

**Study on *In vitro* Assessments of Cytokinin (BAP) and Auxins (NAA, and IAA) on Banana cv Grand Naine Multiplication**

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**Abstract**

*Presently, banana has been grown throughout the world around 150 countries with a region of 4.84 million per hectare producing 95.6 million ton. Banana is mostly preferred to be propagated vegetative through suckers but traditionally process is lengthy time consuming and not much capable as for production of homogenous plant. Plant Tissue culture, are micro propagation systems which are well developed for crop of bananas and can be exploited to multiple genotypes. As quantity trait of performance of yield in banana, the tissue culture developed plants produce 39% of higher yield as compare to conventional methods using suckers as propagating material. In this study in vitro assessments of cytokinin (BAP) and auxins (NAA, and IAA) were used to check shooting and rooting parameters. The most elevated number of shoots 2.5 was recorded in mix of 5.0 mg per L of BAP + 0.0 mg per L of 1-Naphthaleneacetic acid (NAA) where as the most elevated length of shoots 4.6 cm was recorded in 5.0 mg per L of BAP +1.5 mg per L NAA. The most elevated number of leaf 3.68 was recorded in blend of 5.0 mg per L BAP + 0.5 mg per L of NAA. The more drawn out length of leaf 3.7 was recorded in 5.0 mg per L of BAP +1.5 mg per L of NAA. For establishing various groupings of IAA has been utilized in which the most elevated number of roots 6.0 was recorded in convergence of 0.5 mg per L of IAA where as the most elevated length of root 5.5 cm was recorded in centralization of 0.5 mg per L of IAA. Subsequently, the present work prompted the improvement of a reasonable micropropagation protocol for cv. Grand Naine.*

**Keywords:** *Banana, Grand naine, Benzylaminopurine, Indole-3-acetic acid and M. S. media.*

**Introduction**

“Banana” is the most popular berry fruit available to every common person. It is the seedless fruit grown all over the year. The edible banana is originated from *M. accuminata* and *M. balbisiana* from the hot tropical regions of South East Asia. India has been considered as one of the centres of origin for *M. paradisiaca* as *M. balbisiana*. Banana is affluent source of vitamins also, for instance vitamin A (190 IU per 100 g of edible portion), rich source of vitamin B1, B2 and vitamin C (100 mg/ 100g). Fruits also have good source of minerals such as phosphorus, magnesium, potassium, sodium and also rich in iron and calcium. Banana have not only source of food security but it also plays very important role in income source of small holder farmers.

Presently, banana has been grown throughout the world around 150 countries with an area of 4.84 million per hector producing 95.6 million ton (FAOSTAT, 2011). Tamil Nadu is the lead producer of banana which has been followed by other states such as Madhya Pradesh, Andhra Pradesh, Gujarat, and Maharashtra. These states are contributing about 78% of total production of India. Banana is mostly preferred to propagated vegetative through suckers but traditionally process is lengthy time consuming and not much capable as for production of homogenous plant concern by Baneerjee and De Langhe (1985). Plant Tissue culture, are micro propagation systems which are well developed for crop of bananas and can be exploited to multiple genotypes. As quantity trait of performance of yield in banana, the tissue culture plants are estimated to generate 39% of elevated yield as compare to sword suckers (Pardeep *et al.*, 1992) (Farahani *et al.*, 2008). Keeping in consideration the above facts, research has been undertaken with objective to learn the impact of Cytokinin (BAP) and Auxins (NAA, and IAA) on Banana cv Grand Naine multiplication.

### **Material and Method**

An experiment was conducted at Lovely Professional University, Phagwara, Punjab during the year 2018-2019. This study involves the effect of different growth regulators on banana (*Musa paradisiaca*) cv. Grand Naine. In this experiment different type of growth regulators were used such as auxins (NAA, IAA) and cytokine (BAP). The different level of BAP, NAA, and IAA were used to check shooting and rooting parameters. The Glass wares were autoclaved under 121° C at 15 psi pressure for 15 mints. The equipments such as scalpel, spatulas, and forceps are covered with aluminum foil during autoclaving. Murashige and Skoog (1962) media used in experimental study. To get contamination free explants it needs to sterilize with various

chemicals such as Bavistin, tween 20 and with double distilled water. Finally, the plant material made upto 1-2 cm width and the 2-3 cm in length and used as explants for multiplications. The sterilization of explants is very important as the whole experiment success depends on explants. After the inoculation the culture glass wares were then placed in culture room which is under control and aseptic conditions. Temperature of room was  $28 \pm 2^\circ \text{C}$  where the photoperiod light of 16 hours and 8 hour dark period in room. As all the studies were done in laboratory under well-defined conditions of the medium, growth, temperature, light. Completely Randomized Design (CRD) was employed for the experiment. The data was subjected to statistical analysis using OPSTAT (Sheoran *et al.*, 1998) and SPSS (version 19.02) computer software. Means for each trait under stress and non-stress conditions were separated by the least significant difference (LSD) at  $P \leq 0.05$  and  $P \leq 0.01$ .

### **Result and Discussion**

**The combination assessment of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on number of shoots:** The micro propagation is typically dependent at the range of shoots produced during in vitro. To acquire this target, it is necessary to standardize the sort of growth hormone that has notable have an effect on proliferation of shoot. Ali and Mirza, (2006) showed that for direct shoot regeneration (without callusing), there has been specific reaction of explants at diverse growth regulator awareness and mixtures.

The different combination and its effect of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on banana (cv. Grand Naine) explants were recorded at two stages at 30 days and 45 days given in Table-1. The maximum shoot number was recorded 2.5 in  $T_9$  ( $A_3+B_1$ ) followed by 2.2 in  $T_6, T_{10}$  and  $T_{14}$  ( $A_2+B_2$ ) ( $A_3+B_2$ ) ( $A_4+B_2$ ) and the lowest number of shoot 0.66 was recorded in  $T_1$  ( $A_1+B_1$ ) while 3.2 was observed in  $T_9$  ( $A_3+B_1$ ) followed by 2.5 in  $T_{10}$  ( $A_3+B_2$ ) and lowest number of shoot 0.66 was discovered in  $T_1$  ( $A_1+B_1$ ) which were significant at 30 DAS and 45 DAS respectively. The result was supported by Gebeyehu (2015) when better number of shoot (3.17) proliferation was attained at  $5.00 \text{ mg L}^{-1}$  BAP +  $0.5 \text{ mg L}^{-1}$  NAA at 60 DAI which was much higher than that of the (1.75) control. However, our present results were different from, Rahaman *et al.*, (2004) who found the maximum number of shoot (4.52) at the combination of  $1.5 \text{ mg L}^{-1}$  BAP +  $1.5 \text{ mg L}^{-1}$  NAA at 30 DAI.

**Table-1: Impact of various fixations of 6-Benzylaminopurine (BAP) &1-Naphthaleneacetic acid (NAA) on number of shoots at 30 days and 45 days.**

Treatment	Number of shoots	
	30 Days	45 Days
T <sub>1</sub> (A1+B1)	0.66 <sup>a</sup> ± 0.006	0.66 <sup>a</sup> ± 0.015
T <sub>2</sub> (A1+B2)	1.66 <sup>c</sup> ± 0.006	1.92 <sup>c</sup> ± 0.012
T <sub>3</sub> (A1+B3)	1.66 <sup>c</sup> ± 0.006	2.2 <sup>d</sup> ± 0.058
T <sub>4</sub> (A1+B4)	1.0 <sup>b</sup> ± 0.289	1.33 <sup>b</sup> ± 0.015
T <sub>5</sub> (A2+B1)	1.56 <sup>c</sup> ± 0.006	2.2 <sup>d</sup> ± 0.100
T <sub>6</sub> (A2+B2)	2.2 <sup>d</sup> ± 0.058	3.0 <sup>f</sup> ± 0.153
T <sub>7</sub> (A2+B3)	1.66 <sup>c</sup> ± 0.006	2.2 <sup>d</sup> ± 0.100
T <sub>8</sub> (A2+B4)	1.8 <sup>c</sup> ± 0.058	2.2 <sup>d</sup> ± 0.058
T <sub>9</sub> (A3+B1)	2.5 <sup>e</sup> ± 0.058	3.2 <sup>f</sup> ± 0.173
T <sub>10</sub> (A3+B2)	2.2 <sup>d</sup> ± 0.058	2.5 <sup>e</sup> ± 0.058
T <sub>11</sub> (A3+B3)	2.1 <sup>d</sup> ± 0.058	3.0 <sup>f</sup> ± 0.058
T <sub>12</sub> (A3+B4)	1.66 <sup>c</sup> ± 0.010	2.33 <sup>de</sup> ± 0.006
T <sub>13</sub> (A4+B1)	1.66 <sup>c</sup> ± 0.015	2.2 <sup>d</sup> ± 0.100
T <sub>14</sub> (A4+B2)	2.2 <sup>d</sup> ± 0.153	2.1 <sup>cd</sup> ± 0.058
T <sub>15</sub> (A4+B3)	1.66 <sup>c</sup> ± 0.015	2.2 <sup>d</sup> ± 0.058
T <sub>16</sub> (A4+B4)	1.66 <sup>c</sup> ± 0.025	2.1 <sup>cd</sup> ± 0.050
C.D 5% level	0.26	0.24
SE(m)	0.09	0.08
SE(d)	0.13	0.12
C.V.	8.79	6.40

**The combination effect of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) length of shoots:**The highest length of shoots (4.6) was recorded in T<sub>12</sub> (A3+B4 of BAP & NAA) followed by 4.46 in T<sub>11</sub>(A3+B3 BAP & NAA) and the lowest length of shoot 0.00 was recorded in T<sub>1</sub> (A1+B1) while 5.42 was observed in T<sub>12</sub> (A3+B4) followed by 5.28 in T<sub>11</sub> (A3+B3) and lowest length of shoot zero was observed in T<sub>1</sub> (A1+B1) which were significant at 30 DAS and 45 DAS, respectively (Table-2).The similar results were reported by Gebeyehu (2013) who reported the longest shoot length was produce by the concentration of 5.00 mg L<sup>-1</sup> BAP + 1.0 mg L<sup>-1</sup> NAA was showing the maximum shoot proliferation (0.43, 2.42, 2.63 and 3.42 shoots per plantlet) at 10, 20, 30 and 60 DAI, correspondingly.

**Table-2: Impact of various fixations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on length of shoots at 30 days and 45 days.**

Treatment	Length of shoots (cm)	
	30 Days	45 Days
T <sub>1</sub> (A1+B1)	0 <sup>a</sup> ±0.000	0 <sup>a</sup> ± 0.000
T <sub>2</sub> (A1+B2)	1.5 <sup>b</sup> ±0.173	2.08 <sup>b</sup> ± 0.021
T <sub>3</sub> (A1+B3)	2.5 <sup>d</sup> ±0.015	3.2 <sup>c</sup> ± 0.173
T <sub>4</sub> (A1+B4)	2.8 <sup>e</sup> ± 0.100	3.16 <sup>c</sup> ± 0.012
T <sub>5</sub> (A2+B1)	2.7 <sup>e</sup> ± 0.20	3.2 <sup>c</sup> ± 0.173
T <sub>6</sub> (A2+B2)	3.7 <sup>e</sup> ± 0.058	3.61 <sup>d</sup> ± 0.031
T <sub>7</sub> (A2+B3)	2.42 <sup>cd</sup> ± 0.021	3.2 <sup>c</sup> ± 0.153
T <sub>8</sub> (A2+B4)	2.4 <sup>cd</sup> ± 0.038	3.0 <sup>c</sup> ± 0.153
T <sub>9</sub> (A3+B1)	3.78 <sup>i</sup> ± 0.042	4.55 <sup>f</sup> ± 0.015
T <sub>10</sub> (A3+B2)	3.3 <sup>g</sup> ± 0.042	3.92 <sup>e</sup> ± 0.035
T <sub>11</sub> (A3+B3)	4.46 <sup>j</sup> ± 0.026	5.28 <sup>g</sup> ± 0.015
T <sub>12</sub> (A3+B4)	4.6 <sup>j</sup> ± 0.012	5.42 <sup>g</sup> ± 0.021
T <sub>13</sub> (A4+B1)	3.0 <sup>f</sup> ± 0.036	3.55 <sup>d</sup> ± 0.015
T <sub>14</sub> (A4+B2)	3.65 <sup>hi</sup> ± 0.015	5.2 <sup>g</sup> ± 0.021
T <sub>15</sub> (A4+B3)	2.32 <sup>c</sup> ± 0.021	3.19 <sup>c</sup> ± 0.015
T <sub>16</sub> (A4+B4)	3.55 <sup>h</sup> ± 0.026	4.45 <sup>f</sup> ± 0.021
C.D 5% level	0.17	0.24
SE(m)	0.06	0.08
SE(d)	0.09	0.12
C.V.	3.41	4.06

**The combination effect of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) number of leaves:**The highest number of leaf 3.68 was recorded in T<sub>10</sub> (A3+B2) afterward 3.66 in T<sub>9</sub> (A3+B1), T<sub>11</sub> (A3+B3) and the lowest number of leaf 0.68 was recorded in T<sub>1</sub> (A1+B1) while 4.66 was observed in T<sub>11</sub> (A3+B3) followed by 4.55 in T<sub>9</sub>(A3+B1), T<sub>13</sub> (A4+B1) and lowest number of leaf 1.32 was observed in T<sub>1</sub> (A1+B1) which were significant at 30 DAS and 45 DAS, respectively (Table-3). The similar results were reported by Gebeyehu A., (2013) who recorded the maximum numbers of leaves produced were 1.67, 2.67, 3.67 and 4.33 on the medium that was supplemented with 5.0 mg L<sup>-1</sup> BAP + 0.50 mg L<sup>-1</sup> NAA while minimum number of foliage (0.00, 0.00, 0.00 and 0.67 leaves per explants) on the medium which was augmented with 0.0 mg L<sup>-1</sup> BAP + 0.00 mg L<sup>-1</sup> NAA at 10, 20, 30 and 60 DAI respectively.

**Table-3: Impact of various fixations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on number of leaves at 30 days and 45 days.**

Treatment	Number of Leaves	
	30 Days	45 Days
T <sub>1</sub> (A1+B1)	0.68 <sup>a</sup> ± 0.006	1.32 <sup>a</sup> ± 0.035
T <sub>2</sub> (A1+B2)	1.66 <sup>b</sup> ± 0.015	2.6 <sup>b</sup> ± 0.036
T <sub>3</sub> (A1+B3)	1.66 <sup>b</sup> ± 0.010	2.65 <sup>b</sup> ± 0.021
T <sub>4</sub> (A1+B4)	2.3 <sup>c</sup> ± 0.058	3.0 <sup>b</sup> ± 0.306
T <sub>5</sub> (A2+B1)	2.66 <sup>cd</sup> ± 0.015	3.0 <sup>b</sup> ± 0.577
T <sub>6</sub> (A2+B2)	2.37 <sup>c</sup> ± 0.025	3.65 <sup>c</sup> ± 0.015
T <sub>7</sub> (A2+B3)	2.2 <sup>c</sup> ± 0.058	3.66 <sup>c</sup> ± 0.015
T <sub>8</sub> (A2+B4)	2.2 <sup>c</sup> ± 0.100	3.66 <sup>c</sup> ± 0.006
T <sub>9</sub> (A3+B1)	3.66 <sup>f</sup> ± 0.015	4.55 <sup>e</sup> ± 0.006
T <sub>10</sub> (A3+B2)	3.68 <sup>f</sup> ± 0.012	4.0 <sup>cd</sup> ± 0.058
T <sub>11</sub> (A3+B3)	3.66 <sup>f</sup> ± 0.012	4.66 <sup>e</sup> ± 0.006
T <sub>12</sub> (A3+B4)	3.2 <sup>e</sup> ± 0.115	4.33 <sup>de</sup> ± 0.006
T <sub>13</sub> (A4+B1)	2.63 <sup>cd</sup> ± 0.021	4.55 <sup>e</sup> ± 0.006
T <sub>14</sub> (A4+B2)	2.63 <sup>cd</sup> ± 0.015	3.0 <sup>b</sup> ± 0.058
T <sub>15</sub> (A4+B3)	2.63 <sup>cd</sup> ± 0.006	3.66 <sup>c</sup> ± 0.015
T <sub>16</sub> (A4+B4)	3.0 <sup>de</sup> ± 0.577	3.65 <sup>c</sup> ± 0.006
C.D 5% level	0.44	0.48
SE(m)	0.15	0.17
SE(d)	0.21	0.23
C.V.	10.3	8.19

**The combination effect of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) length of leaves:**The highest length of leaf 3.7 was recorded in T<sub>12</sub> (A3+B4) followed by 2.96 in T<sub>9</sub>(A3+B1) and the lowest length of leaf zero was recorded in T<sub>1</sub> (A1+B1) while 4.33 was observed in T<sub>12</sub> (A3+B4) subsequently 3.95 in T<sub>11</sub> (A3+B3) and lowest length of leaf zero was observed in T<sub>1</sub> (A1+B1) which were significant at 30 DAS and 45 DAS respectively (Table-4).The similar results were reported byGebeyehu A., (2013) who recorded the longest leaves 1.52, 2.27, 2.70 and 3.13 cm were produced by the concentration of 5.0 mg/L BAP + 1.0 mg/L NAA at 10, 20, 30 and 60 DAI respectively, which was here statistically noteworthy.

**Table-4: Impact of various fixations of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) on length of leaves at 30 days and 45 days**

Treatment	Length of Leaves (cm)	
	30 Days	45 Days
T <sub>1</sub> (A1+B1)	0 <sup>a</sup> ± 0.000	0 <sup>a</sup> ± 0.000
T <sub>2</sub> (A1+B2)	1.0 <sup>b</sup> ± 0.058	1.33 <sup>b</sup> ± 0.006
T <sub>3</sub> (A1+B3)	1.8 <sup>d</sup> ± 0.058	2.33 <sup>d</sup> ± 0.006
T <sub>4</sub> (A1+B4)	2.15 <sup>g</sup> ± 0.006	2.52 <sup>f</sup> ± 0.006
T <sub>5</sub> (A2+B1)	1.87 <sup>e</sup> ± 0.006	2.12 <sup>c</sup> ± 0.006
T <sub>6</sub> (A2+B2)	2.12 <sup>g</sup> ± 0.006	2.33 <sup>d</sup> ± 0.006
T <sub>7</sub> (A2+B3)	2.01 <sup>f</sup> ± 0.012	2.36 <sup>d</sup> ± 0.006
T <sub>8</sub> (A2+B4)	1.66 <sup>c</sup> ± 0.006	2.33 <sup>d</sup> ± 0.006
T <sub>9</sub> (A3+B1)	2.96 <sup>k</sup> ± 0.006	3.33 <sup>i</sup> ± 0.006
T <sub>10</sub> (A3+B2)	2.66 <sup>i</sup> ± 0.006	3.0 <sup>h</sup> ± 0.058
T <sub>11</sub> (A3+B3)	2.63 <sup>i</sup> ± 0.006	3.95 <sup>k</sup> ± 0.006
T <sub>12</sub> (A3+B4)	3.70 <sup>l</sup> ± 0.006	4.33 <sup>l</sup> ± 0.006
T <sub>13</sub> (A4+B1)	2.34 <sup>h</sup> ± 0.006	2.92 <sup>g</sup> ± 0.006
T <sub>14</sub> (A4+B2)	2.86 <sup>j</sup> ± 0.006	3.66 <sup>j</sup> ± 0.006
T <sub>15</sub> (A4+B3)	1.88 <sup>e</sup> ± 0.006	2.41 <sup>e</sup> ± 0.006
T <sub>16</sub> (A4+B4)	2.82 <sup>j</sup> ± 0.006	3.66 <sup>j</sup> ± 0.006
<b>C.D 5% level</b>	0.06	0.05
<b>SE(m)</b>	0.02	0.02
<b>SE(d)</b>	0.03	0.02
<b>C.V.</b>	1.70	1.00

**The Effect of Indole-3-acetic acid (IAA) on root number:**The highest number of roots 6.0 was recorded in T<sub>2</sub> (C2 for IAA) followed by 4.2 in T<sub>3</sub> (C3) & the minimum number of roots 2.37 was recorded in T<sub>1</sub> (C1) while 7.6 was observed in T<sub>2</sub> (C2) followed by 6.6 in T<sub>3</sub> (C3) and minimal number of roots 3.3 was perceived in T<sub>1</sub> (C1) which were significant at 30 DAS and 45 DAS, respectively (Table-5). The similar results were reported by Iqbal *et al.*,(2013) on different combination of auxin and they reported MS supplemented with 2.0mg/L IAA gave the best result which produced 60% root induction. In case of IBA 1.5mg/L shown maximum number of roots 5.1 and 7.1 in 15 and 30 DAI, respectively on media composition supplemented with 0.5mg/L IBA but, collective effect was not statistically significant.

**Table-5: Impact of various fixations of Indole-3-acetic acid (IAA) on number of roots at 30 days and 45 days**

Treatment	Number of Roots	
	30 Days	45 Days
T <sub>1</sub> (C1)	2.37 <sup>a</sup> ± 0.006	3.3 <sup>a</sup> ± 0.058
T <sub>2</sub> (C2)	6.0 <sup>c</sup> ± 0.058	7.6 <sup>c</sup> ± 0.058
T <sub>3</sub> (C3)	4.2 <sup>b</sup> ± 0.115	6.6 <sup>b</sup> ± 0.058
C.D 5% level	0.26	0.20
SE(m)	0.08	0.06
SE(d)	0.10	0.08
C.V.	3.08	1.72

**The Effect of Indole-3-acetic acid (IAA) on root length:**The highest length of root 5.5 was recorded in T<sub>2</sub> (C2) afterward 3.2 in T<sub>3</sub>(C3) and the minimal number of roots 2.3 was recorded in T<sub>1</sub> (C1) while 6.6 was discovered in T<sub>2</sub> (C2) followed by 4.6 in T<sub>3</sub>(C3) and lowest number of roots 4.2 was perceived in T1 (C1) which were significant at 30 DAS and 45 DAS, respectively (Table-6). The similar consequences were described by Viehmanet *al.*, (2007) who recorded 2.85 cm as longest root at 2.9 mg/L IAA while here the shortest root length 2.23 cm recorded at 14.5 mg/L IAA produced.

**Table-6: Impact of various fixations of Indole-3-acetic acid (IAA) on length of roots at 30 days and 45 days**

Treatment	Length of roots (cm)	
	30 DAYS	45 DAYS
T <sub>1</sub> (C1)	2.3 <sup>a</sup> ± 0.058	4.2 <sup>a</sup> ± 0.058
T <sub>2</sub> (C2)	5.5 <sup>c</sup> ± 0.058	6.6 <sup>a</sup> ± 0.115
T <sub>3</sub> (C3)	3.2 <sup>b</sup> ± 0.058	4.6 <sup>a</sup> ± 0.115
C.D 5% level	0.20	0.35
SE(m)	0.06	0.10
SE(d)	0.08	0.14
C.V.	2.72	3.37

**Conclusion**

The influence of different growth hormone (BAP, NAA and IAA) on stability was studied. Observations were recorded for shoot number, leaf number, shoot length, leaf length, root length,

and root number. The BAP 5.0 mg/L mix with NAA 0.0 mg/L could be utilized for rate of cell division, settling blossoms and high degree of shoot initiation. The IAA 0.5 mg/L mix with BAP 0.0 mg/L could be utilized to start for rate of cell growth, cell division and high degree of root initiation. BAP alone or in combination with NAA and IAA was found essential during shoot and root proliferation and multiplication phase.

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