

Original Research Article

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In vitro Root Development System in Two Popular Cultivars of Banana

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ABSTRACT

Banana is one of the most important and remunerative cash crops grown round the year in Ethiopia. The energy and nutritional status of banana are much higher than other common tropical and subtropical fruits. The objective of this study was to establish *in vitro* root development protocol in Poyo and Grand naine varieties from sucker. For root induction suckers of Poyo and Grand naine were cultured on MS basal medium that contained 30 g/l sucrose, 6 g/l agar and vitamins. Rooting of the shoots was done on full strength MS basal medium supplemented with different concentrations of IBA (0.5, 1.0, 1.5, 2.0 mg/l) and NAA in sole and in combination (0.5 mg/l IBA+ 0.5 mg/l NAA and 1.0 mg/l IBA + 1.0 mg/l NAA). In all cases, MS basal medium without PGRs was used as control. The rooted plantlets were then acclimatized under greenhouse conditions by transplanting the plantlets on moist red soil, sand soil and compost or their mixture in 2:1:1 ratio. Results showed that root formation responses were significantly ($P<0.05$) higher under all levels and combinations of hormones when compared with control treatments. For Poyo variety, 0.5mg/l IBA had higher response for root numbers and root length than at other treatments. Number of days to root emergence was found to be shorter at 20.mg/l NAA of hormonal level than other treatments. In the case of Grand naine variety, the concentration of 1.5mg/l IBA alone treatment had positive effects on multiple root formation and MS + 0.5 mg/l IBA for highest length of roots. The number of days for root emergence was found to be shorter at combination of 1.0 + 1.0mg/l IBA and NAA in grand naine. Compared to the control, both IBA and NAA alone or in combination showed significant positive effect on root formation of shoots of both varieties. The acclimatization experiment showed that plantlets of both varieties survived better on the sterilized mixture of moist red soil, sand and compost soil in a ratio of 2:1:1. Comparison of the performance of both varieties showed that Poyo showed superior performance compared to Grand naine variety with respect to all measured agronomic traits except field survival rate which found better in grand naine, suggesting that varietal difference for the difference in the response to the PGRs.

Keywords

In vitro rooting,
PGR, *Musa* spp,
Poyo, Grand naine,
IBA, NAA

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Introduction

In Ethiopia, as in many parts of the tropical and subtropical developing world, banana is a highly important food crop. However, in the last 20 years, there was a rapid decline in banana production due to widespread of soil degradation and the infestation of banana orchards with pests and diseases. These problems were further aggravated by the common practice of propagating new banana plants using infected suckers. The situation was threatening food security, employment and incomes in banana producing areas. Tissue culture technology is considered worldwide an appropriate option to provide sufficient quality and quantity of such materials (Fanos Mekonnen *et al.*, 2014).

Now a day, although banana plantation is widely spreading in Ethiopia, its quantity and quality are affected by number of biotic and abiotic factors. Its production is threatened by various foliar diseases, nematodes, viruses, and insect pests. Virus diseases are the major problems of banana plantation throughout world including Ethiopia. The impact of these factors is highly affecting the livelihood of poor marginal small-scale farmers (Seifu, 2003). In most parts of the country, production of banana has yet been limited to backyard and small-scale productions.

The report of CSA, (2014) and FAOSTAT, (2012) indicated the average productivity of banana in Ethiopia is 5-8.95 tons/ha, which is far below the world average 15.8 tons/ha. Lack of improved varieties is one of the critical factors that affect its production and productivity in Ethiopia (Tekle *et al.*, 2014).

Since almost all the cultivated or edible banana cultivars are triploid, seedless, or seed sterile, banana is propagated vegetatively. Beside carrying weevils, fungal pathogens, nematodes, and viruses, the materials used for conventional propagation suffer from slow

multiplication, bulkiness, and poor phytosanitary quality (Sagi, *et al.*, 1998). The productivity of banana is highly reduced by transmission of insect pests and diseases caused by the farmer produced suckers as well as the application of high quantities of pesticides that result in additional cost of production and serious consequences for the environment. In general, the traditional clonal propagation method is unable to supply the increasing demand for disease free and healthy planting materials of banana.

According to Lepoivre (2000), virus diseases are major productivity limitations for vegetatively propagated bananas. Therefore, it is crucial to investigate and adopt alternative propagation method of banana that ensures disease free and healthy planting materials. To improve the present scenario, tissue culture technology could be best alternative which can provides rapid and large scale planting material (Stover and Simmonds, 1987).

In micropropagation, rooting of micro-cuttings is often problematic due to unmatched combination of auxins. Losses at this stage have vast economic consequences. In conventional propagation via cutting, many genotypes are also found weak in formation of roots (Hazarika *et al.*, 2006). Thus research on strong root development *in vitro* is highly needed. At same time, strong root formation fascinating scientific subject matter.

Critical prerequisites in the development of banana include efficient and reproducible plant regeneration systems and a successful acclimatization process of tissue cultural plants for field transfer (Sagi *et al.*, 1998). The two major problems faced in developing tissue cultural banana plants are low rooting efficiency of regenerated shoots and low acclimatization rate of rooted *in vitro* raised plants in the field. These problems could probably be due to poor quality roots such as

thickened, callused, and absent lateral roots and root hairs formation, which all affect the uptake of nutrients by the plants after transfer into soil. Therefore, rooting efficiency and quality roots formation are critical in ensuring successful and continuous production of tissue cultural banana. Indole-3-butyric acid (IBA) had been reported by many researchers as the best auxin for root induction in banana at an optimum concentration of 10 μ M. IBA was known to have greater ability to promote rooting with less callus formation (Stickens *et al.*, 1996). Numerous authors reported that rooting in agar medium produced thick, short, and stumpy roots. Many authors reported that vermiculite was able to improve rooting efficiency and produce good quality roots which were finer with abundant lateral branches and root hairs (Singh, and Shetty, 2011).

Based upon these studies which reported positive effects of auxin on rooting in banana outside of the Ethiopia, an effort had been made to improve rooting in most popular commercial cultivars of Ethiopia i.e., Poyo and Grand Naine which was need of hour.

Micropropagation has been widely used for rapid mass multiplication of banana; however, its application on commercial scale is restricted often due to high rate of plant loss when transferred to natural or *ex vitro* conditions due to weak rooting (Hartman *et al.*, 1990).

Every technology has some limitations; Tissue culture technology is not exception to it. However, success at commercial scale is constrained by formation of aberrant plantlets and low survival of the regenerate during transfer to field conditions.

The major problem in field survival of tissue culture raised plantlets is due to poor *in vitro* rooting. Crops with root problems usually have irregular growth and flowering resulted

in to huge economic loss to the farmers especially small-scale farmers (Gaspar *et al.*, 1996; Sahay and Verma, 2000; Bairu and Kane, 2011).

Considering this prelude the present study was undertaken with specific objective to determine the effect of different combination and concentrations of Auxins (ABA and NAA) on root initiation, root numbers, root length and survival rate of *in vitro* raised plantlets of two banana varieties.

Materials and Methods

The experimental material comprised of three banana cultivars Viz. Poyo and Grand naine. From each cultivar sucker was used as explant for *in vitro* rooting.

The suckers of banana (*Musa spp.*) cv. Poyo and grand naine (Collected from Areka tissue culture centre) were treated with 70 % cetrimide for 5 min, rinsed thoroughly with double distilled water followed by treatment with 70 % ethanol for 5 min and 0.1 % mercuric chloride solution for 5 min.

Finally the suckers were washed 5-6times with sterile double distilled water under aseptic conditions

Elongation of shoots

The adventitious buds/multiple shoot induced from the explants were excised from the bunch without any callus or globular structures and cultured on the shoot elongation medium (SEM), consisting of MS medium supplemented with BAP and Kinetin.

Rooting

The elongated shoots were transferred to rooting medium with varying concentrations of IBA and NAA in sole and in combination. After appropriate sub culturing, well grown

shoots were aseptically transferred for root induction in the culture jars treated with five levels (0.0mg/l, 0.5mg/l, 1.0mg/l, 1.5mg/l and 2.0mg/l) of IBA and NAA.

Their combination (IBA+NAA) was taken in arrangements (i.e. IBA 0.5mg/l+NAA0.5mg/l and IBA1.0mg/l+NAA1.0mg/l). The combine effects of auxins with minimal concentration on rooting of banana have been recorded by many researchers (Tilkat, 2009; Arias, 1992). Finally all these were incubated at $25 \pm 2^{\circ}\text{C}$ temperature and photoperiodic cycle of 16 hours with light intensity. In this step there were total of 10 treatments for each banana variety ten cultured tubes for each. The number of cultured tubes in each round was 200 (10 treatments \times 2 varieties \times 10 culture tubes).

Transplantation

After six weeks the plantlets were taken out, roots were washed thoroughly with tap water and transferred to small earthen pots filled with potting mixture consisting of garden soil, sand and bio-manure (Green house, Areka Tissue culture laboratory) in equal proportion. Each pot with plantlet was initially covered with transparent polythene bag for 5 days. However, after 2 days the corners of the polythene bags were cut.

Subsequently, the polythene bags were removed. After allowing the plants to grow further for another 6 days, the plants were carefully transferred to large earthen pots (30 cm diameter \times 37.5 cm height) having garden soil, sand and bi-manure in equal proportion. After two weeks the plantlets were transferred to field and survival rate was measured after 4 weeks.

Statistical analysis

All the quantitative data analysis was done by using excel spread sheet, recorded for all

responses were subjected to two-way Analysis of variance (ANOVA) and significant differences among treatments were determined by Fisher's Least Significance Difference (LSD) at 5% level of significance by using the SAS (Version 9.2) software package for the differentiation of the effect of treatment, genotype and treatment-genotype interaction.

Results and Discussion

Effect of different concentrations of auxins (IBA and NAA) in sole and combination on *in vitro* root induction and duration of root initiation culture in Poyo variety

Highest numbers of roots (7.3 and 8.7) were observed on the medium supplemented with 0.5mg/l BAP, while in the control medium produced 2.1 and 2.8 roots/plant after 18 days and 36 days of inoculation, respectively (Table 1 and Fig. 2). These results are in agreement with the findings of Molla *et al.*, (2004) who obtained 8.28 maximum numbers of roots (8.28 / plant) on 0.5 mg/l IBA followed by 6.33 roots, on 0.6 mg/l IBA. It indicates increasing concentration of IBA doesn't in favor of rooting. The results of current investigation also in line with the findings of Gubbuk and Pekmezci (2001) who obtained 8.28 roots per plantlet on MS medium fortified with 0.5 mg/l IBA.

On the MS Medium provided with NAA, the highest numbers of roots (4.2 and 6.5/ plant) were recorded at the concentration 1.0mg/l NAA, while the significantly low number of roots 1.3 and 2.1 recorded in the control group after 18 days and 36 days of inoculation, respectively (Table 1). These results are similar with the findings of Anbazhagan *et al.*, (2014) who recorded maximum number of roots (6.1/ plant) on the MS medium augmented with 1.0mg/l NAA. The number of roots/plant received in current investigation also comparable with

Viehmanna *et al.*, (2007) who recorded maximum number of roots (14.79 roots /plant) on MS media supplemented with 5.4 μ M NAA (which is equivalent to 1.0 mg/l).

The results are also commensurate with the

findings of Sazedur *et al.*, (2013) who stated, 1.0mg/l NAA best for rooting in the cv. *Agnishwar*. The results also found in line with the findings of Rout G (2004) and Habib (1994) who reported superiority of NAA over other auxins in root development *in vitro*

Table.1 Effect of different concentrations of auxins (IBA and NAA) in sole and combination on *in vitro* root induction culture in *Poyo* variety

Type of growth Regulator	Concentration In mg/l	Number of roots 18 days after the inoculation		Number of roots 36 days after the inoculation	
		Number of roots	Std. Error	Number of roots	Std. Error
MS + IBA	0.0	2.1000	.32240	2.8000	.25517
	0.5	7.3000	.96575	8.7000	1.09128
	1.0	5.3000	.52451	7.2000	.98601
	1.5	4.5000	.44020	6.8300	.84064
	2.0	3.2000	.46092	4.1700	.62112
MS + NAA	0.0	1.3000	.27162	2.1000	.35214
	0.5	3.6000	.54549	4.8000	.40056
	1.0	4.2000	.49643	6.5000	.52557
	1.5	3.5200	.39038	4.9000	.52831
	2.0	3.3000	.44944	4.5000	.54732
MS+ IBA+NAA	0.5+0.5	5.5000	.86049	8.6000	.64274
	1.0+1.0	4.3000	.40661	6.9000	.56431

Table.2 Effect of different concentrations of auxins (IBA and NAA) in sole and combination on *in vitro* root induction culture in *Grand naine* variety

Type of growth Regulator	Concentration In mg/l	Number of roots 18 days after the inoculation		Number of roots 36 days after the inoculation	
		Number of roots	Std. Error	Number of roots	Std. Error
MS + IBA	0.0	3.7500	.50602	4.9000	.73409
	0.5	4.0000	.41096	5.5000	.62414
	1.0	4.7500	.50668	6.2500	.57701
	1.5	5.2500	.57528	6.8500	1.06011
	2.0	3.5500	.43211	4.6500	.78206
MS + NAA	0.0	1.3000	.26119	2.0400	.32971
	0.5	2.0800	.33856	3.6900	.47550
	1.0	3.4700	.37625	3.9000	.50022
	1.5	3.1600	.49826	3.7000	.34928
	2.0	2.8900	.47410	3.3900	.49294
MS+ IBA+NAA	0.5+0.5	3.2500	.50755	3.5000	.34319
	1.0+1.0	2.1500	.29411	2.8500	.34681

Plate.1 Preparation of Explant and Shoot elongation



Plate.2 Rooting after 18 days



Plate.3 Rooting after 36 days



Effect of different concentrations of auxins (IBA and NAA) in sole and combination on *in vitro* root induction in Grand naine variety

In Grand Naine, the maximum number (5.25 and 6.85) and the minimum (3.55 and 4.65) roots/ plant were observed in the MS media supplemented with 1.5mg/l IBA and 2.0mg/l IBA after 18th and 36th days of the inoculation, respectively (Table 2 and Fig. 2). Similar result reported by Million *et al.*, (2013) that IBA 1.5mg/l produced maximum number of roots 5.1 and 7.1 in 15 and 30 days. But in contrast with Shahanawaz *et al.*, (2014) who reported maximum rooting on MS medium of half strength supplemented with IBA 1.0mg/l and activated charcoal 200mg/l.

This might be because of difference in temperature, banana variety and strength of the MS media used. As per the Gubbuk H, (2004) continued rhizogenises is influenced by sugar, Auxin, temperature and light.

The MS medium with NAA, produced (3.47 and 3.90) roots/plant. Whereas, poor response was observed with least number of roots

(1.3and 2.04) on MS media added with 1.0mg/l NAA and the control media respectively (Table 2 and Fig. 2). Similar result was reported by Anbazhagan *et al.*, (2014) with the maximum number of roots 3.4 using NAA at the concentration 1.0mg/l.

The MS medium with combination of IBA and NAA produced maximum number of (3.25 and 3.5) root/plant with the combination of 0.5mg/l IBA+0.5mg/l NAA and 1.0mg/l IBA+1.0mg/l NAA after 18th and 36th days of inoculation, respectively (Table 2 and Fig. 2). This results are in agreement with Arun *et al.*,(2012), who reported 4.56 and 2.30 roots/plant on 0.5mg/l IBA+0.5mg/l NAA and 1.0mg/l IBA+1.0mg/l NAA fortified MS medium respectively. However, the appropriate amount of auxin in the rooting medium is crucial for the induction of the root (Arias 1992; Arinaitwe *et al.*, 2000).

The overall percentage of rooted shoots

The percentages of rooting/ shoots were counted from all treatment tested in the two banana varieties. The MS medium fortified with BAP produced 60, 80, 80, 80, and 70%

in *Poyo* and 50, 70, 80, 90 and 70% in the Grand naine with 0.0, 0.5, 1.0, 1.5 and 2.0mg/l concentration. On the other hand plantlets taken from MS medium supplemented with NAA showed the survival rates of 50, 70, 70, 80 and 90% in *Poyo* and 40, 60, 60, 70 and 90% in Grand naine at 0.0, 0.5, 1.0, 1.5 and 2.0mg/l concentration, respectively. This shows that there is direct proportionality between increase in concentration of growth regulators and the percentage of rooted shoots. These result confirms the previous finding with Sazedur *et al.*, (2013) who achieved 83, 96 and 88% survival of the plantlets taken from MS medium added with 0.5, 1.0 and 2.0 mg/l IBA and 67, 76 and 79% as percentage of rooted shoots in 0.5, 1.0 and 2.0mg/l NAA.

Rapid propagation of banana planting material through sucker culture ensures quick availability of genetically uniform (true to type) diseases free planting materials within short period of time. In the present study, an effective protocol for root development from sucker as explants was developed for two banana varieties *Poyo* and Grand naine. Accordingly, irrespective of the type of carbon source 3% sucrose used in the MS solid medium, the concentration of 0.5 mg/l IBA was found to be the best for root induction and root length for varieties *Poyo*, while the 2.0 mg/l NAA found to be the best for quick ignition of rooting in both cultivars tested. The combination 0.5 mg/l IBA + 0.5 mg/l NAA and 1.0 mg/l IBA + 1.0 mg/l NAA fail to produce promising results for *Poyo*.

Whereas the combination of IBA and NAA (1.0 mg/l each) found best for quick root induction in grand naine but fail to produce multiple roots. The concentrations of IBA 1.5 and 0.5 mg/l found best for multiple rooting and highest length of roots, respectively in grand naine.

The survival rate was recorded highest in the plantlets which were taken or raised in MS medium with 2.0 mg/l IBA and 2.0 mg/l NAA.

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