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Effect of BAP and NAA on *In vitro* Shoot Establishment and Proliferation of Banana (*Musa paradisiaca*) Cv.Grand naine

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Abstract: ***The study aimed to investigate the effect of different concentration of BAP (6-benzylaminopurine) (0.0, 2.5, 5.0, 7.5 and 10.0 mg/l), NAA (α-naphthalene acetic acid) (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) on shoot establishment and multiplication of banana Cv. Grand Naine. Among the different concentrations used 5mg/l BAP showed shortest days to shoot induction (11 days). In NAA levels, zero concentration was observed as fewer days (13.5). As interaction concerned 5 mg/l BAP + 0.5 mg/l NAA found shortest days to induce which was recorded as 10 days. In shoot proliferation, 5 mg/l BAP observed highest shoot number (2.0 and 2.6 shoots at 15 and 30 DAI respectively). 0.5 mg/l NAA showed maximum number of shoots in 15 DAI and 1.0 mg/l NAA at 30 DAI. The interaction of 5.0 mg/l BAP + Controlled NAA showed 2.67 and 3.0 shoots at 15 and 30 DAI respectively. The longest shoot (4.2 and 4.9 cm) at 15 and 30 DAI respectively was produced by the treatment of 5 mg/l BAP. With 1.5 mg/l NAA level 3.0 and 3.7 cm shoot length observed in both 15 and 30 DAI respectively. At interaction 5 mg/l BAP +2.0 mg/l NAA was shown long length of shoot (5.07 and 5.83 cm) in 15 and 30 DAI, respectively. The maximum number of leaves (3.4 and 4.4 during 15 DAI and 30 DAI, respectively in media supplemented 5 mg/l BAP) (2.6 and 3.4 at 15 and 30 DAI respectively in 1.5 mg/l NAA) and (4.0 and 5.3 at 15 and 30 DAI, respectively in interaction of 5.0 mg/l BAP + 2.0 mg/l NAA). The treatment of 5 mg/l BAP was produced longest leaves 3.4 and 3.9 cm at 15 and 30 DAI respectively, while NAA level of 2.0 mg/l produced longest leaves which were 2.5 and 3.0 cm at 15 and 30 DAI, respectively. In interaction 5 mg/l BAP + 2.0 mg/l IAA developed 4.43 and 5.27 cm at 15 and 30 DAI respectively.***

Keywords: Banana, BAP, NAA, Grand naine

1. Introduction

The banana and plantains *(Musa spp.)* belongs to the family *Musaceae* are one of the world's most important subsistence crops. It is originated in Malaysia through a complex hybridization process (Novak, 1992). Presently, banana is grown in around 150 countries across the world on an area of 5.0 million ha producing 103.6 million tonnes. Asia, Africa and Latin America are the major banana producing continents. Among the major banana producing countries India is leading in the production and area coverage nearly 776 thousand ha with 26.5 million tonnes production followed by China producing 10.5 million tones and Philippines, Ecuador, Brazil with production of 9.2, 7.0 and 6.9 million tonnes respectively (FAO STAT 2014).

The extensive basic work on the *in vitro* propagation of banana (Kodym and Zapata, 1999; Nandwani *et al.,* 2000) had led to the technological development of *in vitro* mass production of different cultivars. Plant tissue culture techniques can potentially overcome some of the factors limiting traditional approaches to banana improvement.

Application of tissue culture technique is a tool to produce large number of disease free plants in limited period of time and space (khanam, D *et al.,* 1996). *In vitro* propagation of different cultivar required different culture media for shoot proliferation and root differentiation (Dore, S.R *et al.,* 1983). The productivity of vegetative propagated banana and plantain is greatly reduced by viral disease (Lepoivre, 2000). Moreover, 5-10 suckers can be obtained per plant per year which may be of uniform size and virus free. *In vitro* micro propagation of plants requires special conditions such as high levels of growth regulators, high humidity, excess of nutrition factors, both minerals and carbohydrates, and low light intensity (Dantas de Oliveria *et al.,* 1997).

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 (Demissie, 2013).

Al- amin *et al.,* (2009) studied the effect of different concentration of BAP and NAA on virus free plant regeneration, shoot multiplication. Among different concentrations, 7.5 mg/l BAP + 0.5 mg/l NAA showed highest shoot proliferation. Demissie, (2013) observed highest shoot proliferation at 5 mg/l BAP + 1.0 mg/l NAA among different concentrations. He recorded good numbers of shoots at 5 mg/l BAP +0.5 mg/l NAA at 60 DAI (3.08), longest shoot produced at concentration of 5 mg/l BAP +1.0 mg/l NAA, maximum number of leaves produced on the medium supplemented with 5.0 mg/l BAP and 0.5 mg/l NAA.

2. Materials and Methods

This experiment was undertaken in Mahatma Phule Krishi Vidyapeeth, State Level Biotechnology Centre, Tissue culture Laboratory, Rahuri India, during the period of June 2014 to September 2014. The healthy 4-6 months young sucker of Cv. Grand naine were collected, washed and excised properly. The excised explants treated with bavistin fungicide solution (2.5 g/l) and streptomycin (300 mg/l) for 30 minute. Then, they were washed with distilled water 2-3 times. Further surface sterilization was done by 0.1 mercuric chloride (HgCl2) and few drops of tween -20 followed 4-6 repeated wash were done to remove off detergents and traces. These explants were trimmed and aseptically inoculated on Murashige and Skoog (1962) media for shoot initiation and proliferation with phytohormones of BAP, NAA each at different concentrations alone and in combinations on shoot proliferation.

Five levels of BAP (0.0, 2.5, 5.0, 7.5, and 10.0 mg/l) and 5 levels of NAA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l were used. Individual hormones and combinations of both BAP and NAA were used as treatments. The experiments were arranged in factorial completely randomized design (FCRD) with 4 replications. Data were collected on the effect of different treatments on shoot proliferation. The culture tubes were transferred to growth room and allowed to grow in controlled environment. The temperature of the growth room was maintained within 25±1°C by an air conditioner. A 16-hour light period was maintained with light intensity of 2000 lux for the growth and development of culture.

3. Result and Discussion

3.1 Effect of BAP, NAA and combination on Shoot Induction

Days to induction have been shown in table 1. BAP levels significantly influenced on days to shooting it was observed 17.8 days on hormone exposed to 10mg/l. various reports also showed higher level of cytokinin product can inhibit the acceleration of the shoot establishment. Sazdur *et al.*, (2013) investigated optimum concentration of phytohormones which affected the induction of banana. As regard to NAA, longest day was observed in 2.0 mg/l NAA and shortest days were observed in control treatment. In the case of combination, it was found that longest days were 19.33 at 10 mg/1 BAP +2.0 mg/l NAA and minimum number of days was recorded for induction was 10 days at both 5 mg/l BAP + 0.0 mg/l NAA and 5 mg/l BAP + 0.5 mg/l NAA. Although there was considerable variation in days to shooting among the treatment, their difference due to interaction effect was not statistically significant. In relation to this experiment, Muhammad *et al*., (2013) found best medium for culture establishment was MS + 5.0 mg/l BAP + 1.0 mg/l IAA and they investigated time required to produced shoot let was 15-21 days.

3.2 Effect of BAP, NAA and combination on number of shoots.

The micro propagation is mostly dependant on the number of shoots produced during *in vitro*. To achieve this target it is necessary to standardize the type of growth hormone that has great influence on proliferation of shoot. Ali and Mirza, (2006) confirmed that for direct shoot regeneration (without callusing), there was different response of explants at various growth regulator concentration and combinations.

In this experiment for the BAP level, the maximum number of shoot recorded at 5mg/l BAP during 30 DAI which was 2.6 shoots. Control treatment has produced minimum number, 1.4 shoots during 30 DAI. The effect was significant both in 15 DAI and 30 DAI. Similarly Munguatosh *et al*., (2013) studied BAP levels and indicated 6.0 mg/l BAP significantly increased number of shoots formed i.e. 9.5. Sazdur *et al.*, (2013) found maximum multiplication rate (95%) in MS media containing 4.0 mg/l BAP, which recorded highest average number of shoots for each explants (5.9) found in MS medium fortified with 4.0 mg/l BAP. Bhosale *et al*., (2011) reported that 7mg/l BAP gave 4.5 shoots in selected variety.

While computing NAA level, 1.0 mg/l NAA produced maximum number during 30 DAI, where the control treatment shown less number of shoots. Collectively the effects were not found statistically significant. Viehman *et al*., (2007) reported highest number of shoot 3.73 in 2.7µm and lowest found in media composition of 5.4µm which was 1.37.

The interactions of both hormones (BAP and NAA) at different level were studied on different number of shoot. From the investigation it was observed that the mean number of shoot 2.67 (maximum) at 15 DAI and 3 shoots (maximum) at 30 DAI under concentration of 5mg/l BAP + 0 mg/l NAA. The minimum numbers of shoots were observed in control (0.33) both in 15 DAI and 30 DAI. Similarly and in contrary various experiments were undertaken on the effect of growth hormone on number of shoots generated. Rahman *et al*., (2004) found highest shoot number at 1 .5 mg/l BAP + NAA (4.52) at 30 DAI. The result of current investigation is not fully supported by Rabbani *et al.*, (1996) where they found that highest number of shoots per explants at 28 DAI (3.11 ± 0.66) with 5.0 mg/l of BAP and Kn. This variation might be due to the different concentrations of NAA (auxins) and BAP (cytokinin) and their combinations.

3.3 Effect of BAP, NAA and combination on a length of shoots.

As indicated in table 1, BAP levels were statistically significant and 5 mg/l BAP was given. Highest shoot length 4.2cm and 4.9cm in 15 and 30 DAI, respectively. The minimum shoot length was observed 1.5 and 2.2 cm in 15 and 30 DAI, respectively in control treatment. Similar results were seen by Sazdur *et al*., (2013) where 5.0mg/l BAP had given maximum elongation of shoots (4.9 cm) even though they did not specified on days to inoculation. In relation to this study Munguatosh *et al*., (2013) reported that 6.0 mg/l BAP gave maximum length of shoot which was 4.45cm.

As regard the effect of NAA was not statistically significant, 1.5 mg/l NAA recorded maximum length both at 15 DAI

and 30 DAI. During the study, it was observed that, the maximum shoot length (5.1cm) and (5.8cm) in 15 and 30 DAI, respectively on hormone exposed to 5 mg/l BAP + 2.0 mg/l NAA concentration. Rahaman *et al*., (2004) observed similar results and obtained longest shoot in 5.0mg/l BAP (3.6 cm). The present study differs from the finding 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 treatment. As regard to the shortest shoot length this result agree with Al-Amin *et al*., (2009) where they found (1.1cm) leaves by the control treatment where BAP hormones were absent. The treatment of 2.5 mg/l BAP with various combinations of NAA and 7.5 mg/l BAP and 10.0 mg/l BAP produced shorter shoot length closer to control treatment (1.23cm) at 15 DAI.

Table 1: Effect of different concentrations of BAP, NAA and interaction on number of days to establishment, number of shoots and shoot length**.**

Factors	Days to est.	No. of shoots		Shoot length(cm)	
A:- BAP Level		15 DAI	30 DAI	15 DAI	30 DAI
A1= BAP 0.0 mg/L	14.6(3.83)bc	1.0(1.22)de	1.4 (1.33)de	1.5de	2.2de
A2= BAP 2.5 mg/L	11.8(3.44)de	1.6(1.45)abc	2.2(1.64)abcd	2.6bcd	3.3bc
A3= BAP 5.0 mg/L	11.0(3.32)de	2.0(1.55)abc	2.6 (1.75)ab	4.2a	4.9a
A4= BAP 7.5 mg/L	14.7(3.84)bc	1.6(1.43)abc	2.0 (1.56)bcd	3.1bc	3.9bc
A5= BAP 10 mg/L	17.8 (4.22)a	1.2 (1.29)de	1.9(1.54)bcde	1.9cde	2.3de
SEm (±)	0.46	0.19	0.19	0.31	0.34
CD at 5%	1.31	0.56	0.56	0.88	0.97
B:- NAA Level					
B1= NAA 0.0 mg/L	13.5 (3.67)	1.5 (1.38)	1.8 (1.49)	2.3	2.7
B2= NAA 0.5 mg/L	14.0 (3.72)	1.6 (1.44)	2.1 (1.59)	2.6	3.3
B3= NAA 1.0 mg/L	14.0 (3.76)	1.4 (1.36)	2.2 (1.62)	2.6	3.4
B4= NAA 1.5 mg/L	14.0 (3.74)	1.4 (1.36)	1.9 (1.55)	3.0	3.7
B5= NAA 2.0 mg/L	14.2 (3.75)	1.5 (1.41)	2.0 (1.58)	2.9	3.6
SEm (±)	0.46	0.19	0.19	0.31	0.34
CD at 5%	NS	NS	NS	NS	NS
C:- Interaction A*B					
A1B1	14.00(3.753)	0.33 (0.880)	0.33 (0.880)	0.00	0.00
A1B2	14.67(3.841)	1.33 (1.344)	1.67 (1.440)	1.23	2.07
A1B3	15.00(3.884)	1.33 (1.344)	2.00 (1.581)	2.20	3.17
A1B4	14.33(3.791)	1.00 (1.255)	1.33 (1.344)	2.73	3.70
A1B5	15.00(3.880)	1.33 (1.344)	1.67 (1.440)	1.73	2.50
A2B1	11.33(3.376)	1.67 (1.462)	2.00 (1.559)	2.60	3.17
A2B2	12.00(3.477)	2.00 (1.559)	3.00 (1.858)	3.07	3.67
A2B3	11.67(3.425)	1.33 (1.344)	2.00 (1.559)	2.43	3.17
A2B4	12.67(3.561)	1.67 (1.462)	2.00 (1.581)	2.33	3.00
A2B5	11.33(3.376)	1.67 (1.440)	2.33 (1.678)	3.00	3.60
A3B1	10.00(3.176)	2.67 (1.774)	3.00 (1.871)	3.77	4.53
A3B2	10.00(3.161)	2.00 (1.559)	2.67 (1.774)	3.27	3.93
A3B3	11.67(3.425)	2.00 (1.559)	3.00 (1.871)	4.47	5.27
A3B4	12.33(3.523)	1.67 (1.440)	2.33 (1.678)	4.53	5.40
A3B5	11.00(3.325)	1.67 (1.462)	2.00 (1.581)	5.07	5.83
A4B1	14.33(3.799)	1.67 (1.462)	2.00 (1.581)	2.93	3.57
A4B2	15.67(3.967)	2.00 (1.559)	2.00 (1.559)	3.60	5.23
A4B3	15.67(3.970)	1.33 (1.344)	2.00 (1.559)	2.30	3.17
A4B4	13.67(3.692)	1.33 (1.344)	2.00 (1.581)	3.50	4.40
A4B5	14.33(3.795)	1.67 (1.462)	2.00 (1.559)	3.53	4.50
A5B1	18.00(4.250)	1.33 (1.344)	2.00 (1.581)	2.23	2.60
A5B2	17.67(4.213)	1.00 (1.225)	1.33 (1.344)	2.10	2.77
A5B3	17.00(4.134)	1.00 (1.225)	2.00 (1.559)	2.00	2.67
A5B4	17.00(4.134)	1.33 (1.344)	2.00 (1.581)	2.00	2.33
A5B5	19.33(4.406)	1.33 (1.344)	2.33 (1.678)	1.33	1.60
SEm (±)	1.03	0.44	0.44	0.69	0.76
CD at 5 %	NS	NS	NS	NS	NS
G.M	13.9	1.07	2.04	2.71	3.39

All values in the brackets are transformed: All values with same letter are not significant

3.4 Effect of BAP, NAA and combination on the number of leaves

From the data dictated in table 2; that the BAP levels were significantly influenced the number of leaves. The media supplemented with 5 mg/l BAP was found best on number of leaf 3.4 and 4.4 at 15 and 30DAI respectively. As regard to NAA level, 1.5mg/l NAA shown high number of leaves on both days of inoculation and it was statistically significant.

From the investigation it was observed that hormone combination having 5.0 mg/l + 2.0 mg/l NAA gave the maximum leaf number per micro shoot. In contrast to this,

media containing control (no hormone) were found to produce the minimum leaf number (0.67 and 1.3) in 15 and 30 DAI respectively.

As regard to maximum number of leaves obtained the result relatively agree with Demissie (2013), that he found maximum number of leaves at 10, 20, 30 and 60 DAI produced on the medium supplemented with 5 mg/l BAP and 0.5 mg/l NAA were 1.67, 2.67, 3.67 and 4.33 leaves per explants respectively. Rahman *et al*., (2004) found the maximum number of leaves (3.12 per explants at 30 DAI produced with 5.0 mg/l BAP, which was similar with the present study. In this study lowest number were of leaves obtained by control which does not agree with the finding of Al-Amin *et al*., (2009) but agree with Rabbani *et al*., (1996) and Rahaman *et al*., (2004) where they did not monitored any leaves formation at different dates interval after inoculation.

3.5 Effect of BAP, NAA and combination on a length of leaves.

The mediums supplemented with different BAP levels were statistically significant. The longest leaf recorded in 5 mg/l BAP at both 15 DAI and 30 DAI; whereas NAA levels 2.0 mg/l NAA reported longest leaf length on both days after inoculation in comparison with other treatments. NAA level does not significantly influenced the length of leaves.

As regard to the interaction, the lengths of plantlets were not statistically significant. The longest leaves were produced by combination of 5 mg/l BAP + 2.0 mg/l NAA at 15 and 30 DAI which were 4.43 and 5.27 cm respectively. This investigation disagree with results of Al-Amin *et al*., (2009) that they found the longest leaves by the treatment concentration of 7.5 mg/l BAP +0.5 mg/l NAA treatment (0.85, 2.70, 4.23 cm at 10, 20 and 30 DAI respectively).

But this result slightly agree with Rahman *et al*., (2004) and they obtained longest leaves 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) using BARI-Banana -1. In contrary they found shortest leaves in 2.0 mg/l BAP. But in this experiment the shortest leaf length was produced by control treatment of BAP and 0.5 mg/l NAA (1.07 and 1.30 cm) 15 and 30 DAI, respectively.

Table 2: Effect of different concentrations of BAP, NAA and interaction on number of leaves and leaf length

Factors	No. of Leaves		Leaf length (cm)	
A:- BAP Level	15 DAI	30 DAI	15 DAI	30 DAI
A1= BAP 0.0 mg/L	1.4 (1.35)de	2.4 (1.67)de	1.2de	1.5e
A2= BAP 2.5 mg/L	2.2(1.63)bcd	3.2 (1.93)bc	2.0bc	2.3cd
A3= BAP 5.0 mg/L	3.4 (1.98)a	4.4 (2.22)a	3.4a	3.9a
A4= BAP 7.5 mg/L	2.5 (1.72)bc	3.2 (1.91)bc	2.5bc	3.1b
A5= BAP 10 mg/L	1.9(1.53)cde	2.5 (1.73)de	1.7de	2.0cd
SEm (±)	0.21	0.18	0.26	0.27
CD at 5%	0.59	0.51	0.74	0.77
B:- NAA Level				
B1= NAA 0.0 mg/L	2.0 (1.53)	2.6 (1.75)e	1.8	2.1
B2= NAA 0.5 mg/L	2.3 (1.65)	3.2(1.92)abcd	2.0	2.4
B3= NAA 1.0 mg/L	2.2 (1.64)	3.2(1.91)abcd	2.1	2.6
B4= NAA 1.5 mg/L	2.6 (1.75)	3.4(1.98)abcd	2.4	2.8
B5= NAA 2.0 mg/L	2.3 (1.65)	3.2(1.91)abcd	2.5	3.0
SEm (±)	0.21	0.18	0.26	0.27
CD at 5%	NS	0.51	NS	NS
C:- Interaction A*B				
A1B1	0.67 (1.052)	1.33 (1.290)	0.00	0.00
A1B2	1.33 (1.344)	2.33 (1.678)	1.07	1.30
A1B3	1.67 (1.462)	2.67 (1.774)	1.83	2.30
A1B4	2.00 (1.559)	3.00 (1.858)	2.10	2.50
A1B5	1.33 (1.344)	2.67 (1.774)	1.33	1.80
A2B1	2.67 (1.774)	3.00 (1.871)	1.90	2.10
A2B2	2.33 (1.678)	3.67 (2.038)	2.10	2.43
A2B3	2.00 (1.559)	3.33 (1.954)	2.03	2.37
A2B4	2.00 (1.581)	3.33 (1.954)	1.77	2.20
A2B5	2.00 (1.559)	3.00 (1.858)	2.40	2.77
A3B1	3.33 (1.954)	4.00 (2.112)	2.97	3.37
A3B2	3.67 (2.038)	4.67 (2.270)	2.63	3.03
A3B3	3.33 (1.954)	4.33 (2.196)	3.53	3.97
A3B4	3.00 (1.858)	4.00 (2.112)	3.73	4.23
A3B5	4.00 (2.112)	5.33 (2.413)	4.43	5.27
A4B1	2.33 (1.678)	3.00 (1.871)	2.43	2.90
A4B2	2.33 (1.656)	3.33 (1.941)	2.87	3.33
A4B3	2.33 (1.678)	3.00 (1.871)	1.83	2.40
A4B4	3.33 (1.954)	3.67 (2.038)	2.80	3.33
A4B5	2.33 (1.678)	3.00 (1.871)	3.02	3.57
A5B1	1.00 (1.225)	2.00 (1.581)	2.00	2.33

A5B2	2.00 (1.559)	2.33 (1.678)	1.67	2.00
A5B3	2.00 (1.599)	2.67 (1.774)	1.73	2.20
A5B4	2.67 (1.774)	3.33 (1.954)	1.67	2.07
A5B5	2.00 (1.559)	2.33 (1.678)	1.55	1.80
SEm (±)	0.47	0.40	0.58	0.61
CD at 5 %	NS	NS	NS	NS
G.M	2.3	3.17	2.21	2.62

: - All values in the brackets are transformed: All values with same letter are not significant.

4. Conclusion

From this study, for shoot establishment and proliferation of banana cv. Grand naine; BAP concentration of 5 mg/l was recorded as suitable concentration on days to shoot induction. Also it was observed that 5 mg/l BAP was superior over other concentration on shoot number, shoot length, leaf number and leaf length both at 15 and 30 DAI.

Among different level of NAA concentration used the best performed are, controlled level for days to shoot induction, 0.5 mg/l NAA for shoot number, 1.5 mg/l NAA for length of shoot and number of leaves and 2.0 mg/l NAA for length of leaves were seen best performing treatment as to compare others.

As interaction concerned, 5 mg/l BAP + 0.0 mg/l NAA was best on rapid initiation of shoot and for highest shoot number. In other hand, 5 mg/l BAP + 2.0 mg/l NAA were proved best for length of shoot, number of leaves and length of leaves at both 15 and 30 DAI. It had shown that linear relationship between effects of hormones on the alone and on interaction.

The proliferation rate were shown increasing trend from 15 DAI to 30 DAI in most treatments. Higher and controlled concentration of growth regulators were shown limited growth and proliferation.

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