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RESEARCH PAPER

Effect of different dose of cytokinin for shoot multiplication of banana (*Musa paradisiaca* L.) variety 'GRAND NAINE' under *in-vitro* condition

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Abstract : The maximum callus formation (20.3%) was observed in treatment BAP 8 mgl^{-1} while the minimum (4.6%) was noted under control. With the combination of BAP and BA, the maximum callus formation (27.0%) was recorded under BAP 8 mgl^{-1} + BA 4 mgl^{-1}; however, it was at par with BAP 8 mgl^{-1} + BA 3 mgl^{-1} at 75 days after inoculation.At 90 days after inoculation, maximum callus percentage (29.3) was found under BAP 8 mgl^{-1}+ BA 4 mgl^{-1}.At 105 days after inoculation, callus percentage was maximum callus (33.0%) was noted under the treatment combination of BAP 8 mgl^{-1}+ BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1}+ BA 3 mgl^{-1}, while the minimum (9.3%) was recorded under control again. The earliest shoot initiation (21.0, 22.0 days, respectively) was noted under BAP 8 mgl^{-1} and BA 5 mgl^{-1}, separately; while it was statistically earliest *i.e.* 20.66 days in combination with BAP 2 mgl^{-1}+ BA 5 mgl^{-1}. Maximum shoot length (0.76 cm) was recorded in the treatment of BAP 8 mgl^{-1} at 20 days after shoot initiation.Maximum shoot length (3.06 cm) was noted under BAP 8 mgl^{-1} alone which statistically superior to other under BAP alone treatments while it recorded minimum under control at 40 days after shoot initiation. Under BA treatments, the maximum shoot length (2.26 cm) was noted with BA 4 mgl^{-1} and 5 mgl^{-1} both; however, it was at par with BA 2 mgl^{-1} and 3 mgl^{-1} at 40 days after shoot initiation. With the effect of BAP and BA combinations, the maximum shoot length (3.23cm) was recorded under BAP 8 mgl^{-1}+ BA 5 mgl^{-1}at 40 days after shoot initiation. The minimum duration of root initiation (14.66 days) was noted under the treatment of Indole Butyric acid 4 mgl^{-1} ; however, it was significantly at par with indole butyric acid 2 mgl^{-1} and 3 mgl^{-1}. The maximum duration (34.33 days) was observed under control. Minimum number of roots (4.0 roots) were recorded under the treatment applied 1 mgl^{-1} IBA in culture medium. Further, number of roots was found maximum 10.33 roots under the treatment of 5 mgl^{-1} IBA followed by 4 mgl^{-1} IBA concentrations. Culture medium with IBA 5 mgl^{-1} showed maximum root length (1.66 cm) followed by IBA 4 mgl^{-1}, 3 mgl^{-1} and 2 mgl^{-1} with 1.56 cm, 1.40 cm and 1.06 cm, respectively. It was concluded that BAP 8 mgl^{-1} and BA 5 mgl^{-1} separately performed better results on account of callus formation, shoot initiation and multiplication of shoots whereas with their combination *viz.*, BAP 8 mgl^{-1} +BA 4 mgl^{-1} showed best result on the above parameters. For root initiation and its development IBA 5 mgl^{-1} was found to be the best among all the treatments.

Key Words : Cytokinin, Shoot multiplication, Banana, *In- vitro* condition

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INTRODUCTION

Banana, an antique fruit crop of the world is known as 'apple of the paradise' and botanically named as *Musa paradisiaca* L. It is the cheapest and plentiful fruit. This fruit is the food of sages since ancient time. Banana is the staple food in Uganda, Bukoba and Tanzania. It is the most important world traded tropical fruit. Banana is basically a tropical crop, grows well in temperature range of 15ºC – 35ºC with RH regime of 75-85 per cent. Soil for banana should have good drainage, adequate fertility and moisture. Deep, rich loamy soil with pH 6.0-7.5 is most preferred for banana cultivation. Banana is a globally important fruit crop with 97.5 million tonnes of production. Banana ranks first in production with 33.40 per-cent share among the fruits in India.

This fruit covers an area of 8.03 million hectare, producing 29.72 million tonnes with a productivity of 37.0 MT/ha during the year 2013-14with national average of 33.5 T/ha. Tamil Nadu ranks first in production with 5.65 million tonnes with an area of 1.18 million hectares followed by Maharashtra with 4.83 million tonnes with 0.83 million hectares, Gujarat with 4.25 million tonnes with 0.66 million hectares and Andhra Pradesh with 3.16 million tonnes with 0.90 million hectares.; however, the productivity was recorded highest 66.0 tonnes /hectare in Madhya Pradesh followed by Gujarat (63.0T/ha), Maharashtra (58.2T/ha) and Tamil Nadu (47.9T/ha). Banana contributes 33.4 per cent to total production in India.(Indian Horticulture data base -2014). In India, banana is the second most important crop next to the mango. It remains available throughout the year, affordability, varietal range, tastes, nutritive and medicinal value makes it the favorite among all class of the people. In addition to fruit plantains and banana provide many cultivars with medicines, beverages, fibres, edible floral parts, dyes, fuel, stream for cooking cordage etc. Banana is the rich source of carbohydrates and vitamins particularly vitamin B complex.

For rapid *in-vitro* multiplication of banana, male inflorescence of banana are most commonly used as explants. The male flower buds of banana were collected 1 to 10 weeks after flowering.The present investigation was carried out to study the callus formation, shoot emergence and shoot length with different doses of cytokinin and root intiation with different doses of auxin.

MATERIAL AND METHODS

The present study was conducted in Tissue Culture Laboratory of Department of Horticulture in Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut during 2014-15. The male flower buds of banana were collected 1 to 10 weeks after flowering. The flower buds were kept upto 24 hours before inoculation of the immature flowers. In non-sterile conditions, reduced the size of the male bud until the explant is 0.8 cm x 2 cm. The reduced bud is kept in non- dehydrating conditions until sterilization, e.g. in a container with a few drops of water and sealed with a plastic film. These flower buds were thoroughly washed with double distilled water. Then the flowers were kept in 70 per cent ethyl alcohol for a period of 5 minutes. After that they were washed with double distilled water stirring well. This process was applied five times just to wash out the adhering chemicals on the surface of the flower buds. Then the explants were put on the floor of the Laminar Flow and they were gently surrounded by few drops of ethyl alcohol through smearing with sterilized cotton and were gently by opening of front lid simultaneously with air flow. In this way whole of explants were thoroughly surface sterilized. The whole process was done in the Laminar Flow. Then the lower and upper portion of male flower bud was cut and removed through sterilized knife and placed as "initial explant" in a callus induction medium with addition of BAP (Benzylaminopurine) @ 1, 2, 4, 6 and 8 mg/$l^{-1}$and BA (*N*6-benzyladenine) @ 1, 2, 3, 4 and 5mg mg / $l^{-1}$of 5 containers/pot represent one treatment with three replications under two- way factorial experiment to develop into an embryogenic callus under the flame spirit lamp to keep the surrounding free from micro-organisms.Then the inoculated culture containers were sealed with paraffin film to make the container free from germs. The inoculated containers were placed into the growth room for initiation of callus under 25ºC and 60 per cent RH. Timely observation was taken and contaminated containers were picked up and kept out of the growth room.

The contaminated containers were sterilized through autoclaving regularly. The explants were initiated in a suitable medium and maintained in dark conditions without subculture for 3 months. Embryogenic callus was initiated in suitable medium to develop embryogenic cell suspension with repeated media replenishments. The data of suspension were recorded at different intervals according to treatment.Timely observations were taken as per the schedule.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Callus formation percentage :

Significant increase in average callus formation percentage were recorded from 4.6 per cent to 27.0 per cent, 6.3 per cent to 29.3 per cent and 9.3 per cent to

Table: 1: Effect of different concentrations of BAP, BA and their combinations on calli formation, shoot initiation, number of shoots and shoot length of banana cv. GRAND NAINE

Treatments (mgl^{-1})	Calli formation (%)			Shoot initiation after callus formation (days)	Number of shoots			Shoot length at different intervals after shoot initiation (cm)		
	75 DAI	90DAI	105 DAI		45 DASI	60 DASI	75 DASI	20 DASI	30 DASI	40 DASI
Control	4.67	6.33	9.33	47.66	3.33	4.33	7.33	0.46	0.67	1.60
BAP 1	5.33	8.00	12.33	35.00	3.33	11.67	21.67	0.46	0.70	0.93
BAP 2	9.00	11.00	14.67	31.00	5.67	14.33	23.00	0.56	1.60	2.23
BAP 4	13.00	15.67	18.67	25.00	6.67	16.33	25.33	0.56	2.00	2.60
BAP 6	19.00	21.67	24.33	25.00	8.00	17.67	27.00	0.63	1.90	2.50
BAP 8	20.33	23.00	28.33	21.00	9.67	16.67	25.33	0.76	2.50	3.06
BA 1	5.00	7.33	9.33	39.33	3.33	12.00	21.00	0.40	0.56	0.83
BA 2	8.33	11.00	14.33	33.33	5.00	13.67	22.67	0.63	1.40	2.10
BA 3	12.00	14.67	17.00	27.33	5.67	14.67	23.67	0.63	1.70	2.13
BA 4	13.33	15.33	18.00	27.66	6.67	15.67	25.00	0.46	1.50	2.26
BA 5	15.33	18.00	20.00	22.00	7.67	15.00	25.33	0.46	1.50	2.26
BAP 1 + BA 1	7.33	10.00	13.00	33.33	4.33	12.33	22.33	0.56	0.76	0.96
BAP 1 + BA 2	10.33	12.67	17.00	30.00	6.00	14.00	23.67	0.73	1.76	2.30
BAP 1 + BA 3	13.33	15.67	18.00	23.00	6.33	16.33	24.00	0.76	1.90	2.40
BAP 1 + BA 4	14.33	16.67	20.00	22.66	7.33	16.67	24.00	0.80	2.06	2.46
BAP 1 + BA 5	16.67	19.33	22.00	21.00	8.33	16.00	24.67	0.80	2.13	2.46
BAP 2 + BA 1	11.33	14.00	17.00	31.66	7.33	17.33	25.67	0.63	1.70	2.30
BAP 2 + BA 2	12.00	15.00	18.67	29.00	8.00	18.00	28.00	0.767	1.76	2.36
BAP 2 + BA 3	13.67	16.33	19.00	21.33	8.67	18.67	27.67	0.83	1.83	2.43
BAP 2 + BA 4	15.00	18.00	21.33	21.66	9.00	19.00	30.00	0.86	1.86	2.50
BAP 2 + BA 5	18.00	20.67	23.33	20.66	10.67	21.33	32.00	0.90	1.93	2.53
BAP 4 + BA 1	14.67	17.00	20.00	30.33	8.00	18.00	25.33	0.66	2.06	2.63
BAP 4 + BA 2	16.67	19.67	22.67	27.33	10.00	19.33	26.00	0.73	2.10	2.66
BAP 4 + BA 3	19.00	22.00	24.67	22.00	11.33	21.33	27.67	0.76	2.20	2.70
BAP 4 + BA 4	20.67	23.67	26.33	22.33	12.00	22.00	29.67	0.83	2.30	2.73
BAP 4 + BA 5	22.00	24.67	28.00	23.00	12.33	22.33	31.00	0.83	2.30	2.73
BAP 6 + BA 1	22.33	24.33	27.33	30.00	8.67	18.67	28.00	0.70	2.00	2.56
BAP 6 + BA 2	22.67	25.67	28.00	25.67	10.67	20.67	28.00	0.76	2.06	2.63
BAP 6 + BA 3	23.00	26.33	30.00	21.66	10.67	20.67	28.00	0.83	2.13	2.66
BAP 6 + BA 4	24.33	28.00	31.00	22.00	11.67	21.67	30.00	0.83	2.20	2.63
BAP 6 + BA 5	25.33	29.00	31.33	21.66	12.67	22.67	33.00	0.86	2.20	2.70
BAP 8 + BA 1	23.67	27.33	31.00	29.00	10.67	20.67	29.67	0.80	2.60	3.13
BAP 8 + BA 2	25.00	27.67	30.00	24.00	11.00	21.00	31.00	0.83	2.56	3.13
BAP 8 + BA 3	26.67	28.67	32.00	21.66	12.67	22.67	32.67	0.83	2.50	3.16
BAP 8 + BA 4	27.00	29.33	33.00	22.66	13.00	23.00	33.33	0.86	2.56	3.16
BAP 8 + BA 5	24.33	25.33	29.00	22.00	13.33	23.33	33.33	0.83	2.56	3.23
C.D. (P=0.05)	1.62	1.89	2.072	2.29	1.70	2.02	2.25	0.15	0.31	0.23

33.0 per cent at 75, 90 and 105 days after inoculation, respectively (Table 1).

Maximum callus formation (20.3%) was observed in treatment BAP 8 mgl^{-1} while the minimum (4.6%) was noted under control at 75 days after inoculation. BA 5 mgl^{-1} showed best performance on account of callus formation *i.e.* 15.3 per cent. With the combination of BAP and BA, the maximum callus formation (27.0%) was recorded under BAP 8 mgl^{-1} + BA 4 mgl^{-1} ; however, it was at par with BAP 8 mgl^{-1} + BA 3 mgl^{-1} at 75 days after inoculation.

At 90 days after inoculation the maximum callus percentage (29.3) was found under BAP 8 mgl^{-1}+ BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1}+ BA 3 mgl^{-1}, while the minimum (6.3%) was recorded under control again.

At 105 days after inoculation the maximum callus (33.0%) was noted under the treatment combination of BAP 8 mgl^{-1}+ BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1}+ BA 3 mgl^{-1}, while the minimum (9.3%) was recorded under control again. The same trend of observation was noted under BAP and BA treatments alone.

Time of shoot initiation after callus formation (days) of banana cv. GRAND NAINE :

The earliest shoot initiation (21.0, 22.0 days, respectively) was noted under BAP 8 mgl^{-1} and BA 5 mgl^{-1}, separately; while it was statistically earliest *i.e.* 20.66 days in combination with BAP 2 mgl^{-1} + BA 5 mgl^{-1} (Table 1). But, there were no statistical difference among the treatments with BAP 1 mgl^{-1}+ BA 4 mgl^{-1}, BAP 1 mgl^{-1}+ BA 5 mgl^{-1}, BAP 4 mgl^{-1}+ BA 3 mgl^{-1}, BAP 4 mgl^{-1}+ BA 4 mgl^{-1}, BAP 6 mgl^{-1}+ BA 5 mgl^{-1}, BAP 6 mgl^{-1}+ BA 4 mgl^{-1}, BAP 6 mgl^{-1} + BA 5 mgl^{-1} , BAP 8 mgl^{-1} + BA 3 mgl^{-1}, BAP 8 mgl^{-1} + BA 4 mgl^{-1} and BAP 8 mgl^{-1} + BA 5 mgl^{-1}. The maximum duration was (47.66 days) observed under control on shoot initiation after callus formation.

Effect of different concentrations of BAP, BA and their combinations on number of shoots:

Maximum number of shoots (9.6) were emerged under the treatment of BAP 8 mgl^{-1} whereas it was minimum under control at 45 days after shoot initiation. Under BA treatment, maximum shoots (7.6) were recorded in BA 5 mgl^{-1} ; however, it was significantly at par with BA 4 mgl^{-1}. The maximum shoots (13.3) was recorded under the treatment of BAP 8 mgl^{-1}+BA 5 mgl^{-1} (Table 1).

At 60 days after shoot initiation, the maximum shoots (17.6) were noted under the treatment of BAP 6 mgl^{-1} ; however, it was significantly at par with BAP 4 mgl^{-1} and BAP 8 mgl^{-1}. With the combination of BAP and BA, the maximum shoots (23.3) were noted under BAP 8 mgl^{-1} + BA 5 mgl^{-1}; however, it was at per with BAP 8 mgl^{-1} + BA 4 mgl^{-1}.

At 75 days after shoot initiation, the maximum shoots (27.0) were noted under BAP 6 mgl^{-1} alone in the culture medium; while it was significantly at par with BAP 4 mgl^{-1} and BAP 8 mgl^{-1}. Under BA treatments, the maximum shoots (25.3) were recorded in the BA 5 mgl^{-1}; however, it was significantly at par with BA 3 mgl^{-1} and BA 4 mgl^{-1} treatments. Further, it observed maximum shoots (33.3) under BAP 6 mgl^{-1} + BA 5 mgl^{-1}; however, it non-significant with BAP 8 mgl^{-1} + BA 3 mgl^{-1} and BAP 8 mgl^{-1} + BA 4 mgl^{-1}.

Shoot length :

Maximum shoot length (0.76cm) was recorded in the treatment of BAP 8 mgl^{-1}; however, it was significantly at par with BAP 6 mgl^{-1} while the minimum (0.46 cm) was noted under control at 20 days after shoot initiation. Further, shoot length was recorded maximum (0.63 cm) under BA 2 and BA 3 treatments while it was minimum under BA 1 treatment at 20 days after shoot initiation.

Under BAP and BA treatments combinations, the maximum shoot length (0.90 cm) was noted under BAP 2 mgl^{-1} + BA 5 mgl^{-1} treatment; however, it was at par with BAP 2 mgl^{-1} + BA 3 mgl^{-1} , BAP 2 mgl^{-1} + BA 4 mgl^{-1}, BAP 4 mgl^{-1} + BA 4 mgl^{-1} , BAP 4 mgl^{-1} + BA 5 mgl^{-1} and BAP 6 and 8 mgl^{-1} with all the combinations of BA at 20 days after shoot initiation.

Maximum shoot length (2.50cm) was noted under BAP 8 mgl^{-1} alone while it recorded minimum under control at 30 days after shoot initiation. Under BA treatments, the maximum shoot length (1.70cm) was noted with BA 3 mgl^{-1}; however, it was at par with BA 4 mgl^{-1} and BA 5 mgl^{-1}.

Further, with the effect of BAP and BA combinations, the maximum shoot length (2.60cm) was recorded under BAP 8 mgl^{-1} + BA 1 mgl^{-1}; however, it was significantly at par with BAP 8 mgl^{-1} combined with BA 2 to 5 mgl^{-1} concentrations at 30 days after shoot initiation.

Maximum shoot length (3.06 cm) was noted under BAP 8 mgl^{-1} alone which staistically superior to other under BAP alone treatments while it recorded minimum under control at 40 days after shoot initiation. Under BA treatments, the maximum shoot length (2.26 cm) was noted with BA 4 mgl^{-1} and 5 mgl^{-1} both; however, it was at par with BA 2 mgl^{-1} and 3 mgl^{-1} at 40 days after shoot initiation.

Further, with the effect of BAP and BA combinations, the maximum shoot length (3.23 cm) was recorded under BAP 8 mgl^{-1}+ BA 5 mgl^{-1}; however, it was significantly at par with BAP 8 mgl^{-1} combined with BA 1 to 4 mgl^{-1} concentrations at 40 days after shoot initiation. The minimumshoot length (1.60 cm) was recorded under control (Table 1).

Time of root initiation after shoot development :

It is evident from the Table 2 that incorporation of IBA from 1 to 5 mg/l concentrations in the culture medium found great variability among all the treatments under study for the time of root initiation after shoot development. The minimum duration of root initiation (14.66 days) was noted under the treatment of indole butyric acid 4 mgl^{-1} ; however, it was significantly at par with indole butyric acid 2 mgl^{-1} and 3 mgl^{-1}. The maximum duration (34.33 days) was observed under control.

Number of roots of banana plants cv. GRAND NAINE:

The explant of banana was inoculated in culture medium with IBA 1 to 5 mgl^{-1} showed maximum number of roots (10.33) with indole butyric acid 5 mgl^{-1}; however, it was at par with IBA 4 mgl^{-1} concentrations. The minimum roots (3.00) were recorded under cntrol.

Effect of different concentrations of IBA on root length of banana cv. GRAND NAINE:

In this experiment the incorporation of IBA from 1 to 5 mgl^{-1} concentrations in the culture medium observed significant variability on root length of banana (Table 2). The explant of Grand Naine banana inoculated in the culture medium with IBA 5 mgl^{-1} showed maximum root length (1.66cm) followed by IBA 4 mgl^{-1} , 3 mgl^{-1} and 2 mgl^{-1} with 1.56 cm, 1.40 cm and 1.06 cm, respectively.

The minimum root length (0.46 cm) was found under control which was at par with IBA 1mgl^{-1}.

Effect of various concentrations of BAP, BA and IBA on explant development :

Explants that survived and exhibited some degree of growth were maintained and used for further studies. When cultures were shifted to the medium containing different combination of BAP alone, there was a significant increase in the mean number of shoot after 30 days of culture in MS medium containing 6 mgl^{-1}BAP.

Callus formation percentage :

The treatments under study for a period upto 75 days showed that significantly the maximum callus formation (20.3%) was observed in treatment BAP 8 mgl^{-1} while the minimum (4.6%) was noted under control. Under BA alone treatments, BA 5mgl^{-1} showed best performance on account of callus formation *i.e.*15.3 per cent. With the combination of BAP and BA, the maximum callus formation (27.0%) was recorded under BAP 8 mgl^{-1} + BA 4 mgl^{-1} ; however, it was at par with BAP 8 mgl^{-1} + BA 3 mgl^{-1} at 75 days after inoculation. Maximum callus percentage (29.3) was found under BAP 8 mgl^{-1} + BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1} + BA 3 mgl^{-1}, while the minimum (6.3%) was recorded under control at 90 days after inoculation.

The callus percentage at 105 days after inoculation was found great variability on account of treatments either with BAP, BA alone or with their combinations. The maximum callus (33.0%) was noted under the treatment combination of BAP 8 mgl^{-1}+ BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1}+

Table 2: Effect of different concentrations of IBA ontime of root initiation, number of roots and root length at 20 days after root initiation

Treatments (mg /l)	Time of root initiation (days)	Number of roots	Root length at 20 days after root initiation (cm)
IBA 1	18.66	4.00	0.70
IBA 2	17.00	5.66	1.06
IBA 3	15.33	7.00	1.40
IBA 4	14.66	9.00	1.56
IBA 5	24.33	10.33	1.66
Control	34.33	3.00	0.46
C.D. (P=0.05)	2.875	2.13	0.24

BA 3 mgl^{-1}, while the minimum (9.3%) was recorded under control again. The same trend of observation was noted under BAP and BA treatments alone. It showed that as time increased the percentage of callus initiation increased under aseptic condition. The same pattern of observations were recorded by Frison and Sharrock (1998), Darvari *et al.* (2010) and Wirakarnain *et al.* (2008). The explantsas male flower of banana variety GRAND NAINE inoculated in the culture medium concentrations of 8 mg BAP and BA 5 shifted. The highest percentage of callus formation rise 38.00 per cent at 105 days of inoculation, which was significantly superior and rest of the treatments. Minimum callus formation was recorded under the treatment applied BAP 8 + BA 5 and BA 1 (6.66) in culture medium. It showed that the combination of BAP and BA resulted better performance on callus formation. The same pattern of observations were reported by Wirakarnain *et al.* (2008); Hernandez and Garcia (2008); Rashid *et al.* (2012); Kumar *et al*. (2011); Sultan *et al.* (2011) and Jafari *et al*. (2011).

Time of shoot initiation after callus formation (days) of banana plantlet :

BAP (6mgl^{-1}) showed the greater performances of time of shoot initiation which was observed as minimum duration (20 days) followed by 5 BA (mgl^{-1}) treatment observe at 22 days. The long duration of shoot initiation was recorded (39.33 days) in the applied treatment of 1 BA followed by 35.33 days at 1 BAP, 33.33 days at 2 BA, 27.33 days at 3 BA, 26.66 days at 2 BAP (mgl^{-1}) concentrations.

The minimum time of shoot initiation (22 days) was observed under the treatment applied with 5 BA and 4 BAP (mgl^{-1}) in culture medium which showed great variability among the treatments. The minimum time of shoot initiation (20.0 days) was found under the treatment applied 6 mgl^{-1} BAP followed by 22.0 days, 23.0 days, 25.33 days and 26.0 days under the applied treatments 5 BA and 4 BAP, BAP 4+BA 5, BAP 6+BA 4 and BAP 4 + BA 4 concentrations, respectively.

Effect of different concentrations of BAP and BA on time of shoot initiation after callus formation (days) of banana plantlet cv. GRAND NAINE at different days showed great variability. The same pattern of performance were noted by Hernandez and Garcia (2008); Kumar *et al.* (2011); Sultan *et al.* (2011) and Jafari *et al.* (2011).

Effect of different concentrations of BAP on number of shoots:

The earliest shoot initiation (21.0, 22.0 days) was noted under BAP 8 mgl^{-1} and BA 5 mgl^{-1}, separately; while it was statistically earliest *i.e.* 20.66 days in combination with BAP 2mgl^{-1}+ BA 5 mgl^{-1}. But, there were no statistical difference among the treatments with BAP 1mgl^{-1}+ BA 4 mgl^{-1}, BAP 1mgl^{-1}+ BA 5 mgl^{-1}, BAP 4 mgl^{-1}+ BA 3 mgl^{-1}, BAP 4 mgl^{-1}+ BA 4 mgl^{-1}, BAP 6 mgl^{-1}+ BA 5 mgl^{-1}, BAP 6 mgl^{-1}+ BA 4 mgl^{-1}, BAP 6 mgl^{-1} + BA 5mgl^{-1} , BAP 8 mgl^{-1} + BA 3 mgl^{-1}, BAP 8 mgl^{-1} + BA 4 mgl^{-1} and BAP 8 mgl^{-1} + BA 5mgl^{-1}. The maximum duration was (47.66 days) observed under control on shoot initiation after callus formation.The same line of studies was conducted by Mahadev *et al. (*2011); Harirah and Khalid (2006); Resmi and Nair (2007) and Meenakshi *et al*. (2011).

Shoot length of banana plantlet :

Maximum shoot length (0.76cm) was recorded in the treatment of BAP 8 mgl^{-1}; however, it was significantly at par with BAP 6 mgl^{-1} while the minimum (0.46cm) was noted under control at 20 days after shoot initiation. Further, shoot length was recorded maximum (0.63cm) under BA 2 and BA 3 treatments while it was minimum under BA 1 treatment at 20 days after shoot initiation.

Under BAP and BA treatments combinations, the maximum shoot length (0.90 cm) was noted under BAP 2 mgl^{-1}+ BA 5 mgl^{-1} treatment; however, it was at par with BAP 2 mgl^{-1} + BA 3 mgl^{-1}, BAP 2 mgl^{-1}+ BA 4 mgl^{-1} , BAP 4 mgl^{-1} + BA 4 mgl^{-1}, BAP 4 mgl^{-1}+ BA 5 mgl^{-1} and BAP 6 and 8 mgl^{-1} with all the combinations of BA at 20 days after shoot initiation. Maximum shoot length (2.50 cm) was noted under BAP 8 mgl^{-1} alone while it was recorded minimum under control at 30 days after shoot initiation. Under BA treatments, the maximum shoot length (1.70 cm) was noted with BA 3 mgl^{-1}; however, it was at par with BA 4 mgl^{-1} and BA 5 mgl^{-1}.

Further, with the effect of BAP and BA combinations, the maximum shoot length (2.60 cm) was recorded under BAP 8 mgl^{-1} + BA 1 mgl^{-1}; however, it was significantly at par with BAP 8 mgl^{-1} combined with BA 2 to 5 mgl^{-1} concentrations at 30 days after shoot initiation.

Maximum shoot length (3.06 cm) was noted under BAP 8 mgl^{-1} alone which staistically superior to other under BAP alone treatments while it recorded minimum under control at 40 days after shoot initiation. Under BA

treatments, the maximum shoot length (2.26 cm) was noted with BA 4 mgl^{-1} and 5 mgl^{-1} both; however, it was at par with BA 2 mgl^{-1} and 3 mgl^{-1} at 40 days after shoot initiation. Further, with the effect of BAP and BA combinations, the maximum shoot length (3.23cm) was recorded under BAP 8 mgl^{-1} + BA 5 mgl^{-1}; however, it was significantly at par with BAP 8 mgl^{-1} combined with BA 1 to 4 mgl^{-1} concentrations at 40 days after shoot initiation. The minimum shoot length (1.60 cm) was recorded under control. The same pattern of study were recorded by Resmi and Nair (2007); Meenakshi *et al.* (2011) and Krikorian *et al*. (1993).

Time of root initiation after shoot development :

The minimum duration of root initiation (14.66 days) was noted under the treatment of indole butyric acid 4 mgl^{-1} ; however, it was significantly at par with indole butyric acid 2 mgl^{-1} and 3 mgl^{-1}. The maximum duration (34.33 days) was observed under control. The same pattern of performance were noted by Kumar *et al.* (2011); Sultan *et al.* (2011); Jafari *et al.* (2011) and Mahadev *et al.* (2011).

Number of roots of banana plantlet :

The explant of banana was inoculated in culture medium with IBA 1 to 5 mgl^{-1} showed maximum number of roots (10.33) with indole butyric acid 5 mgl^{-1}; however, it was at par with IBA 4 mgl^{-1} concentrations. The minimum roots (3.00) were recorded under control.

Minimum number of roots (4.0 roots) was recorded under the treatment applied 1 mgl^{-1} IBA in culture medium. Further, number of roots was found maximum 10.33 roots under the treatment of 5 mgl^{-1} IBA followed by 4 mgl^{-1} IBA concentrations. The minimum number of roots was noticed in the treatment 1mgl^{-1} IBA (4.0 roots). The maximum number of roots was observed (10.33 roots) under applied 5 mgl^{-1} treatment of IBA followed by 9.0, 7.0 and 5.66 numbers of roots under the treatments of 4 mgl^{-1} IBA, 3 mgl^{-1} IBA and 2 mgl^{-1} IBA, respectively. The same pattern of root development was noted by Kumar *et al.* (2011); Sultan *et al*. (2011); Jafari *et al.* (2011) and Mahadev *et al.* (2011).

Root length of multiple shoots of banana:

The explant of Grand Naine banana inoculated in the culture medium with IBA 5 mgl^{-1} showed maximum root length (1.66cm) followed by IBA 4 mgl^{-1}, 3 mgl^{-1} and 2 mgl^{-1} with 1.56 cm, 1.40 cm and 1.06 cm, respectively. The minimum root length (0.46 cm) was found under control which was at par with IBA1 mgl^{-1}. The same pattern of observations were noted by Darvari *et al*. (2010); Wirakarnain *et al.* (2008); Hernandez and Garcia (2008); Kumar *et al.* (2011); Sultan *et al*. (2011); Mahadev *et al.* (2011) and Harirah and Khalid (2006).

Conclusion :

Successful shoot multiplication depends upon the appropriate combination of growth regulators in culture media.The callus percentage at 105 days after inoculation was found with great variability on account of treatments either with BAP, BA alone or with their combinations. The maximum callus (33.0%) was noted under the treatment combination of BAP 8 mgl^{-1}+ BA 4 mgl^{-1}, however, it was significantly at par with BAP 8 mgl^{-1} + BA 3 mgl^{-1}, while the minimum (9.3%) was recorded under control again. Significant time of shoot initiation. BAP (6 mgl^{-1}) showed the greater performances of time of shoot initiation which was observed in minimum duration (20 days) followed by 5 BA (mgl^{-1}) treatment (22 days). The long duration of shoot initiation was recorded (39.33 days) in the applied treatment of 1 BA followed by 35.33 days at 1 BAP, 33.33 days at 2 BA, 27.33 days at 3 BA, 26.66 days at 2 BAP (mgl^{-1}) concentrations. Maximum shoot length (0.76 cm) was recorded in the treatment of BAP 8 mgl^{-1}; however, it was significantly at par with BAP 6 mgl^{-1} while the minimum (0.46cm) was noted under control at 20 days after shoot initiation. Maximum shoot length (2.50 cm) was noted under BAP 8 mgl^{-1} alone while minimum was under control at 30 days after shoot initiation. Under BA treatments, the maximum shoot length (1.70 cm) was noted with BA 3 mgl^{-1}; however, it was at par with BA 4 mgl^{-1} and BA 5 mgl^{-1}. The minimum duration of root initiation (14.66 days) was noted under the treatment of indole butyric acid 4 mgl^{-1}; however, it was significantly at par with indole butyric acid 2 mgl^{-1} and 3 mgl^{-1}. The maximum duration (34.33 days) was observed under control. Minimum number of roots (4.0 roots) was recorded under the treatment applied 1 mgl^{-1} IBA in culture medium. Further, number of roots was found maximum (10.33 roots) under the treatment of 5 mgl^{-1} IBA followed by 4 mgl^{-1} IBA concentrations.

The explant of Grand Naine banana inoculated in the culture medium with IBA 5 mgl^{-1} showed maximum root length (1.66 cm) followed by IBA 4 mgl^{-1} , 3 mgl^{-1}

and 2 mgl^{-1} with 1.56 cm, 1.40 cm and 1.06 cm, respectively. The minimum root length (0.46 cm) was found under control which was at par with IBA 1mgl^{-1}.

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