HgCl_2$ in laminar air flow chamber for 5 min as reported by Sumalatha (2016). After each step of sterilization, the explants were rinsed with sterile double distilled water four times to remove traces of surface sterilizing chemicals. Finally the surface sterilized shoot-tip explants were reduced to 2 cm size by removing the outer layers of corm and leaf sheaths using sterilized forceps and scalpel and inoculated under clean conditions.

***Establishment of the Explant in Culture Medium***

After complete sterilization, the surface sterilized and excised shoot tips were inoculated to shoot initiation media comprising of MS basal medium supplemented with different concentrations of BAP (0.5, 1.0, 1.5 and 2.0 mg/l). MS basal medium without plant growth regulator (PGR) was used as a control. Therefore, the total number of treatments used at this stage were 5 with 3 replications arranged randomly. The cultures were kept in a dark room for three days to prevent the blackening of the initiated sucker produced during the step wise removal of the covering leaf primordial. Then the initiated cultures were incubated and maintained for four weeks with a photoperiod of 16/8h light/dark using cool white fluorescent lamps (photon flux density, 40µmol $m^{-2}$ $s^{-1}$ irradiance), at a temperature of 25±2°C and relative humidity of greater than 75% in a growth room. Finally the initiation response, initiated shoot numbers per explant and average days taken for shoot initiation were recorded.

***Multiplication Stage***

For multiple shoot formation, the clean and healthy shoots regenerated from the initiated shoot tip sword sucker were divided longitudinally into two or three parts and transferred to full strength MS medium supplemented with different concentrations (1, 1.5, 2mg/L) of BAP each combined with 0.25 and 0.5mg/L of IBA. As a carbon source and solidifying agent, 30g/L of sucrose and 6 g/L of agar, respectively were also added. MS medium without PGRs was used as a control. Therefore, 7 treatments containing each 9 bottles with 3 replications arranged randomly on the culture media were used. Then the cultures were incubated and maintained in a growth room for four weeks at a temperature of 25±2°C with a photoperiod of 16 hr per day provided by white florescent tube and each required data of this stage was recorded.

***Rooting Stage***

For root induction, the *in vitro* developed healthy banana shoots were separated and transferred individually to half strength MS medium supplemented with 30 g/L of sucrose and various concentrations of IBA (1.0, 2.0, 3.0 and 4.0 mg/L). Activated charcoal at 0.3 g/L for each rooting treatments were also added (Gubbuk and Pekmezci, 2004). Half-strength MS medium devoid of any plant growth regulator was used as a control. The total number of treatments that was used in rooting stage was 5 with 3 replications arranged randomly. The inoculated cultures were then incubated and maintained for four weeks in a growth room at a temperature of 25±2°C and 16/8 hour photoperiod provided by cool-white fluorescent tube. After four weeks, the rooting percentage, the number and length of the regenerated roots were recorded.

***Acclimatization Stage***

The *in vitro* regenerated and rooted banana plantlets were taken out from culture bottles using sterilized forceps with great care to avoid any damage to the plantlets root system. Then the roots were carefully washed thoroughly with slightly warm running tap water to remove the adherent traces of agar and medium attached to the roots. Immediately after washing, the *in vitro* raised banana plantlets were covered by wet newspaper and transferred to green house for primary hardening and transplanted carefully using sharp wooden sticks on trays containing moisture coco peat and covered with transparent plastic bags in the green house for seven days in order to maintain high humidity (80 to 90%) and reduce light intensity. After seven days, the plastic cover was removed and the humidity was gradually decreased to 50 -60%, light intensity raised up to normal and temperatures were increased to 27 ± 2°C up to 40 days. The survival rate of the successfully acclimatized plants at primary hardening were computed. Then, plantlets were later transferred to bigger plastic pots for secondary hardening that was filled with forest soil, sand and manure in different ratios. Then the percentage of successfully survived banana plantlets from the total transplanted banana plantlets in green house were computed.

***Data Analysis***

The experiment was arranged in a completely randomized design and data was subjected to one way analysis of variance (ANOVA) using the SAS software packages (version 9.2) and the significant differences among the treatment mean values were evaluated using Fisher's least significant differences (LSD) at 5% level of significance.

## Results and Discussion

***Effects of Different Concentration of BAP on Number of Days for Shoot Initiation and Percent Shoot Initiated***

In the present study, the properly surface sterilized shoot tips obtained from the sword suckers of banana cv. *Giant Cavendish* were directly inoculated on full strength MS medium supplemented with different concentrations of BAP (0.5 1.0, 1.5 and 2.0mg/l) for *in-vitro* shoot initiation. Results showed that percent of shoot initiated per explant was significantly ($P<0.001$) varied between treatments with PGRs treatment at all concentration levels significantly surpassing values observed in control (Table 1). Among the different concentrations of BAP tested, significantly highest shoot initiation percentage (93.40%) was observed on full strength MS medium fortified with 1.0 mg/L BAP followed by 73.40% shoot initiation MS medium supplemented with 1.5 mg/L BAP (Table 1). This indicates that shoot initiation from banana shoot tip explants is cytokinin concentration dependent. Similar research done by Ali et al. (2011) supports the result of this study as they obtained best shoot formation response (100%) using banana shoot tip meristem explant cultured on MS medium fortified with 1.0 mg/L BAP. On the contrary, authors such as Venkatachalam et al. (2007) and Bairu et al. (2008) reported that 5mg/L BAP was the most effective concentration for *in vitro* shoot induction and proliferation of many banana cultivars. Variation between

the present result and theirs may be due to difference in the genotypes we used. Arinaitwe et al. (2000) previously reported that same explants from different genotypes can differentially respond to different concentrations of the same hormone. Moreover, Sipen and Davey (2012); Rahman et al. (2013) and Iqbal et al. (2013) reported that the *in vitro* shoot regenerative capacity of banana is highly genotype specific and greatly influenced by cytokinin concentrations in shoot inducing medium.

Likewise, days to shoot initiation was significantly ($P<0.001$) varied between treatments with explants treated with PGR at all concentration levels took fewer days for shoot initiation than the control (MS without PGR) (Table 1). Among the various concentrations of BAP, shoot tip explants cultured on MS medium supplemented with 1.0 mg/L BAP took lesser number of days (11.00 days after inoculation) compared to the rest of the treatments. On the other hand, the exogenous BAP free MS basal medium took significantly more days (22 days) for shoot initiation and establishment (Table 1). This indicates that the endogenous plant growth hormones available in the explants may not be sufficient to induce shoots. The present result is in accordance with the findings of Shagufta et al. (2011) and Ali et al. (2011) who recorded shoot initiation within 10.6 days after inoculation using MS medium supplemented with 1.0 mg/L of BAP.

***Effects of PGRs on Shoot Multiplication***

Different concentrations and combinations of cytokinin and auxin in culture media are key factors which determine successful shoot multiplication. Previously, trials on multiplication of several cultivars of banana have been reported (Al-Amin et al., 2009; Hapsoro et al., 2010; Ali et al., 2011). However, the optimum medium for shoot multiplication of each cultivar was different. Results of this study showed that shoot multiplication was varied significantly ($P<0.05$) between PGR treated shoots and control (with no PGR) (Table 2). The result revealed that the percentage of shoot response under various concentrations and combinations of BAP and IBA was found in the range of 22.22 to 92.60% (Table. 2). Significantly highest shoot response (92.60%) was found on the treatment combination of 1.5 mg/L BAP and 0.5 mg/L of IBA (with mean of shoot formed/explants of 8.33); followed by MS medium fortified with 1.0 mg/L BAP + 0.25 mg/L IBA which accounted for 66.67% (with mean 6.00 of shoot formed/explants) compared to the control and other treatments. The lowest mean shoot percentage (22.22 %) was observed on the MS medium devoid of PGRs. Benzyl amino purine (BAP) combined with auxins (indole acetic acid and indole butyric acetic acid) exhibit synergistic effect on shoot formation and hence has also been used by a number of researchers (Jafari et al., 2011; Sipen and Davey, 2012; Ngomuo et al., 2013).

Variable number of shoots were produced in MS media supplemented with different concentrations of BAP and IBA. Among the different concentrations analyzed, significantly maximum mean number of shoots per explant (7.67±0.58) were obtained on the treatment combination of MS medium supplemented with 1.5 mg/L BAP and 0.5 mg/L IBA; followed by MS medium fortified with 1.0 mg/L BAP + 0.25 mg/L IBA (4.67±0.58) (Table 2). In addition, the shoots obtained on these treatments were morphologically better having well differentiated and relatively taller and deep green appearance. Although MS + 2.0 mg/L BAP + 0.25 mg/L IBA, and MS + 2.0 mg/L BAP + 0.5 mg/L IBA resulted in less mean number of shoot, they were also significantly better than the control.

Overall, the present study showed that BAP was effective when combined with IBA for optimum shoot multiplication from shoot tips of the banana cultivar used in this experiment. These findings are similar with that of Gubbuk and Pekmezcu (2004) and Ngomuo et al. (2013) who reported that apart from the genotypes of the cultivar, shoot multiplication and proliferation are also influenced by exogenous cytokinin concentration in growth medium. On the contrary to the present result, Rabbani et al. (1996) and Ali et al. (2011) found highest number of shoots per explants (3.11 ±0.66 and 5.8±0.33) with 5.0 mg/l of BAP + Kn, and 1.5 mg/l of BAP after four weeks, respectively. The source of variation may be due to the different concentrations of BAP (cytokinin) and IBA (auxins), their combinations and genotype of the cultivar. This result is in agreement with the findings of Arinaitwe et al. 2000; Rahman et al. (2013) and Iqbal et al. (2013) who reported that shoot formation and multiplication in banana plants depend on the concentration of cytokinin used and genotype of the cultivar.

With respect to shoot length, the maximum length (5.27±0.11 cm) was observed in the treatment combinations of full strength MS medium supplemented with 1.5 mg/l BAP + 0.5 mg/l IBA followed by the treatment combinations of full strength MS medium augmented with 1.0 mg/L BAP + 0.25 mg/ L IBA, which produced 3.92±0.32 cm. In contrast, the shorter shoot length (1.38±0.34cm) was recorded on MS medium devoid of growth hormones (control) similar to the finding of AL-Amin et al. (2009) who recorded 1.05 cm shoot length on hormone free MS medium.

Table 1. Effect of different concentration of BAP (Mean ± SD, n=3) on *in vitro* shoot initiation and establishment of Banana cv. Giant Cavendish

| BAP concentration (mg/L) | Average Shoot Initiation (%) | Mean of Initiated shoots/explant | Average Days taken for shoot initiation |
|---|---|---|---|
| 0.00 | 20.00±0.00$^{d}$ | 1.00±0.00$^{d}$ | 22.00±1.00$^{a}$ |
| 0.5 | 46.60±0.58$^{c}$ | 2.33 ±0.58$^{c}$ | 18.00±0.00$^{b}$ |
| 1.0 | 93.40±0.58$^{a}$ | 4.67±0.58$^{a}$ | 11.00±1.00$^{d}$ |
| 1.5 | 73.40±0.57$^{b}$ | 3.00±0.00$^{b}$ | 15.00 ±1.00$^{c}$ |
| 2.0 | 40.00±0.68$^{c}$ | 2.00±0.00$^{c}$ | 16.00±2.00$^{bc}$ |
| CV | | 14.04 | 7.21 |
| LSD | | 0.66 | 2.15 |

Means in the column that are followed by the same letter (s) are not significantly different at 5% significance level using Fisher's LSD test, CV= coefficient of variation (%), LSD=Least Significant Difference, %= Percentage.

Table 2. Effect of Different Concentrations and Combination of BAP and IBA on *in vitro* Shoot Multiplication of Banana CV. Giant Cavendish.

| Concentration of PGRs ( mg/l) | | Mean Percent of shooting | Mean of shoot №/ shoot let | Mean of Shoot Length/shoot let ( cm) |
|---|---|---|---|---|
| BAP | IBA | | | |
| 0 | 0 | 22.22 ± 0.00$^{e}$ | 1.00 ±0.00$^{e}$ | 1.38±0.34$^{f}$ |
| 1 | 0.25 | 66.67 ± 1.00$^{b}$ | 4.67±0.58$^{b}$ | 3.92±0.32$^{b}$ |
| 1.5 | 0.25 | 37.04 ± 0.58$^{cd}$ | 2.33±1.15$^{cd}$ | 2.21±0.36$^{d}$ |
| 2 | 0.25 | 25.93 ± 0.58$^{de}$ | 2.00±0.00$^{d}$ | 1.67±0.18$^{ef}$ |
| 1 | 0.5 | 40.74 ± 0.58$^{c}$ | 3.00±0.00$^{c}$ | 2.87±0.15$^{c}$ |
| 1.5 | 0.5 | 92.60 ± 0.58$^{a}$ | 7.67±0.58$^{a}$ | 5.27±0.11$^{a}$ |
| 2 | 0.5 | 25.33± 0.58$^{de}$ | 2.00±0.00$^{d}$ | 2.03±0.15$^{de}$ |
| | CV (%) | | 6.50 | 9.13 |
| | LSD | | 0.94 | 0.44 |

Means in the column that are followed by the same letter (s) are not significantly different at 5% significance level using Fisher's LSD test, CV= coefficient of variation (%), LSD=Least Significant Difference.

The treatment 2. 0 mg/ L BAP + 0.25 mg/ L IBA (1.67±0.18 cm) produced shorter shoot length which is not significantly different from control treatment (Table 2). Reddy et al. (2014) obtained 5.68±0.3cm mean shoot length when shoot tip explants were cultured in MS medium fortified with 2.0 mg/L BAP which is partially similar to the present result.

***Effect of IBA on In Vitro Rooting of Regenerated Banana Plantlets***

A good and healthy root system is essential for successful acclimatization of the *in vitro* derived shoots and subsequent growth in the fields as roots assist the absorption of nutrients from the soil (Xiansong, 2010). Therefore, the *in vitro* raised and proliferated shoots were excised and transferred in to half strength MS medium supplemented with different concentration of IBA (1.0, 2.0, 3.0 and 4.0 mg/l) for healthy root induction and development. Results showed that root induction was varied significantly between PGR treated shoots and control (with no PGR). Among the various concentrations of IBA investigated, the highest root induction percentage (93.40 %) was achieved on ½ strength MS medium supplemented with 2.0 mg/L IBA and the lowest (33.40 %) was found on ½ strength MS medium supplemented with 4.0 mg/L IBA (Table 3). Previously, Rahman et al. (2013) and Saraswathi et al. (2014) reported the effectiveness of IBA on development of roots in banana cultivars. Raut and Lokhande (1989) mentioned the importance of exogenous auxin application for root formation, though degree of effectiveness varies with cultivar and concentration used. Result of the present study is more or less similar with that of Rahman et al. (2013) and Devendrakumar et al. (2013) who found highest rooting percentage (96 % and 94.30 %, respectively) when shoots were cultured on a ½ MS medium fortified with 1.0 mg/L IBA and 0.5 mg/L IBA in cv. Agnishwar and *Cavendish dwarf*, respectively. However, the slight difference seen between theirs and the current result may be due to the variation of genotype of the plant material. Completely, no root formation was observed on the hormone free half strength MS medium, suggesting that exogenous application of auxin is mandatory for effective rooting as endogenous auxin could be too low for root induction.

Results of rooting experiment also showed that number of roots per shoot and root length per shoot were significantly ($P<0.05$) varied between treatments (Table 3). Among the different concentrations of IBA tested, the highest mean root number (7.67±0.58) and root length (11±1.00) were observed at 2mg/L of IBA followed by 5.33±0.58 of root number and 8.00±00 root length at 3mg/L of IBA. The lowest values of both root number and root length per shoot were obtained at 4mg/L of IBA with treatment without PGR actually was totally non-responsive (Table 3). The present result is in agreement with Rai et al. (2014) who noted that 2 mg/L IBA produced maximum number of roots with a lot of root hairs. Similarly, Lohidas and Sujin (2015) also found 8.66 ±0.58 number of roots on ½ strength MS medium supplemented with 2.0 mg/L IBA. Moreover, the present result is at a par with the finding of Rashmid and Babu (2017) who obtained 7.62 roots per plantlet on Banana cv. Rajapuri Bale (AAP) on a medium supplemented with 2.0 mg/L of IBA. With respect to root length the present result is nearly similar with that of Uzirabera et al. (2015) who found the highest root length (8.99 cm) at 1.5 mg/L IBA on banana cv. Kamalapur Red. However, the current result is not in agreement with that of Davendrakumar et al.*(* 2013) who reported that 0.5mg/L IBA produced the highest root number (6 root/plantlet) in *Cavendish dwarf* variety. The cause of variation may be the difference in cultivars we used as different cultivars may respond to varying concentration of the same hormone. Dihiz et al. (2007) also reported that consistently higher number of roots are produced when MS media is augmented with moderate concentration of IBA, NAA and activated charcoal. In this study, 2.0 mg/L IBA supplemented to ½ MS media showed maximum number of roots per plantlets. Overall, from the present result of rooting parameters, half strength MS medium augmented with 2.0 mg/L of IBA showed good rooting performance and was found to be the most effective for *in vitro* rooting of banana which is in line with the report of Lohidas and Sujin (2015).

***Acclimatization of the In Vitro Raised Plantlets***

Pati et al. *(*2013) reported that maximum mortality of micro propagated plants occurs during acclimatization phase as plantlets undergo rapid and extreme changes in morphological and physiological functioning, histological

and biochemical changes in *in vivo*. To resolve this problem, acclimatization is essential for the survival and successful establishment of *in vitro* raised plantlets (Deb and Imchen, 2010). Therefore, it is necessary to acclimatize the *in vitro* raised banana plantlets in two stages *viz.* primary and secondary hardening. Results showed that the survival rate of *in-vitro* regenerated plantlets at primary acclimatization was 96.00% (Table 4)) in green house. The present result is in line with the finding of Uzaribara et al. (2015) who recorded 95% survival rate of *in vitro* produced red banana (*Musa acuminata*) plantlets on a coco peat at primary acclimatization. Similarly, Rai et al. (2014) also recorded 96% survival rate of *in vitro* raised plantlets on a mixture of coco peat and sand under greenhouse conditions which is at a par with the current result. However, mortality of the 4% of plantlets at the primary hardening may be due to the injuries to the root system, sensitivity of the *in vitro* plantlets to sudden exposure to harsh environmental conditions and excessive evaporation.

After primary hardening, the *in vitro* raised banana plantlets were transferred to a bigger plastic pot that contained a mixture of forest soil, sand and manure in different ratios for further hardening before field transfer and they were successfully acclimatized. Result showed that survival rate of *in-vitro* regenerated plantlets on the pot mixtures of forest soil: sand: manure in the ratio of 3:2:1; 3:2:2 and 3:1:2 were 97.92%, 93.75% and 95.83%, respectively.

Though highest survival percentage (97.92%) was found on the substrate mixture of forest soil: sand and manure in the ratio of 3:2:1, there was good plantlet survivability in secondary hardening under all substrate mixtures which may be because of the *in vitro* produced plantlets were reduced their sensitivity to sudden exposure of tough environmental conditions in the primary acclimatization stage. The present result was in agreement with Devalakere et al. (2019) who recorded 97.17% survival rate during secondary hardening processes of banana cv. Kamalapur Red (AAA). Moreover, successful acclimatization and survival percentage of *in vitro* produced banana plantlets ranging from 80-100% under greenhouse conditions have been reported by several workers (Rahman et al., 2013; Ahmed et al ., 2014; Hossain et al., 2016).

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Table 3. Effect of different concentrations of IBA on *in vitro* rooting of Giant Cavendish Banana along with ½ strength MS medium

| Concentration of IBA ( mg/L) | Mean Percent of Rooting Response | Mean Root No/plantlets | Mean Root Length( cm) |
|---|---|---|---|
| 0.00 | 0.00 ±0.00$^{e}$ | 0.00±0.00$^{d}$ | 0.00±0.00$^{d}$ |
| 1.00 | 73.40±0.58$^{b}$ | 4.67±0.57$^{b}$ | 7.89±1.17$^{b}$ |
| 2.00 | 93.40±0.58$^{a}$ | 7.67±0.58$^{a}$ | 11.00±1.00$^{a}$ |
| 3.00 | 53.40±0.58$^{c}$ | 5.33±0.58$^{b}$ | 8.00±0.00$^{b}$ |
| 4.00 | 33.40±0.58$^{d}$ | 1.33±0.57$^{c}$ | 3.22±0.96$^{c}$ |
| CV (%) | | 13.58 | 13.45 |
| LSD | | 0.93 | 1.47 |

Means in the column that are followed by the same letter (s) are not significantly different at 5% significance level using Fisher's LSD test, CV= coefficient of variation (%), LSD=Least Significant Difference, %= Percentage.

Table 4. Survival rate of the *in vitro* raised plantlets at primary and secondary acclimatization

| Substrate used | Acclimatization stage | Total No of plantlets used | No of plantlets survived | Survival rate (%) |
|---|---|---|---|---|
| Coco peat | Primary | 150 | 144 | 96.00 |
| forest soil: sand: manure( 3:2:1) | Secondary | 48 | 47 | 97.92 |
| Forest soil: sand: manure(3:2:2) | Secondary | 48 | 45 | 93.75 |
| Forest soil: sand: manure(3:1:2) | Secondary | 48 | 46 | 95.83 |

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