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Effects of Different Concentrations of BAP (6-Benzyl Amino Purine) and NAA (Naphthalene Acetic Acid) on Banana (*Musa spp.*) cv. Giant Cavendish Shoot Proliferation

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Abstract

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 May 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 a green globular hard coat mass after 25 days and adventitious plantlets were developed from this ball like structure. 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 + 0.5 mg/l NAA showed highest proliferation of 1.00, 1.5, 1.75 and 3.17 shoots per clump at 10, 20, 30 and 60 DAI, respectively. The longest shoot (0.42, 2.34, 2.64 and 3.46 cm shoots per plantlet) was produced with the concentration of 5.0 mg/l BAP + 0.5 mg/l NAA at 10, 20, 30 and 60 DAI respectively. The maximum number of leaves (2.33, 3.00, 3.33 and 4.33 leaves per explant at 10, 20, 30 and 60 DAI) produced on the medium supplemented with the same treatment. The second highest number of leaves (2.00, 2.67, 3.67 and 4.00 leaves per explant at 10, 20, 30 and 60 DAI) was produced on the medium supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. The longest leaves were produced by the concentration of 5.0 mg/l BAP + 0.5 mg/l NAA (1.43, 2.27, 2.80 and 3.23 cm) at 10, 20, 30 and 60 DAI respectively.

Keywords

Banana, Regeneration, Micro Propagation, Plantlet

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1. Introduction

Banana belongs to the genus *Musa*, which is one of the two genera in the family Musaceae and order Zingiberales. It is one of the major staple foods, as well as, a valuable source of income through local and international trade for millions of people. In gross production value, banana is the fourth most important food crop in sub-Saharan Africa (FAO STAT, 2003).

In Ethiopia, banana is the second major fruit crop next to citrus. It is produced throughout the country wherever there

is adequate rainfall or irrigation. Banana plantation is wide spreading in southwestern Ethiopia. But, fruit size, bunch weight and fruit quality of most banana plantations in the country are reduced from time to time due to the prevalence of biotic and abiotic stresses, which eventually limit its production and productivity to affecting particularly the livelihood of resource poor marginal smallholder farmers. A complex of foliar diseases, nematodes, viruses, and insect pests threatens the production of Ethiopian bananas (Seifu Gebre-mariam, 2003).

Mainly virus diseases are the major problems of banana in

Ethiopia. The traditional clonal propagation method with suckers commonly practiced in the country aggravates the problem of virus infestations in most banana plantations. Lepoivre (2000) also recognized virus diseases as major productivity limitations for vegetatively propagated banana and plantain. This traditional propagation method with suckers appears to be unable to supply disease free and healthy planting materials of banana. Therefore, it is crucial to investigate and adopt alternative propagation method(s) of banana that ensures disease free and healthy planting materials

Micro propagation could be used as a potential method for production and supply of disease free and healthy planting materials of banana. Apart from its function of eliminating pathogens, micro propagation offers advantages of rapid clonal propagation, avoidance of environmental hazards caused by pesticides application, year round availability of planting materials, long term maintenance of germplasm and reduction in labor cost over that of traditional propagation methods plants (Cronauer and Krikorian, 1984a; Hwang et al., 2000 and Helloit et al., 2002). 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). As regards yield performance, tissue cultured plants have been reported to produce 39% higher yield than plants from sword suckers (Pradeep et al., 1992).

Plant growth regulators are inevitable for *in vitro* regeneration of crop plants in any artificial medium. Generally, cytokinin helps in shoot proliferation and auxins helps in rooting of proliferated shoots. However, the requirement of cytokinin and auxins depends on the variety of banana and culture conditions (Cronauer and Krikorian, 1984a). Giant Cavendish variety plays a vital role in our national economy due to its popularity and acceptability to marginal and commercial farmers. To obtain virus and disease free healthy planting materials, optimization of a protocol for meristem culture of banana cv. Giant Cavendish are of prime importance. Therefore, considering the above fact, the present study was undertaken with the following major objective:

 To study the effect of BAP (6-benzylaminopurine) and NAA (naphthalene acetic acid) on shoot proliferation of banana cv. Giant Cavendish.

2. Materials and Methods

The study was conducted in the Tissue Culture Laboratory of Amhara Region Agricultural Research Institute (ARARI), Bahir Dar, Ethiopia. Materials and methods used for the study are presented in details here below.

2.1. Stock Solution and Media Preparation

The MS (Murashige and Skoog, 1962) media were prepared by dissolving the appropriate amount of macro and micro nutrients, and organic supplements. Similarly, stock solutions of growth regulators (BAP and NAA) were prepared at the ratio of 1mg: 1ml, respectively, 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 for shoot initiation and multiplication. Combinations of three levels of BAP (0.0, 2.5, and 5.0mg/l) and three levels of NAA (0.0, 0.5, and 1.0mg/l) were added separately to the media to study its effect on shoot proliferation. The experiments were arranged in completely randomized design (CRD) with four replications. Each treatment consisted of 10 culture tubes per replication. Data on number of shoots per clump, length of shoots per plantlets, number of leaves per explants and length of leaves per explants were collected. The jars with media were then dispensed 35 ml each and autoclaved at 1.06 kg/cm² pressure at 121°C for 25 minutes after adjusting the pH to 5.7 with 1 N NaOH or 1 N HCl.

Table 1. Treatment combinations of 3 x 3 levels of BAP & NAA used for the preparation of stock solutions.

BAP (mgl ⁻¹)	NAA (mg/l ['])		
	0.0	0.5	1.0
0.0	0-0 (T1)	0.5-0 (T2)	1-0 (T3)
2.5	0-2.5 (T4)	0.5-2.5 (T5)	1-2.5 (T6)
5.0	0-5 (T7)	0.5-5 (T8)	1-5 (T9)

2.2. Plant Material Preparation

A commercial variety of banana called Giant Cavendish (AAA) was used for the study. Mother plants of the selected banana variety, grown with other many varieties in Woramit Horticultural Research Station of ARAR, were used as the source of explants. Sword suckers of about four months of age grown under field conditions were detached from mother plants and brought into 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 attains about 1.0-2.0 cm length and 1.0 cm width at the base. The pale white tissue block $(1.0 \times 2.0 \text{ cm})$ containing meristem and corm base was transferred into a beaker. Surface sterilization of explants was done first under laminar Airflow Cabinet with 70% ethyl alcohol, and then with 0.1% mercuric chloride and a few drops of Tween 20 for 15 minutes. Finally, the explants were then rinsed three times for 10 minutes with distilled water.

2.3. Initiation

Shoot cultures of banana can be started conventionally from

any plant part that contains a shoot meristem such as the parental pseudo stem, small suckers, peepers and lateral buds (Vuylsteke, 1989). From the selected sucker, a cube of tissue of about 1-2 cm³ containing the apical meristem was excised and then dipped in 70% ethanol for 10 minutes. Then, it the tissue was surface sterilized in a 2% sodium hypochlorite solution for 20 minutes, and followed by rising three times for 10 minutes in sterile water. The explants were then placed directly on a multiplication-inducing culture medium, specifically prepared for banana micro propagation known as MS-based media (Murashige and Skoog, 1962) which was supplemented with sucrose as a carbon source at a concentration of 30 g/l. Stock solutions of a cytokinin (BAP) and an auxin (NAA) with different levels of combination as indicated in Table 1 were added to the banana initiation medium.

Since using semi-solid media is recommended in most banana micro propagation systems, a gelling agent agar (7 g/l) was added to the culture medium of the present study. Banana shoot-tip cultures were then incubated at an optimal growth temperature of $28 \pm 2^{\circ}\text{C}$ in a light cycle of 12-16 hr with a photosynthetic photon flux (PPF) of about $60\mu\text{E/m}^2\text{s}^1$ (Al-amin *etal.* 2009).



Fig. 1. Initiation stage of banana cv. Giant Cavendish

2.4. Multiplication

Initial sub-culturing was done when the explants produced some shoots. Sub-culturing was done by cutting the entire samples of *in vitro* shoot 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 about 3-5 cm in length with 3-6 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.



Fig. 2. Multiplication stage of banana cv. Giant Cavendish

2.5. Data Collection and Analysis

Number of shoots per clump, length of shoots per plantlets, number of leaves per explants and length of leaves per explants were considered as the main parameters of the study. Shoot and leaf length was measured with linear meter in cm, while shoot and leaf number was determined by simple counting. All these parameters were measured at the interval of 10, 20, 30 & 60 days after inoculation. Collected data were entered into computer and processed with Microsoft Excel program for further analysis. ANOVA of each parameter was then computed with SAS. Treatment means showing significance difference at 0.01 or 0.05 level of confidence were separated by list significant difference (LSD).

3. Results and Discussion

3.1. Shoot Number of Giant Cavendish Banana as Affected by Concentrations of BAP and NAA

The effects of different concentrations of BAP and NAA on shoot number were statistically very significant at 30 & 60 days after inoculation (DAI) (Table 2). However, significant differences among treatments were not observed for shoot number at early culture stages, specifically at 10 & 20 DAI (Fig. 3). A combination of 5 mg/l BAP and 0.5 mg/l NAA resulted in the highest proliferation of 1.00, 1.5, 1.75 and 3.17 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 (3.17) which was much higher than that of the control (1.75). Different from the present results, Rahaman et al. (2004) found highest shoot number (4.52) at the combnation of 1.5 mg/l BAP and 1.5mg/l NAA at 30 DAI. In disagreement of the present result, Rabbani et al. (1996) also found highest number of shoots per explant at 28 DAI (3.11 \pm 0.66) with 5.0 mg/l of BAP and 0.5 mg/l NAA.

Table 2. Mean shoot number of Giant Cavendish banana as affected by BAP and NAA concentrations and combinations

D A D N A A (/I)	No of days after inoculation								
BAP-NAA(mg/l)	10	20	30	60					
0-0	0.42	0.92	1.08c	1.75d					
0-0.5	0.92	1.17	1.50ab	2.42c					
0-1	0.83	1.17	1.50ab	2.33c					
2.5-0	0.92	1.17	1.58ab	3.00ab					
2.5-0.5	0.83	1.08	1.67ab	3.16a					
2.5-1	0.75	1.08	1.33bc	2.58bc					
5-0	1.00	1.42	1.67ab	3.16a					
5-0.5	1.00	1.5	1.75a	3.17a					
5-1	0.91	1.25	1.58ab	3.08ab					
LSD	ns	Ns	0.34	0.51					
CV (%)	27.68	17.09	12.97	10.68					
Significance	ns	ns	*	**					

Means followed by the same letter(s) are not significantly different at P > 0.05;*= significant at (p < 0.05);**= significant at (P < 0.01); ns= none significant

The findings of the present study did not also agree with the results of Khanam *et al.* (1996) and Rabbani *et al.* (1996) that did not monitor any shoot formation in the control treatment. In the present study, unless it was contaminated with fungus or bacteria, an explant cultured on MS medium without growth regulators produced shoot, commonly only single shoot. Shirani *et al.* (2009) reported that a response of banana and plantain to micro propagation is influenced by hormone type, concentration and cultivar. Therefore, the number of shoots produced per explant varied in MS media supplemented with different concentrations of BAP and NAA.

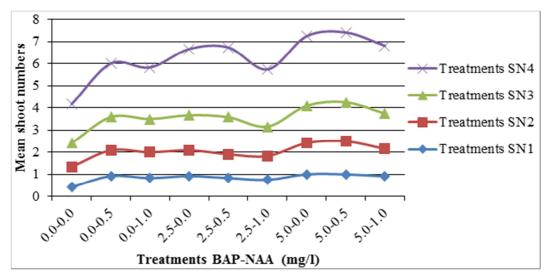


Fig. 3. Effects of different concentrations of BAP and NAA on shoot number at different DAI.

Key: SN1 = Number of shoots per clump10 days after inoculation; SN2 = Number of shoots per clump 20 days after inoculation; SN3 = Number of shoots per clump30 days after inoculation; SN4 = Number of shoots per plantlet (cm) 60 days after inoculation

3.2. Shoot Length of Giant Cavendish Banana as Affected by Different Concentrations of BAP and NAA

The MS medium supplemented with BAP and NAA showed different results for shoot length mainly due to their concentration differences (fig. 4). The longest shoot was produced with the concentration of 5 mg/l BAP + 0.5 mg/l NAA. This particular combination of BAP & NAA showed highest mean shoot length of 0.42, 2.34, 2.64 and 3.46 cm at 10, 20, 30 and 60 DAI, respectively (Table 3). Rahaman et al. (2004) reported similar result. They obtained longest shoot with 5.0 mg/l BAP (3.62 cm), as well as, shortest leaf with 2.0 mg/l BAP. The results of the present study, indeed, differed from the findings of Khanam et al. (1996) and Alamin et al. (2009) who obtained the longest shoot in banana on MS medium supplemented with 7.5 mg/l BAP treatments.

Table 3. Mean shoot length of Giant Cavendish banana as affected by BAP and NAA concentrations and combinations

BAP-NAA(mgL ⁻¹)	No of days after inoculation									
DAT-NAA(IIIgL)	10	20	30	60						
0-0	0.67f	0.83d	1.10c	1.38c						
0-0.5	0.19e	1.46c	2.09ab	2.39b						
0-1	0.23de	1.48c	1.95b	2.32b						
2.5-0	0.21de	1.62c	2.17ab	2.74ab						
2.5-0.5	0.27cd	1.81bc	2.18ab	2.83ab						
2.5-1	0.32bc	1.91abc	1.93b	2.43b						
5-0	0.39ab	2.10ab	2.42ab	3.27a						
5-0.5	0.42a	2.34a	2.64a	3.46a						
5-1	0.4a	2.21ab	2.48ab	3.41a						
LSD	0.07	0.46	0.61	0.79						
CV (%)	15.52	15.10	16.63	16.92						
Significance	**	**	**	**						

Means followed by the same letter(s) are not significantly different at P > 0.05;**= significant at (P < 0.01)

3.3. Leaf Number of Giant Cavendish Banana as Affected by Different Concentrations of BAP and NAA

The maximum number of leaves (2.33, 3.00, 3.33 and 4.33 leaves per explant at 10, 20, 30 and 60 DAI, respectively) was produced on the medium supplemented with 5.0 mg/l BAP and 0.50 mg/l NAA. The second highest number of leaves (2.00, 2.67, 3.67 and 4.00 leaves per explant at 10, 20, 30 and 60 DAI, respectively) was produced on the medium supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA (fig. 5). In agreement with the present results, Rahman *et al.* (2004) found the maximum number of leaves (3.12 per plantlet) with 5.0 mg/l BAP at 30 DAI. Rabbani *et al.* (1996) obtained also similar results with 5.0 mg/l BAP.

Table 4. Mean leaf number of Giant Cavendish as affected by BAP and NAA combinations

BAP-NAA(mgL ⁻¹)	No of days after inoculation								
DAT-NAA(IIIgL)	10	20	30	60					
0-0	0.00d	0.00e	0.00d	0.67e					
0-0.5	0.67cd	1.33d	2.33c	3.00cd					
0-1	0.67cd	1.33d	2.33c	2.33d					
2.5-0	0.67cd	1.67cd	2.00c	3.00cd					
2.5-0.5	1.00c	2.00bcd	2.67bc	3.33bc					
2.5-1	1.33bc	2.00bcd	2.67bc	3.67abc					
5-0	1.33bc	2.33abc	3.33ab	3.67abc					
5-0.5	2.33a	3.00a	3.33ab	4.33a					
5-1	2.00ab	2.67ab	3.67a	4.00ab					
LSD	0.79	0.95	0.78	0.84					
CV (%)	41.07	30.22	18.19	15.62					
Significance level	**	**	**	**					

Means followed by the same letter(s) are not significantly different at P > 0.05**= significant at (P < 0.01)

The lowest number of leaves (0.00, 0.00, 0.00 and 0.67 leaves per explant at 10, 20, 30 and 60 DAI) were obtained from control treatment (Table 4) which did not agree with the finding of Al-amin *et al.* (2009), but agreed with the results of Rabbani *et al.* (1996) and Rahman *et al.* (2004) who did

not monitor any leaves formation at 10, 20 and 30 DAI.

Table 5. Mean effects of BAP and NAA combinations on leaf length of Banana cv. Giant Cavendish at different days after inoculation

DAD NAA(I1)	No of days after inoculation								
BAP-NAA(mgL ⁻¹)	10	20	30	60					
0-0	0.00d	0.00c	0.00d	0.57e					
0-0.5	0.19d	1.17b	1.50c	1.77d					
0-1	0.21d	1.43b	1.80bc	2.20cd					
2.5-0	0.27d	1.47b	1.87b	2.37bc					
2.5-0.5	0.75c	2.10a	2.63a	2.97a					
2.5-1	0.73c	2.03a	2.50a	2.77ab					
5-0	1.03bc	2.03a	2.53a	2.80ab					
5-0.5	1.43a	2.27a	2.80a	3.23a					
5-1	1.25ab	2.07a	2.57a	2.93a					
LSD	0.37	0.41	0.33	0.47					
CV (%)	33.21	14.54	9.42	11.35					
Significance	**	**	**	**					

Means followed by the same letter(s) are not significantly different at P > 0.05;**= significant at (P < 0.01).

3.4. Leaf Length of Giant Cavendish Banana as Affected by Different Concentrations of BAP and NAA

The MS medium supplemented with different concentrations and combinations of BAP and NAA showed different results for leaf length (fig. 6). The longest leaves were produced with the concentration of 5.0 mg/l BAP + 0.5 mg/l NAA 1.43, 2.27, 2.80 and 3.23 cm at 10, 20, 30 and 60 DAI, respectively (Table 4). Rahaman $\it et~al.~(2004)$ 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). They also found shortest leaf in 2.0 mg/l BAP. The results of present experiment agreed with the findings of Khanam $\it et~al.~(1996)$. But, it didn't agree with Al-amin $\it et~al.~(2009)$ who obtained longest leaves in banana on MS medium supplemented with 25 μ M (5mg/1) BAP and 7.5 mg/l BAP treatments.

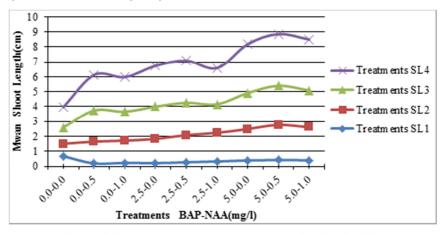


Fig. 4. Effects of different concentrations of BAP and NAA on shoot length at different DAI

Key: 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.

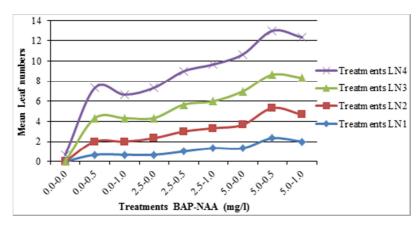


Fig. 5. Effects of different concentrations of BAP and NAA on Leaf number at different DAI.

LN1 = Number of leaves per explants ($N_{\underline{0}}$) 10 days after inoculation; LN2 = Number of leaves per explants ($N_{\underline{0}}$) 20 days after inoculation; LN3 = Number of leaves per explants ($N_{\underline{0}}$) 30 days after inoculation; LN4= Number of leaves per explants ($N_{\underline{0}}$) 60 days after inoculation.

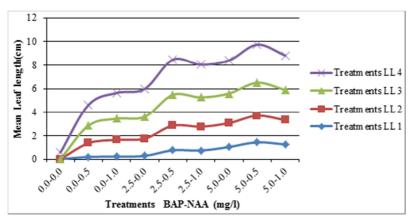


Fig. 6. Effects of different concentrations of BAP and NAA on Leaf length at different DAI

Key: LL1 = Length of leaves per explants (cm) 10 days after inoculation; LL2 = Length of leaves per explants (cm) 20 days after inoculation; LL3 = Length of leaves per explants (cm) 30 days after inoculation; LL4 = Length of leaves per explants (cm) 60 days after inoculation.

 Table 6. Simple correlation coefficients (r) among shoot proliferation of Banana cv. Giant Cavendish at different days after inoculation.

Parameter	SN1	SL1	LN1	LL1	SN2	SL2	LN2	LL2	SN3	SL3	LN3	LL3	SN4	SL4	LN4	LL4
SN1	-	0.65**	0.38 ^{ns}	0.39*	0.70**	0.71**	0.55**	0.58**	0,78**	0.83**	0.58**	0.57**	0.67**	0.79**	0.58**	0.61**
SL1		-	0.79**	0.88**	0.71**	0.93**	0.85**	0.88**	0.66**	0.80**	0.85**	0.88**	0.76**	0.85**	0.84**	0.88**
LN1			-	0.89**	0.50**	0.72**	0.88**	0.69**	0.42*	0.62**	0.79**	0.69**	0.55**	0.69**	0.76**	0.67**
LL1				-	0.57**	0.79**	0.88**	0.76**	0.49*	0.63**	0.77**	0.76**	0.64**	0.73**	0.74**	0.75**
SN2					-	0.59**	0.64**	0.55**	0.80**	0.60**	0.58**	0.54**	0.65**	0.60**	0.54**	0.49**
SL2						-	0.80**	0.83**	0.62**	0.89**	0.79**	0.84**	0.80**	0.91**	0.84**	0.89**
LN2							_	0.80**	0.66**	0.74**	0.90**	0.82**	0.72**	0.76**	0.84**	0.80**
LL2								-	0.66**	0.73**	0.85**	0.99**	0.76**	0.77**	0.86**	0.97**
SN3									-	0.76**	0.65**	0.67**	0.81**	0.72**	0.68**	0.67**
SL3										_	0.76**	0.74**	0.77**	0.97**	0.83**	0.81**
LN3											-	0.86**	0.71**	0.76**	0.87**	0.82**
LL3												-	0.78**	0.77**	0.87**	0.98**
SN4													-	0.79**	0.75**	0.78**
SL4														-	0.81**	0.83**
LN4															-	0.86**
LL4																-

*= Significant at P<0.05;*** = Significant 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 clump30 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; LL4 = Length of leaves per explants (Cm) 60 days after inoculation; LL4 = Length of leaves per explants (Cm) 60 days after inoculation

3.5. Correlation Analysis Between Shoot Proliferation Parameter of Giant Cavendish

Correlation coefficients among parameters of Banana cv. Giant Cavendish are presented in Table 6. All parameters were positively and significantly correlated to each other (P<0.01) except SN1 and LN1 (ns) indicating that number of shoots and number of leaves didn't show significant difference at 10DAI and SN1 and LL1 (P<0.05) indicating that as number of shoots increases, probability of getting longest leaves became increased 10 DAI.

4. 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 2-5 mg/l cytokinin (mostly 6-benzylaminopurine) or by incising or fragmenting the shoot tips. Rates of multiplication range from two to ten or more shoots or 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 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. 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).

According to Ortiz and Vuylsteke (1996), 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. Based on the findings of the present study shoot proliferation of banana cv. Giant Cavendish were best on MS medium supplemented with 5 mg/l BAP + 0.5 mg/l NAA.

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