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Effect of different plant growth regulators on shoot initiation of banana varieties

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Abstract
Plant growth regulators are important components for the healthy growth and development of plants in-vitro under aseptic conditions. Banana is a highly nutritious fruit with large number of extra benefits such as fiber, vitamins, and carbohydrates. It is the best remedy for stomach-related problems such as cramps and constipation. Invitro micropropagation being best method to produce disease and virus free healthy banana plantlets present experiment also performed using invitro micropropagation of banana under aseptic conditions. The present study focuses on studying effect of different plant growth hormones on banana varieties viz., Red banana, Monthan, Grand-naine, and Elakkibale. Suckers with shoot tips were used as a starting material for the micropropagation. Surface sterilized explant was inoculated on MS medium containing different combinations of growth hormones (2 mg/l BAP, 3 mg/l BAP + 0.5 mg/l IAA and 4 mg/l BAP + 1 mg/l IAA and 5 mg/l BAP + 1.5 mg/l IAA) for the shoot initiation and development. Inoculated explant was kept under 16 hr light and 8 hr dark conditions for shoot initiation. Different parameters i.e., days to tissue swelling starting, colour change from white to green, days to first shoot emergence, number of shoots obtained, observing shoot growth and shoot length. Statistical analysis of obtained results showed that 3 mg/l BAP and 0.5 mg/l IAA had best results on all the four banana varieties whereas, Grand naine showed maximum number of shoots in comparison to all the varieties studied in the experiment. In conclusion, accurate combination of growth hormones is important for the establishment of large number shoots. The higher concentration of BAP and IAA result in the reduced growth and development of inoculated explants.

Keywords: Plant growth hormones, micropropagation, suckers, explants, surface sterilization

1. Introduction
Bananas are a full package of nutritional diets with many fibres and vitamins. It is the oldest largely grown fruit crop in India and other countries equally. Crop originated from Malaysia, Indonesia, the Philippines, and New Guinea (Li *et al*., 2013) [9]. All edible banana varieties are derived from hybridization between the wild species *Musa acuminata* and Musa balbisiana. These edible banana varieties are parthenocarpic and seedless. Banana cultivation in world done on 5.6 million hectare and produced 86-million-ton bananas at global level. In India at 9 lakh hectare land banana are cultivated. India is the largest producer of bananas and according to 2023 food statistics in India 30.5 million tons of bananas are produced (2023-24). In Uttar Pradesh alone 1.2 million metric ton production is recorded (2023-24). In India, twenty varieties are cultivated throughout the country viz. Dwarf C avendish, Robusta, Monthan, Poovan, Nendran, Red banana, Nyali, Safed Velchi, Basarai, Ardhapuri, Rasthali, Karpurvalli, Karthali, Elakkibale and Grandnaine etc. Traditionally, banana plants are cultivated with suckers and rhizomes using mother plants. But this method passes bacteria and viruses from one generation to another i.e., do not produce virus-free banana plants (Simmonds *et al*., 1995) [14]. Modern techniques made it possible for healthy disease and virus-free plant generation on a commercial scale for example- tissue culture, micropropagation, meristem culture, and callus culture (Sastry *et al*., 2014) [13]. Traditional approaches of breeding are very hectic time-consuming and costly also planting material used for the propagation of banana for example suckers are good transmitters of disease and viruses. Whereas, plant tissue culture has proved its importance in the production of healthy plants.

Micropropagation is a technique of developing disease and virus free genetically similar banana plantlets on a large scale in which single shoot tips used to grow thousand number of plantlets under aseptic condition using different plant growth regulators (Justine *et al*., 2022) [8]. Different concentration auxin and cytokinin with MS medium are mainly used for the micropropagation of banana (Sujin, 2020) [16].

In the present study, different concentration of plant growth regulators is used for the development of banana shoots and roots. The aim of study is to use best concentration of auxin and cytokinin for the development of shoot and root through micropropagation of banana to obtain maximum growth. The planting material of four different varieties of banana used for the *in vitro*-micropropagation under aseptic conditions.

2. Material and method

2.1. Source of plant material- Suckers about 30-60 cm of four varieties of banana viz., Red banana, Monthan, Grand Naine, and Elakkibale were used as explant in the present research methodology which were taken from the experimental field of Division of Agricultural Biotechnology, College of Agriculture, SVPUAT, Meerut, U.P.

2.2. Explant selection- Healthy suckers about 30-60 cm containing shoot tips were used as an explant in the present experiment.

2.3. Plant hormone stock preparation- Hormone stock solutions were prepared which were further used in media for micropropagation. In this experiment, 10 mg hormones are weighed and dissolved in 90 ml distilled water. Then, 1ml of 1N NaoH is used to dissolve the hormones in distilled water and makeup volume up to 100 ml followed by filter sterilization of hormones. Store in refrigerator for future use. Photo sensitive hormones were stored in dark bottles. Each 1 ml stock contained 0.1 mg of hormone. Hormones utilized in the present research work- Auxin (IAA, IBA) and Cytokinin (BAP, Kinetin, Thidiazuron). Modified MS media (Murashige and Skoog, 1962) [18] used as basal media.

Components of stock solution in MS medium were prepared as follows

S.no.	Chemical constituents	Concentration for 1 ltr stock solution (g/l)	Amount of stock required for final 1 ltr MS medium (ml)
1.	MS-1 (Macrosalts)	(20X)	50
	KNO_3	38	
	NH_4NO_3	33	
	$MgSO_4.7H_2O$	7.4	
	KH_2PO_4	3.4	
	$CaCl_2.2H_20$	8.8	
2.	MS-2 (Microsalts)	(200X)	5
	KI	0.166	
	H_3BO_3	1.24	
	$MnSO_4.4H_2O$	4.46	
	$ZnSO_4.7H_2O$	1.72	
	$Na_2MoO_4.2H_2O$	0.05	
	$CuSO_4.5H_2O$	0.005	
	$CoCl_2.6H2O$	0.005	
3.	MS-3 (Iron Salts)	(200X)	5
	$FeSO_4.7H_2O$	5.56	
	$Na_2EDTA.2H_2O$	7.46	
4.	MS-4 (Vitamins)	(200X)	5
	Thiamine HCL	0.1	
	Pyridoxine HCL	0.1	
	Nicotinic acid	0.1	
	Glycine	0.1	

2.4 Preparation of explants- In the present experiment suckers of four different varieties of banana washed to remove attached soil with tap water, carefully removed outer dead leaves using sharp stainless-steel knife and again washed 2- 3 times with running tap water followed by removal of one another layer.

2.5 Pre-treatment of explant- Placed all the shoot tips in labolene for 15-20 minutes, washed with distilled water for 2-3 times then placed in tween 20 for 5 minutes again washed with distilled water carefully. Now, brought the explants to the laminar air flow for further treatment.

2.6 Surface sterilization of explants- Surface sterilization of explant was performed under laminar air flow in order to maintain aseptic conditions. Under blue flame of laminar trimmed another layer of explant and placed them in distilled water. Now, dipped the explants in 70% ethanol for 60 seconds. Washed with distilled water to remove traces of ethanol and placed on tissue paper. Then, placed in 0.1% $HgCl_2$ for 5 minutes and washed with distilled water. Placed on tissue paper for drying and removal of traces of chemicals.

2.7 Inoculation for shoot initiation and Experimental design- For shoot initiation one litre growth medium prepared in which MS stock solution added one by one using measuring cylinder and after addition of all stock solutions 30 gm/l sucrose, 100 mg/l inositol and 100 mg/l antimicrobial supplement was added. pH was adjusted to 5.8 and then 7 gm agar was added followed by heating for 15-20 minutes in microwave. Autoclave the media at 121℃ for 30 minutes. After autoclaving at

121℃ for 30 minutes, under laminar air flow added four different concentrations of BAP and IAA (T1= 2 mg/l BAP, T2= 3 mg/l BAP + 0.5 mg/l IAA and T3= 4 mg/l BAP + 1 mg/l IAA and T4= 5 mg/l BAP + 1.5 mg/l IAA) hormones for shoot initiation were added. Poured the 30 ml media in each jam bottles. Placed the jam bottles under laminar air flow under observation for 2-3 days for contamination. Now, inoculated the surface sterilized explants on prepared MS media under blue flame of laminar air flow. Inoculated explants were placed under complete darkness for one weak and after one weak placed them under 16 h light and 8 h dark conditions for shoot initiation. Observed them every day for changes in cultured media. The experiment was conducted on completely randomized design (CRD) design with six replicates of each banana variety. For shoot multiplication 4 mg BAP + 2.5 mg thidiazuron + 0.5 mg IAA was used for shoot proliferation.

2.8 Observations- Effect of different treatments on shoot multiplication on four banana varieties after 4 weeks was recorded by calculating days to tissue swelling starting, colour change from white to green, days to first shoot emergence, number of shoots obtained, observing shoot growth and shoot length. Statistical analysis was done using OPSTAT online software.

3. Results and Discussion

Effect of different growth regulators on shoot initiation and shoot proliferation of four banana genotypes

Four different banana genotypes viz., Red banana, Monthan, Grand-naine, and Elakkibale were studied in a series of tests to find the best combination and concentration of plant growth regulators for promoting shoot initiation and multiplication. Daily data collection included factors like the days to tissue swelling, colour change from white to green, days to first shoot emergence, and quantity of shoots per explant.

3.1. Days to tissue swelling

Tissue swelling in banana micropropagation is an important most early phenomenon for successful shoot induction. Swelling of explants in basal media indicates the beginning of shoot development this due to the swelling of adventitious buds at the bases of the explants before shoot emergence (Sujin, 2020) [16]. After some days of explant inoculation, they increased in size, resulting in tissue swelling (Jaisy and Ghai, 2011) [7]. The effect of different plant growth regulators on days to tissue swelling before shoot initiation observed for the four banana genotypes was given in Table 1. Statistical analysis of Table 1 showed that observations in current experiments were significant at 1% and 5% levels. Among all the treatment 3 mg/l BAP + 0.5 mg/l IAA showed the best results for tissue swelling in all the four banana genotypes followed by 4 mg/l BAP + 1 mg/l IAA, 5 mg/l BAP + 1.5 mg/l IAA and 2 mg/l BAP. Whereas, banana genotype Grand Naine showed fast tissue swelling as compared to all the genotypes studied followed by Red banana, Monthan and Elakkibale (Table 1). Therefore, results obtained showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed best response in tissue swelling after four weeks of inoculation in Grand Naine (28.4 days) followed by Red banana (30.8 days), Monthan (34 days) and Elakkibale (40.6 days). Similar findings were observed by the Uddin *et al*., (2006) [17] and reported that tissue swelling in micropropagation was important for shoot initiation. Accurate concentration of BAP and IAA important for the tissue showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed best response in tissue swelling after four weeks of inoculation in Grand Naine (28.4 days) followed by Red banana (30.8 days), Monthan (34 days) and Elakkibale (40.6 days).

Table 1: Effect of different plant growth regulators on days to tissue swelling

	Varieties of banana			
Treatments	**Red banana (mean ±SD)**	**Monthan (mean ±SD)**	**Grand Naine (mean ±SD)**	**Elakkibale (mean ±SD)**
2 mg/l BAP	42.8±0.89	42.2±0.42	36.8±0.16	52.4±0.25
3 mg/l BAP + 0.5 mg/l IAA	30.8±0.23	34.0±0.26	28.4±0.11	40.6±0.15
4 mg/l BAP + 1 mg/l IAA	33.8±0.22	37.2±0.17	30.4±0.21	43.4±0.18
5 mg/l BAP + 1.5 mg/l IAA	36.6±0.23	39.8±0.18	34.2±0.15	47.6±0.19
SE(m)	1.54	0.77	0.71	0.86
CV	4.79	3.52	4.85	4.85

showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed best response in tissue swelling after four weeks of inoculation in Grand Naine (28.4 days) followed by Red banana (30.8 days), Monthan (34 days) and Elakkibale (40.6 days). Similar findings were observed by the Uddin *et al*., (2006) [17] and reported that tissue swelling in micropropagation was important for shoot initiation. Accurate concentration of BAP and IAA important for the tissue swelling followed by the shoot initiation. Previous studies reported that 3 mg/l BAP and 0.5 mg/l IAA combination of hormones was sufficient for the development of large number shoots (Mahmoud *et al*., 2020) [12].

3.2. Days to colour change from white to green

After tissue swelling step in micropropagation of banana change of white colour of explants inoculated into green colour was another important factor in successful shoot initiation process (Sujin, 2020) [16]. The change of white colour to green in 4 weeks of inoculation is the starting of the emergence of shoot buds in the basal medium (Al-Amin *et al*., 2009) [1]. Effect of different plant growth regulators on days to colour change from white to green before shoot initiation observed for the four banana genotypes were given in table 2. Statistical analysis of table 2 showed that observations in current experiments were significant at 1% and 5% levels. Among all the treatments 3 mg/l BAP + 0.5 mg/l IAA showed a fastest change in colour to green from white in all the four banana genotypes followed by 4 mg/l BAP + 1 mg/l IAA, 5 mg/l BAP + 1.5 mg/l IAA and 2 mg/l BAP. Whereas, banana genotype 4 mg/l BAP + 1 mg/l IAA took least number of days for change in colour from white to green as compared to all the genotypes studied followed

by Red banana, Monthan and Elakkibale (Table 2). Therefore, results obtained showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed best response for colour change after four weeks of inoculation in genotype Grand Naine (32.4 days) followed by Red banana (34.2 days), Monthan (37 days) and Elakkibale (44.4 days). The results showed that the 3 mg/l BAP + 0.5 mg/l IAA combination of hormones are best for all the banana varieties. Dagnew *et al* (2012) [5] and Hossain *et al* (2016) [6] reported the 3 mg/l BAP with 0.5 mg/l IAA showed the early change in green colour from white colour.

Table 2: Effect of different plant growth regulators on days to change in colour of explant from white to green

Varieties of banana				
Treatments	**Red banana (mean ±SD)**	**Monthan (mean ±SD)**	**Grand Naine (mean ±SD)**	**Elakkibale (mean ±SD)**
2 mg/l BAP	45. 0 ±0.48	47.6±0.25	42. 0 ±0.18	53.8±0.12
3 mg/l BAP + 0.5 mg/l IAA	34.2±0.24	37. 0 ±0.21	32.4±0.27	44.4±0.13
4 mg/l BAP + 1 mg/l IAA	37.6±0.31	40.0±0.33	35.8±0.16	46.8±0.12
5 mg/l BAP + 1.5 mg/l IAA	40.8±0.22	44.2±0.27	39.2±0.14	50.4±0.17
SE(m)	1.38	1.04	0.75	0.63
CV	5.63	4.85	5.87	6.58

3.3. Days to first shoot emergence

Shoot emergence in micropropagation is very initial phenomenon and indicator of successful inoculation of explants (Sujin, 2020) [16]. Cronauer and Krikorian (1984) reported that multiple shoots could be produced from sliced shoot tips of banana and plantain. Effect of different plant growth regulators on days to first shoot emergence was observed for the four banana genotypes were given in the table 3. Statistical analysis of table 3 showed that observation in experiments were significant at 1% and 5% levels. Among all the treatment 3 mg/l BAP + 0.5 mg/l IAA was first in which shoot was emerged in all the four banana genotypes followed by 4 mg/l BAP + 1 mg/l IAA, 5 mg/l BAP + 1.5 mg/l IAA and 2 mg/l BAP. Whereas, Grand Naine took least number of days to develop first shoot after inoculation as compared to all the genotypes studied followed by Red banana, Monthan and Elakkibale (Table 3). Therefore, results obtained showed that treatment 3 mg/l BAP + 0.5 mg/l IAA has showed best response for shoot emergence in Grand Naine (35.6 days) followed by Red banana (38.4 days), Monthan V2 (41.2 days) and Elakkibale (50 days). The results showed that the 3 mg/l BAP + 0.5 mg/l IAA combination of hormones are best for all the banana varieties.

Table 3: Effect of different plant growth regulators on days to first shoot emergence

Varieties of banana				
Treatments	**Red banana (mean ±SD)**	**Monthan (mean ±SD)**	**Grand Naine (mean ±SD)**	**Elakkibale (mean ±SD)**
2 mg/l BAP	49.0 ±0.98	52.8±0.18	46. 0 ±0.28	66. 0 ±0.17
3 mg/l BAP + 0.5 mg/l IAA	38.4±0.38	41.2±0.28	35.6±0.19	50. 0 ±0.28
4 mg/l BAP + 1 mg/l IAA	43.4±0.91	44.6±0.24	39.6±0.18	54.8±0.12
5 mg/l BAP + 1.5 mg/l IAA	47. 0 ±0.39	48.4±0.23	42.8±0.14	56. 0 ±0.56
SE(m)	3.37	1.11	0.79	0.85
CV	6.98	7.85	6.58	4.52

MS medium supplemented with 3 mg/l BAP and 0.5 mg/l IAA showed early shoot emergence in Grand Naine variety of banana. Similar results were reported by Dagnew *et al* (2012) [5] and Mahanthesh *et al* (2022) [22]. The present research showed that higher concentrations of BAP should be avoided for the micropropagation of bananas because it can be toxic for the plants. To generate multiple shoots, it is important to generate a single shoot therefore, the emergence of the first shoot from the explant inoculated was an important parameter (Hossain *et al*., 2016) [6].

3.4. Number of shoots obtained

Different combinations of plant growth regulators were greatly affected the number of shoots obtained in the present experiment. The correct combination of growth hormones is important for the establishment of large number shoots (Justine *et al*., 2022) [8]. The effect of different plant growth regulators on number of shoots for the four banana genotypes were given in the table 4. Statistical analysis of Table 4 showed that observations in experiments were significant at 1% and 5% levels. Among all the treatments a maximum number of shoots were obtained in 3 mg/l BAP + 0.5 mg/l IAA in all the four banana genotypes followed by 4 mg/l BAP + 1 mg/l IAA, 5 mg/l BAP + 1.5 mg/l IAA and 2 mg/l BAP. Whereas, the maximum number of shoots were observed in Grand Naine as compared to all the genotypes studied followed by Red banana, Monthan, and Elakkibale (Table 4). So, the results obtained showed that treatment 3 mg/l BAP + 0.5 mg/l IAA showed the best response for shoot proliferation in the Grand Naine (11.0 shoots) followed by Red banana (9.0 shoots), Monthan (8.0), and Elakkibale (7.0). The results showed that the 3 mg/l BAP + 0.5 mg/l IAA combination of hormones is best for all the banana varieties for shoot proliferation in banana micropropagation. The inappropriate concentration of plant growth hormones leads to plant death, reduced growth and development (Madhulatha *et al*., 2004) [10]. Grand Naine varieties of banana at 3 mg/l BAP and 0.5 mg/l IAA showed the best results with a maximum number of shoots in comparison to other varieties. Parallel findings were reported by Baysal (2022) [3], Singh *et al* (2021) [15] and Mahanthesh *et al* (2022) [11].

Table 4: Effect of different plant growth regulators on number of shoots obtained

Varieties of banana				
Treatments	**Red banana (mean ±SD)**	**Monthan (mean ±SD)**	**Grand Naine (mean ±SD)**	**Elakkibale (mean ±SD)**
2 mg/l BAP	2.0±0.31	1.5±0.13	5. 0 ±0.10	1. 0 ±0.09
3 mg/l BAP + 0.5 mg/l IAA	9.0 ±0.18	8.0 ±0.19	11.0 ±0.15	7. 0 ±0.08
4 mg/l BAP + 1 mg/l IAA	6.2±0.17	5.6±0.22	6.8±0.09	1.9 ±0.11
5 mg/l BAP + 1.5 mg/l IAA	3.5±0.25	3. 0 ±0.09	4. 0 ±0.15	2. 0 ±0.08
SE(m)	0.79	0.48	0.60	0.20
CV	5.29	4.85	6.25	5.23

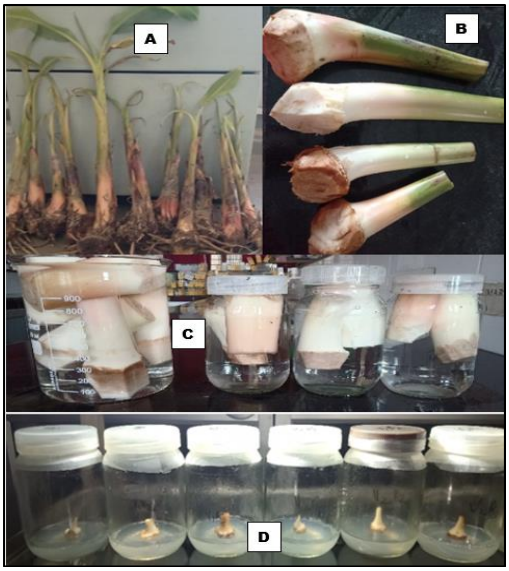

Fig 1: Steps in micropropagation of banana (A) Collection of banana suckers as explant (B) Pre- treatment of explants (C) Surface sterilization of explants (D) Inoculation of explants under aseptic conditions

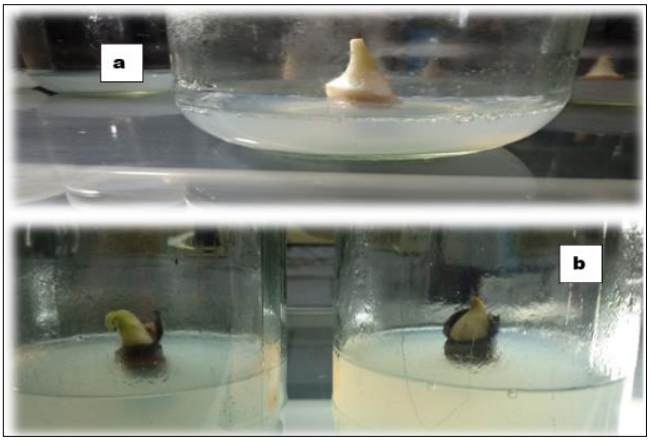

Fig 2: Tissue swelling after inoculation of banana explants (a) Inoculated explant (b) Swelled explants

Fig 3: Change in colour of explants to green from white

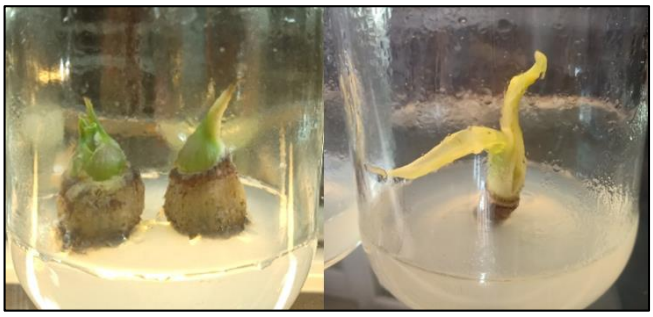

Fig 4: Emergence of shoots from the explant in the MS medium

Fig 5: Formation number of shoots from the MS media

4. Conclusion

The present study indicates that accurate concentration of plant growth hormones is important factor in success of banana micropropagation. Current experiment concludes that 3 mg/l BAP with 0.5 mg/l IAA is best treatment to obtain early shoot initiation and maximum number of shoot establishment from the banana explants. Different combination of cytokinin and auxin hormones provides maximum growth. Alone BAP is not sufficient for the fast growth and development of inoculated explants. Also, higher quantity of hormones can reduce growth and development of invitro plants. Grand Naine banana variety showed best results in the present experiment. Therefore, it is important to optimize best combination of plant growth hormones for commercial establishment of disease and virus-free banana plantlets.

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6. Conflict of Interest: None

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