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Full Length Research Paper

Effect of different combinations of 6-benzyl amino purine and naphthalene acetic acid on multiple shoot proliferation of plantain (*Musa* spp.) cv. Matoke from meristem derived explants

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This study was conducted at the Amhara Region Agricultural Research Institute Tissue culture Laboratory, Ethiopia from April to June 2012 to investigate the effect of different concentrations of 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA) on virus free plant regeneration and shoot multiplication. The culture meristem grew into callus after 28 days then adventitious plantlets were developed. Among the different concentrations, 5 mg/l BAP + 1.0 mg/l NAA showed highest shoot proliferation (1.00, 1.67, 1.75 and 3.08 shoots per clump) at 10, 20, 30 and 60 days after induction (DAI), respectively. Good numbers of shoots were achieved at 5 mg/l BAP + 0.5 mg/l NAA at 60 DAI (3.08). The longest shoot was produced by the concentration of 5 mg/l BAP + 1.0 mg/l NAA showed (0.43, 2.42, 2.63 and 3.42 shoots per plantlet) at 10, 20, 30 and 60 DAI, respectively. The maximum number of leaves (1.67, 2.67, 3.67 and 4.33 leaves per explant) at 10, 20, 30 and 60 DAI produced on the medium supplemented with 5.0 mg/l BAP and 0.50 mg/l NAA. The lowest number of leaves was obtained from the control treatment. The longest leaves were produced by the concentration of 5.0 mg/l BAP + 1.0 mg/l NAA (1.52, 2.27, 2.70 and 3.13 cm) at 10, 20, 30 and 60 DAI, respectively.

Key words: Plantain, regeneration, micro propagation.

INTRODUCTION

Banana and plantains (*Musa* spp.) are the second largest food-fruit crops of the world produced in the tropical and subtropical regions of mostly the developing countries. The two together are positioned fourth in terms of gross value. Bananas are consumed as ripe fruit, where as plantains, which remain starchy even when fully ripe, need cooking for palatability and consumption. Originally, crops from humid tropics, they have acclimatized to abroad range of climatic conditions. While bananas have come to occupy the status of a high value, commercial crop, plantains have remained a staple food of many ethnic groups.

Irrespective of their commercial status, banana and plantains are referred as ‘poor man’s apple’. Banana is globally ranked fourth, next to rice, wheat and maize in terms of gross value of production. Plantain is one of the staples in most West African countries. It is estimated that about 70 million people in West and Central Africa derive more than a quarter of their food energy requirement from plantain (Singh et.al., 2011; IITA, 1990).

One of the differences between plantain and banana is the high levels of phenolic substances in the plantain, a substance that influences the proliferation of plantain under *in vitro* culture. Plantain is vegetatively propagated because almost all cultivated types are triploid, seedless or produce sterile and non viable seeds. *In vitro* propagation of bananas provides excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability

of disease-free material all year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants and faster growth in the early growing stages compared to conventional materials (Daniells and Smith, 1991; Arias, 1992).

The East African highland bananas that make up 18% of all banana fruit produced are either cooked to prepare “matooke” or used to make banana beer. The ABB genomic group produces starchy fruit that is cooked in many countries and also used as a beer-producing banana in East Africa. The cooking banana types generally contain more starch when ripe and are boiled, fried, or roasted to make them palatable. In this regard, many alternative edible forms of banana, such as sundried banana figs, fried chips, flour and banana sauce are being processed in many countries (De Langhe, 1964).

Banana (*Musa* spp.) is an important food crop in sub-Saharan Africa and ranks the fourth most important staple crop in developing countries. In Ethiopia, banana is the second major fruit crop next to citrus. The cooking and beer bananas are adapted to relatively high altitude and humid regions, whereas AAB and ABB types are produced in more arid regions due to their resistance to drought. Concerning the introduction of banana to Ethiopia, no one knows for sure when it entered to the country. All sorts of varieties (dessert, cooking, brewing) are grown throughout Ethiopia. These materials are not studied well (Seifu, 1999).

The productivity of vegetatively propagated banana and plantain is greatly reduced by virus disease (Lepoivre, 2000). Unfortunately, expansion of banana production is frequently limited by costly high quality planting materials. The farmer-produced suckers are good transmitters of insect pests and diseases. This has prompted interest in the use of *in vitro* tissue culture technique. Through meristem micro propagation, pathogen free clones are obtained. Virus is one of the major problems in Ethiopia. The traditional clonal propagation method appears to be unable to supply the increasing demand for disease free and healthy planting materials of banana.

To minimize the previous mentioned problems, micro propagation could be an alternative which offers advantages of rapid clonal propagation, pathogen elimination, avoidance of environmental hazards, year round availability of planting materials, long term maintenance of germplasm and reduction in labor cost as compared to traditional methods.

Banana plantlets regenerated through tissue culture have higher survival rate, reduce the cost of disease and pest control, show vigorous growth and have a shorter harvesting period (Ortiz and Vuylsteke, 1996). Therefore, considering the previous fact; the present study was undertaken with the following major objective: to study the effect of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) on shoot proliferation of plantain cv. Matoke.

MATERIALS AND METHODS

Stock solution and media preparation

The media namely Murashige and Skoog (MS) media (1962) were prepared by dissolving the appropriate amount of macro and micro nutrient; and organic supplements. Similarly, growth regulators (BAP and NAA) stock solutions were prepared using the proportion of 1 mg: 1 ml and stored in refrigerator at 4°C. The MS culture media were prepared from its respective stock solutions using the appropriate amount of sucrose, plant growth regulators and agar (7 g/l) and were used as culture medium for shoot initiation and multiplication. All combinations of both growth regulators at three levels of BAP (0.0, 2.5, and 5.0 mg/l) and three levels of NAA (0.0, 0.5, and 1.0 mg/l) were added separately to the media to study its effect on shoot proliferation. The jars with media were then dispensed 35 ml each and autoclaved at 1.06 kg/cm^2 pressure at 121°C for 25 min after adjusting the pH to 5.8 with 1 N NaOH or 1 N HCl.

Plant material preparation

A variety of plantain namely Matoke (AAB) was obtained from *Woramit* Agricultural research center. Sword suckers of about four months of age grown under field conditions were brought to the preparation room. The suckers were washed thoroughly under running tap water. The roots and outer tissues of the suckers were removed with the help of a sharp knife. A number of outer leaves were removed until the shoot measured about 1.0 to 2.0 cm in length and 1.0 cm width at the base. The pale white tissue block (1.0 × 2.0 cm) containing meristem and corm base were taken in a beaker. Surface sterilization was done under laminar airflow cabinet with 70% ethyl alcohol, the explants were surface sterilized with 0.1% mercuric chloride and a few drops of Tween 20 for 15 min. Finally, the explants were then rinsed three times with distilled water (Figure 1).

Initiation

Shoot cultures of banana start conventionally from any plant part that contains a shoot meristem, that is, the parental pseudo stem, small suckers, peepers and lateral buds (Vuylsteke, 1989). From the selected sucker, a cube of tissue of about 1 to 2 cm^3 containing the apical meristem was excised and then dipped in 70% ethanol for 10 min, surface sterilized in a 2% sodium hypochlorite solution, and after 20 min rinsed three times for 10 min in sterile water. The explants were placed directly on a multiplication-inducing culture medium. For banana micropropagation, MS-based media (Murashige and Skoog, 1962) supplemented with sucrose as a carbon source at a concentration of 30 g/l were widely adopted.

Usually, two types of growth regulators; a cytokinin (BAP) and an auxin (NAA), were added to the growth medium. Three levels of BAP (0.0, 2.5 and 5.0 mg/l) and three levels of NAA (0.0, 0.5, and 1.0 mg/l) of all combinations were used as treatments to the banana initiation medium.

In most banana micropropagation systems, semi-solid media was used. As a gelling agent agar (7 g/l) was frequently added to the culture medium. Banana shoot-tip cultures were incubated at an optimal growth temperature of 28 ± 2°C in a light cycle of 12 to 16 h with a photosynthetic photon flux (PPF) of about 60 µE/m^{2}s^1 (Al-amin et al., 2009) (Figure 2).

Figure 1. Plantain cv. Matoke plant and its suckers.

Figure 2. Initiation stage of banana cv. Giant Cavendish.

Figure 3. Multiplication stage of plantain cv. Matoke.

Multiplication

Initial sub-culturing was done when the ex-plants produced some shoots. For sub-culturing, the entire samples of *in vitro* shoot were cut into small pieces so that each piece was containing about one shoot. Leaf and blackish or brownish basal tissues were removed to expose the meristems. Each piece was inoculated into a similar fresh medium. It was practiced at the interval of every one month. When the shoots grew to about 3 to 5 cm in length with three to six well developed leaves, they were rescued aseptically from the culture tubes and were separated from each other and again cultured on freshly prepared medium containing different combinations of hormonal supplements (Figure 3).

Analysis of variance (ANOVA) of Matoke shoots proliferation parameters

Analysis of variance (ANOVA) of plantain cv. Matoke for complete block design revealed significant difference ($P < 0.01$) due to the main effect of different concentrations of BAP and NAA for the means of number of shoots per clump 10 days after inoculation (SN1); length of shoots per plantlet (cm) 10 days after inoculation (SL1); length of leaves per explants (cm) 10 days after inoculation (LL1); number of shoots per clump 20 days after inoculation (SN2); length of shoots per plantlet (cm) 20 days after inoculation (SL2); number of leaves per explants (No) 20 days after inoculation (LN2); length of leaves per explants (cm) 20 days after inoculation (LL2); length of shoots per plantlet (cm) 30 days after inoculation (SL3); number of leaves per explants (No) 30 days after inoculation (LN3); length of leaves per explants (cm) 30 days after inoculation (LL3); length of shoots per plantlet (cm) 60 days after inoculation (SN4); length of shoots per plantlet (cm) 60 days after inoculation (SL4); number of leaves per explants (No) 60 days after inoculation (LN4) and length of leaves per explants (cm) 60 days after inoculation (LL4).

RESULTS AND DISCUSSION

Effect of different concentrations of BAP and NAA on shoot number of Matoke

The effects of different concentrations of BAP and NAA on shoot number were statistically significant at $P < 0.01$ at all days after induction (DAI). Among the different concentrations, 5 mg/l BAP + 1.0 mg/l NAA showed highest shoot proliferation (1.00, 1.67, 1.75 and 3.08 shoots per clump) at 10, 20, 30 and 60 DAI, respectively. A good number of shoot proliferations was achieved at 5 mg/l BAP + 0.5 mg/l NAA at 60 DAI (3.08) which was superior to the control treatments (1.17).

Okole and Schultz (1996), realized that high concentration of BAP led to poor shoot formation. The explants cultured on MS medium without growth regulator produces only single shoot at 20, 30 and 60 DAI (Table 1). These findings did not agree with the results of Khanam et al. (1996) and Rabbani et al. (1996) where they did not monitor any shoot formation in case of control treatment. This variation might be due to the genotype, culture environments and the different concentrations of NAA and BAP and their associations (Farahani et al., 2008).

Effect of different concentrations of BAP and NAA on shoot length of Matoke

The MS medium supplemented with BAP and NAA showed different results for increasing shoot length which was significantly influenced by different concentrations. The longest shoot was produced by the concentration of 5 mg/l BAP + 1.0 mg/l NAA showed highest shoot proliferation (0.43, 2.42, 2.63 and 3.42 shoots per plantlet) at 10, 20, 30 and 60 DAI, respectively (Table 2). Rahaman et al. (2004) observed similar result. They obtained longest shoot in 5.0 mg/l BAP (3.62 cm). The results of the present study differs from the findings of Khanam et al. (1996) and Al-amin et al. (2009) who obtained longest shoot in banana on MS medium supplemented with 7.5 mg/l BAP treatments.

Effect of different concentrations of BAP and NAA on leaf number of Matoke

The results show that the maximum number of leaves (1.67, 2.67, 3.67 and 4.33 leaves per explant) at 10, 20, 30 and 60 DAI produced on the medium supplemented with 5.0 mg/l BAP and 0.50 mg/l NAA. Rahman et al. (2004) found the maximum number of leaves (3.12 per plantlet) at 30 DAI produced with 5.0 mg/l BAP, which was similar with the present study. Rabbani et al. (1996) obtained same results from 5.0 mg/l BAP. The lowest number of leaves (0.00, 0.00, 0.00 and 0.67 leaves per

Table 1. Mean effects of BAP and NAA combinations on shoot number of Plantain cv. Matoke at different days after inoculation.

Treatment (BAP-NAA, mgL^{-1})	Parameter			
	SN1	SN2	SN3	SN4
0-0	0.17^{b}	0.75^{c}	1.00^{d}	1.17^{e}
0-0.5	0.75^{a}	$1.08b^{c}$	1.08^{cd}	2.17^{d}
0-1	1.00^{a}	1.14^{b}	1.50^{ab}	2.25^{cd}
2.5-0	0.83^{a}	1.16^{b}	1.58^{ab}	2.42^{bcd}
2.5-0.5	0.92^{a}	1.08^{bc}	1.58^{ab}	2.67^{abc}
2.5-1	0.83^{a}	1.08^{bc}	1.33^{bc}	2.50^{bcd}
5-0	0.92^{a}	1.17^{b}	1.75^{a}	2.75^{ab}
5-0.5	0.92^{a}	1.25^{b}	1.58^{ab}	2.33^{bcd}
5-1	1.00^{a}	1.67^{a}	1.75^{a}	3.08^{a}
LSD	0.37	0.34	0.32	0.45
CV (%)	26.00	17.20	12.84	11.09
Significance level	**	**	**	**

Means followed by the same letter(s) are not significantly different at $P > 0.05$. **, significant at ($P<0.01$); SN1, number of shoots per clump 10 days after inoculation; SN2, number of shoots per clump 20 days after inoculation; SN3, number of shoots per clump 30 days after inoculation; SN4, length of shoots per plantlet (cm) 60 days after inoculation.

explant) at 10, 20, 30 and 60 DAI were obtained from control treatment (Table 3) which does not agree with the finding of Al-amin et al. (2009) but agree with Rabbani et al. (1996) and Rahman et al. (2004) where they did not monitor any leaves formation at 10, 20 and 30 DAI.

Effect of different concentrations of BAP and NAA on leaf length of Matoke

The MS medium supplemented with BAP and NAA showed different results for leaf length, which was significantly influenced by different concentrations. The longest leaves were produced by the concentration of 5.0 mg/l BAP + 1.0 mg/l NAA (1.52, 2.27, 2.70 and 3.13 cm) at 10, 20, 30 and 60 DAI, respectively, which was statistically significant. Statistically identical results were observed in 5.0 mg/l BAP + 0.5 mg/l NAA at 20 DAI (2.10 cm), 30 DAI (2.5 cm) and 60 DAI (2.97 cm) as presented in Table 4. The results of present experiment agree with the findings of Khanam et al. (1996) and did not agree with Al-amin et al. (2009) who obtained longest leaves in banana on MS medium supplemented with 25 µM (5 mgL^{-1}) BAP and 7.5 mg/l BAP treatments, respectively. Rahaman et al. (2004) observed different result. They obtained longest leaf in the treatment 5.0 mg/l BAP (3.62 cm) followed by 1.5 mg/l NAA and 4.0 mg/l BAP (3.40 cm).

Mean value comparison of plantain cultivars on shoot proliferation

The effects of different concentrations of BAP and NAA on shoot number 10 DAI were statistically significant at ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (1.00).

The effects of different concentrations of BAP and NAA on shoot length 10 DAI were statistically significant at $P < 0.01$. Among the different concentrations, 5 mg/l BAP + 1.0 mg/l NAA showed highest shoot proliferation (0.43 cm). The effects of different concentrations of BAP and NAA on leaf number 10 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (1.67).

The effects of different concentrations of BAP and NAA on leaf length 10 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (1.52 cm) (Figure 4). The effects of different concentrations of BAP and NAA on shoot number 20 DAI were significant at $P < 0.01$. Among the different concentrations 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (1.67).

The effects of different concentrations of BAP and NAA on shoot length 20 DAI were statistically significant at $P<0.01$. Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (2.42 cm). The effects of different concentrations of BAP and NAA on leaf number 20 DAI were statistically significant at $P < 0.01$. Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (2.67).

The effects of different concentrations of BAP and NAA on leaf length 20 DAI were statistically significant $P < 0.01$. Among the different concentrations, 5 mg/l BAP +

Table 2. Mean effects of BAP and NAA combinations on shoot length of Plantain cv. Matoke at different days after inoculation.

Treatment (BAP-NAA, mgL^{-1})	Parameter			
	SL1	SL2	SL3	SL4
0-0	0.03^{e}	0.67^{f}	0.98^{d}	1.36^{c}
0-0.5	0.10^{e}	1.28^{e}	1.90^{c}	2.27^{b}
0-1	0.21^{d}	1.53^{e}	2.03^{bc}	2.47^{b}
2.5-0	0.21^{d}	1.59^{de}	2.03^{bc}	2.53^{b}
2.5-0.5	0.31^{c}	1.87^{cd}	2.06^{abc}	2.90^{ab}
2.5-1	0.32^{bc}	1.88^{cd}	2.17^{abc}	2.53^{b}
5-0	0.34^{bc}	1.99^{bc}	2.36^{abc}	3.08^{ab}
5-0.5	0.39^{ab}	2.32^{ab}	2.49^{ab}	3.39^{a}
5-1	0.43^{a}	2.42^{a}	2.63^{a}	3.42^{a}
LSD	0.07	0.33	0.58	0.83
CV (%)	15.86	11.11	16.05	18.10
Significance level	**	**	**	**

Means followed by the same letter (s) are not significantly different at $P > 0.05$;**, significant at ($P < 0.01$); SL1, length of shoots per plantlet (cm) 10 days after inoculation; SL2, length of shoots per plantlet (cm) 20 days after inoculation; SL3, length of shoots per plantlet (cm) 30 days after inoculation; SL4, length of shoots per plantlet (cm) 60 days after inoculation.

Table 3. Mean effects of BAP and NAA combinations on leaf number of Plantain cv. Matoke at different days after inoculation.

Treatment (BAP-NAA, mgL^{-1})	Parameter			
	LN1	LN2	LN3	LN4
0-0	0.00^{d}	0.00^{c}	0 0.00^{d}	0.67^{c}
0-0.5	0.33^{cd}	1.00^{bc}	1.33^{c}	2.33^{b}
0-1	0.67^{bcd}	1.67^{ab}	2.67^{ab}	2.67^{b}
2.5-0	0.67^{bcd}	1.67^{ab}	2.67^{ab}	3.33^{ab}
2.5-0.5	1.00^{abc}	1.67^{ab}	2.67^{ab}	3.33^{ab}
2.5-1	1.00^{abc}	2.00^{ab}	2.33^{bc}	3.33^{ab}
5-0	1.33^{ab}	2.33^{a}	2.67^{ab}	3.33^{ab}
5-0.5	1.33^{ab}	2.33^{a}	3.33^{ab}	4.00^{a}
5-1	1.67^{a}	2.67^{a}	3.67^{a}	4.33^{a}
LSD	0.84	1.03	1.16	1.13
CV (%)	54.66	35.04	28.42	21.50
Significance level	*	**	**	**

Means followed by the same letter(s) are not significantly different at $P > 0.05$**, significant at ($P<0.01$); LN1, number of leaves per explants (No) 10 days after inoculation; LN2, number of leaves per explants (No) 20 days after inoculation; LN3, number of leaves per explants (No) 30 days after inoculation; LN4, number of leaves per explants (No) 60 days after inoculation.

1.00 mg/l NAA showed highest shoot proliferation (2.27 cm) (Figure 5). The effects of different concentrations of BAP and NAA on shoot number 30 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (1.75).

The effects of different concentrations of BAP and NAA on shoot length 30 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (2.63 cm). The effects of different concentrations of BAP and NAA on leaf number 30 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (3.67).

The effects of different concentrations of BAP and NAA on leaf length, 30 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (2.70 cm) (Figure 6). The effects of different concentrations of BAP and NAA on shoot number 60 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (3.42).

The effects of different concentrations of BAP and NAA on shoot length 60 DAI were statistically significant ($P <$

Table 4. Mean effects of BAP and NAA combinations on leaf length of plantain cv. Matoke at different days after inoculation.

Treatment (BAP-NAA, mgL^{-1})	Parameter			
	SL1	SL2	SL3	SL4
0-0	0.03^{e}	0.67^{f}	0.98^{d}	1.36^{c}
0-0.5	0.10^{e}	1.28^{e}	1.90^{c}	2.27^{b}
0-1	0.21^{d}	1.53^{e}	2.03^{bc}	2.47^{b}
2.5-0	0.21^{d}	1.59^{de}	2.03^{bc}	2.53^{b}
2.5-0.5	0.31^{c}	1.87^{cd}	2.06^{abc}	2.90^{ab}
2.5-1	0.32^{bc}	1.88^{cd}	2.17^{abc}	2.53^{b}
5-0	0.34^{bc}	1.99^{bc}	2.36^{abc}	3.08^{ab}
5-0.5	0.39^{ab}	2.32^{ab}	2.49^{ab}	3.39^{a}
5-1	0.43^{a}	2.42^{a}	2.63^{a}	3.42^{a}
LSD	0.07	0.33	0.58	0.83
CV (%)	15.86	11.11	16.05	18.10
Significance level	**	**	**	**

Means followed by the same letter(s) are not significantly different at $P > 0.05$; **significance at ($P < 0.01$); SL1, length of shoots per plantlet (cm) 10 days after inoculation; SL2, length of shoots per plantlet (cm) 20 days after inoculation; SL3, length of shoots per plantlet (cm) 30 days after inoculation; SL4, length of shoots per plantlet (cm) 60 days after inoculation.

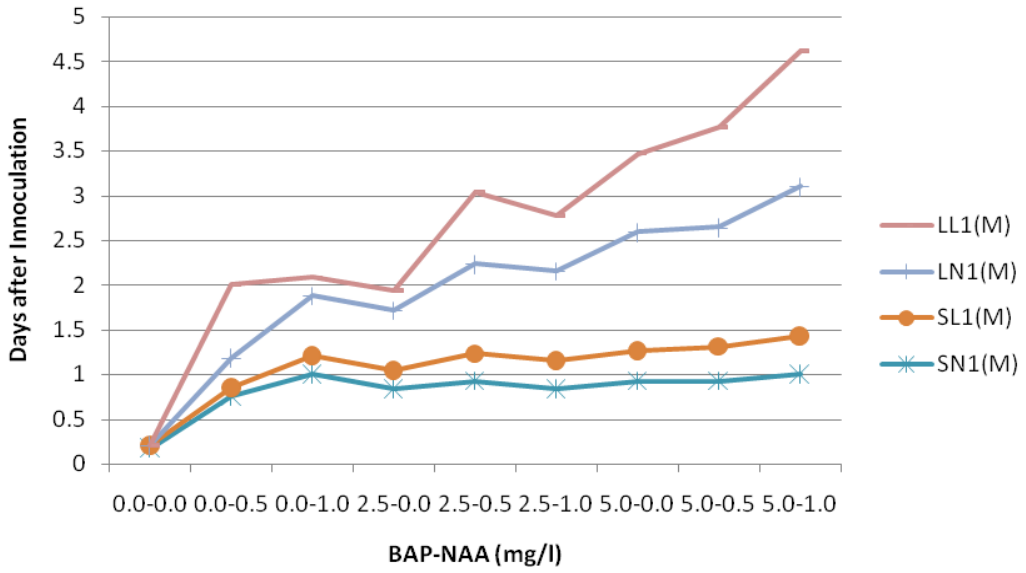

Figure 4. Effects of different concentrations of BAP and NAA on shoot proliferation 10 DAI.

0.01). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (3.42 cm).

The effects of different concentrations of BAP and NAA on leaf number 60 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (4.33).

The effects of different concentrations of BAP and NAA on leaf length 60 DAI were statistically significant ($P < 0.01$). Among the different concentrations, 5 mg/l BAP + 1.00 mg/l NAA showed highest shoot proliferation (3.13 cm) (Figure 7).

Correlation analysis between shoot proliferations of Matoke

Correlation coefficients among parameters of plantain cv. Matoke was shown in Table 5. As expected, all parameters were positively and significantly correlated to each other ($P < 0.01$) except SN1 and LL1 ($P < 0.05$) indicating that as number of shoots increases, probability

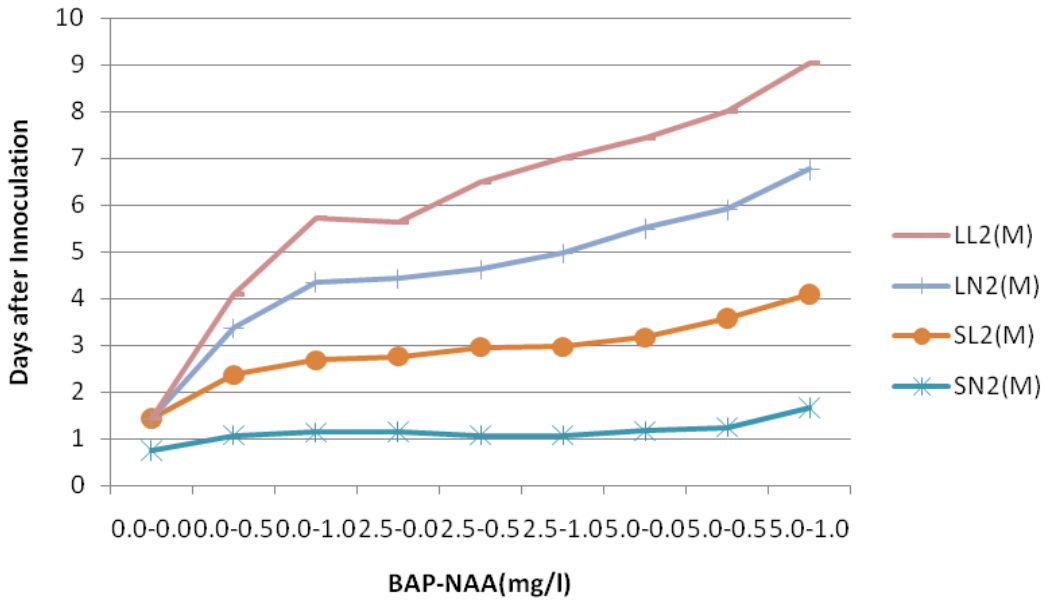

Figure 5. Effects of different concentrations of BAP and NAA on shoot proliferation 20 DAI.

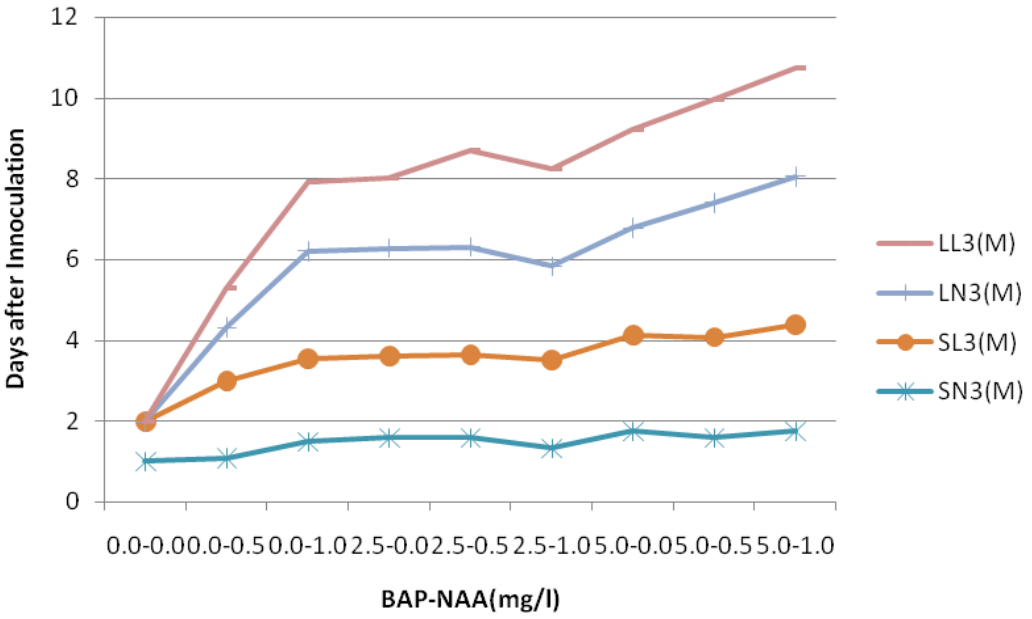

Figure 6. Effects of different concentrations of BAP and NAA on shoot proliferation 30 DAI.

of getting longest leaves became increased at 10 DAI.

Conclusions

Micro propagation techniques were developed during the past two decades and are now well established (Israeli et al.,1995). Shoot-tip culture is simple, easy and applicable to wide range *Musa* genotypes (Vuylsteke, 1989). Multiple shoot or bud formation is easily achieved by culturing shoot tips on standard nutrient media containing 2 to 5 mg/l cytokinin (mostly 6-benzylaminopurine) or by incising or fragmenting the shoot tips. Rates of multiplication range from two to 10 or more shoots or

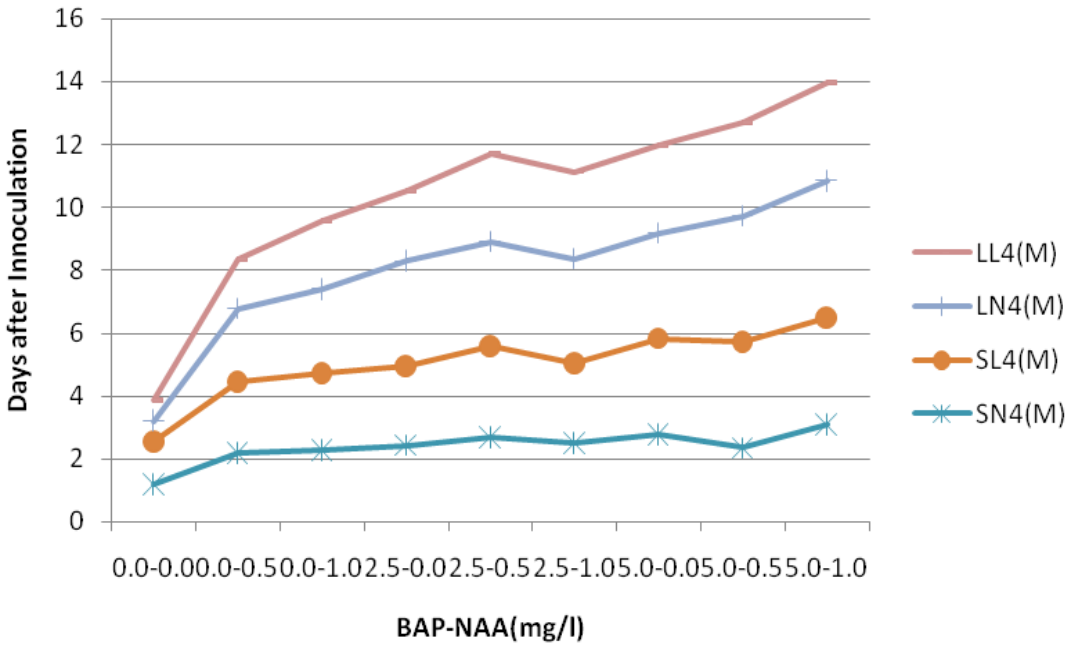

Figure 7. Effects of different concentrations of BAP and NAA on shoot proliferation 60 DAI.

bud propagules per month, resulting in potential propagation rates of several thousands or millions of plants per year. Such rates are several orders of magnitude greater than achievable through conventional propagation.

In vitro propagation has many advantages, such as higher rates of multiplying clean (pest and disease-free) planting material and the small amount of space required to multiply large numbers of plants, clonal propagation, uniform production, and breeding. Micro propagation has played a key role in plantain and banana improvement programs worldwide (Rowe and Rosales, 1996; Vuylsteke et al., 1997). Maximum yield gains from *in vitro* derived plants range from 20% in bananas to 70% in plantains. However, this superior field performance does not appear to be consistent and requires optimal crop husbandry (Vuylsteke, 1998).

The present study was conducted at the Tissue Culture Laboratory of Amhara Region Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia, during the period from March to August 2012 to investigate the effect of different concentrations of BAP and NAA on virus free plant regeneration and shoot multiplication. The culture meristem grew into callus after 25 days and adventitious plantlets were developed. Combinations of three levels of BAP (0.0, 2.5, and 5.0 mg/l) and three levels of NAA (0.0, 0.5, and 1.0 mg/l) were added separately to the media to study its effect on shoot proliferation.

Among the different concentrations, 5.0 mg/l BAP + 1.0 mg/l NAA showed highest shoot proliferation of 1.00, 1.67, 1.75 and 3.08 shoots per clump at 10, 20, 30 and 60 DAI, respectively, on plantain cv. Matoke. The longest shoot produced was 0.43, 2.42, 2.63 and 3.42 shoots per plantlet at 10, 20, 30 and 60 DAI, respectively, with the concentration of 5.0 mg/l BAP + 1.0 mg/l NAA. The maximum number of leaves was 1.67, 2.67, 3.67 and 4.33 leaves per explant at 10, 20, 30 and 60 DAI, on the medium supplemented with the same treatment. The longest leaves of 1.52, 2.27, 2.70 and 3.13 cm were produced at 10, 20, 30 and 60 DAI, respectively, by the concentration of 5.0 mg/l BAP +1.0 mg/l NAA.

According to the findings of the present study shoot proliferation of Plantain cv. Matoke were best on MS medium supplemented with 5.0 mg/l BAP + 1.0 mg/l NAA. Bearing in mind the importance of banana in Ethiopia, and the difficulties in traditional propagation of the crop, as outlined previously, an experiment has been conducted using suckers as a source. This research allows for conservation of banana clones, rapid propagation of selected disease-free planting materials and more efficient breeding procedures.

The experiment raised in the present attempt could also be used for the massive *in vitro* production of banana plantlets. Although, studies were made for different banana and plantain cultivars, only limited banana tissue culture experiments were conducted to date. Hence, future research needs to optimize protocols for each variety of banana using different tissue culture techniques in the region.

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Table 5. Simple correlation coefficients (r) among shoot proliferation of plantain cv. Matoke at different days after inoculation.

Parameter	SN1	SL1	LN1	LL1	SN2	SL2	LN2	LL2	SN3	SL3	LN3	LL3	SN4	SL4	LN4
LL4	0.71**	0.88**	0.66**	0.75**	0.58**	0.94**	0.77**	0.96**	0.70**	0.88**	0.81**	0.97**	0.78**	0.86**	0.87**
LN4	0.66**	0.82**	0.73**	0.76**	0.67**	0.87**	0.88*	0.83**	0.72**	0.87**	0.91**	0.88**	0.75**	0.83**	-
SL4	0.73**	0.81**	0.62**	0.71**	0.55**	0.93**	0.69**	0.76**	0.72**	0.94**	0.70**	0.77**	-0.66**	-	
SN4	0.72**	0.78**	0.58**	0.65**	0.76**	0.75**	0.70**	0.80**	0.76**	0.69**	0.69**	0.82**	-		
LL3	0.68**	0.88**	0.68**	0.76**	0.61**	0.88**	0.82**	0.98**	0.72**	0.81**	0.85**	-			
LN3	0.61**	0.78**	0.76**	0.71**	0.63**	0.79**	0.93**	0.80**	0.69**	0.74**	-				
SL3	0.72**	0.78**	0.60**	0.69**	0.56**	0.93*	0.73**	0.79**	0.70**	-					
SN3	0.64**	0.76**	0.63**	0.66**	0.68**	0.71**	0.70**	0.69**	-						
LL2	0.65**	0.87**	0.67**	0.76**	0.60**	0.87**	0.78**	-							
LN2	0.64**	0.82**	0.86**	0.72**	0.65**	0.79**	-								
SL2	0.71**	0.93**	0.72**	0.83**	0.62**	-									
SN2	0.64**	0.67**	0.59**	0.67**	-										
LL1	0.46*	0.88**	0.76**	-											
LN1	0.54**	0.79**	-												
SL1	0.70**	-													
SN1	-														

*Significance at P<0.05;**significance at P<0.01; ns, non significant; SN1, number of shoots per clump10 days after inoculation; SL1, length of shoots per plantlet (cm) 10 days after inoculation; LN1, number of leaves per explants (No) 10 days after inoculation; LL1, length of leaves per explants (cm) 10 days after inoculation; SN2, number of shoots per clump20 days after inoculation; SL2, length of shoots per plantlet (cm) 20 days after inoculation; LN2, number of leaves per explants (No) 20 days after inoculation; LL2, length of leaves per explants (cm) 20 days after inoculation; SN3, number of shoots per clump 30 days after inoculation; SL3, length of shoots per plantlet (cm) 30 days after inoculation; LN3, number of leaves per explants (No) 30 days after inoculation; LL3, Length of leaves per explants (cm) 30 days after inoculation; SN4, length of shoots per plantlet (cm) 60 days after inoculation; SL4, length of shoots per plantlet (cm) 60 days after inoculation; LN4, number of leaves per explants (No) 60 days after inoculation; LL4, length of leaves per explants (cm) 60 days after inoculation.

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