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SHORT COMMUNICATION

OPTIMIZATION OF BAP AND IAA CONCENTRATION ON SHOOT INDUCTION, PROLIFERATION AND ROOTING IN SHOOT-TIP CULTURE OF BANANA CV. DWARF CAVENDISH

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The effect of BAP (6-benzylaminopurine) and IAA (indole-3-acetic acid) alone and in combination was investigated on shoot initiation, multiplication and root induction for Dwarf Cavendish banana. Shoot-tips (1-1.2 cm) were isolated from field collected suckers to initiate the culture. Concentration of BAP ranged from 0-6 mg L^{-1} for shoot induction and from 0-8 mg L^{-1} for shoot multiplication. The concentration of IAA in both initiation media and multiplication media ranged from 0-2 mg L^{-1}. The explants responded best on shoot induction media containing MS media supplemented with BAP (6 mg L^{-1}) and IAA (1 mg L^{-1}). Highest multiplication rate was observed on the same medium. Best root induction and proliferation was observed on half strength MS media supplemented with 1 mg L^{-1} IAA.

***Key words*: Banana, multiplication, rooting, shoot induction, shoot-tip culture**

Banana is a monocotyledonous herb, cultivated in more than 130 countries of tropics and sub-tropics. It is the fourth most important food commodity in the world, providing a well balanced diet to millions of people and contributing to the livelihood through crop production, processing and marketing. The cultivation of banana in northern parts of India is still confined to certain pockets. In *tarai* region, Gorakhpur farmers are doing large scale cultivation of Grand Nain banana through tissue culture plants. Dwarf Cavendish is mostly preferred by the local farmers. Its short stature makes it stable, wind resistant and easier to manage thus, plants may be grown without propping. Also they are relatively tolerant to the low temperature, experienced during winter season in northern India. Dwarf Cavendish is widely distributed in different parts of the world where tall clones lodge in high wind and difficult to harvest. Tissue culture propagation of banana through shoot-tip as well as floral apices has been utilized to increase production in various parts of world. *In vitro* propagation of banana provides high multiplication rate, physiological uniformity, year round availability of disease-free material, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval and faster growth in the early growing stages compared to conventional materials (Vuylsteke 1989, Arias 1992) which are inevitable for commercialization of banana. Banana growers of Varanasi and adjoining areas generally use conventional planting materials of Dwarf Cavendish. For this purpose, sword suckers are used for new plant establishment and reestablishment of old plantations. There are no enough government organizations or private banana companies producing banana planting material in Varanasi and adjoining area.

Micropropagation of various cultivars of banana through shoot-tip explants is well documented (Cronauer and Krikorian 1984, Wong 1986, Vuylsteke 1989,

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Muhammad *et al.* 2007). In almost all cases, different combinations of cytokinin and auxin in various concentrations were reported for multiple shoot regeneration. The physiological state of explants, seasonal and cultivar differences are the reasons, perhaps that, different workers have reported different media compositions for plantlets regeneration in banana. Thus, in this study, to optimize BAP and IAA concentrations for micropropagation of Dwarf Cavendish banana, shoot proliferation rate and elongation affected by interaction of cytokinin (BAP) and auxin (IAA) concentrations have been investigated. The effect of full and half strength MS media was investigated at different levels of IAA for rooting. The optimization of multiplication and rooting of Dwarf Cavendish cultivar will aid in producing large number of disease free planting material not only in Varanasi but also in other regions of Uttar Pradesh.

Young healthy suckers of banana cv. Dwarf Cavendish were collected from the Experimental Field of Department of Horticulture, Banaras Hindu University, Varanasi. The suckers were washed under tap water and trimmed to a block of 50 mm^3 containing shoot-tip and rhizomatous base. Field suckers were processed within two hours of uprooting by chopping down the rhizome and pseudostem parts, soaking the excised tissue block in 0.1% bavistin (broad spectrum fungicide) + 0.05% streptocycline solution for 30 minutes at 150 rpm in a rotary shaker followed by rinsing in sterile water. After trimming to 3–4 cm, tissue were treated with 1% Cetrimide for 30 minutes, the external sheaths were removed and finally treated with mercuric chloride (0.1%) for 5 minutes under laminar air flow cabinet. After six-eight rinses with sterile water, the external oxidized tissues were removed and shoot-tips of 10–12 mm with almost half shoot and corm tissues (i.e. explants) were excised aseptically and given a quick dip in ethanol.

For culture initiation, MS medium (Murashige and Skoog 1962) was used with different concentrations of BAP (0-6 mg L^{-1}) either alone or in combination with IAA (0-2 mg L^{-1}). Vertically cut shoot-tips were utilized as explants and cultured on MS semisolid medium. The pH of media was adjusted to 5.85 and gelled with 0.7% Agar prior to autoclaving at 121°C and 1.2 kg cm^{-2} pressure for 30 minutes. Cultures were incubated in growth chambers at day-night temperature varying from 25±2°C and 3000 lux fluorescent light for 16 hours and 8 hours of dark period. After initiation, explants were transferred on multiplication media consisting of BAP (0-8 mg L^{-1}) and IAA (0-2 mg L^{-1}). The data on shoot multiplication was recorded after 4 weeks. When shoots were 4-5 cm long, they were detached from clump and transferred to rooting medium, which consisted of MS (full and half) solidified with 0.7% agar and supplemented with IAA (0, 0.5, 1.0 mg L^{-1}).

The data taken from the effect of PGRs on different parameters of shoot induction, multiplication and rooting were analyzed by Completely Randomized Design (CRD) as suggested by Panse and Sukhatme (1967). The shoot induction media consisted of 12 treatments with three replications and observations were recorded on 8 explants per replication. Multiplication media consisted of 15 treatments with 6 replications containing single explants per replication. The multiplication rate of explants was calculated as the ratio of shoot number at the end of subculture (4 weeks) to the initial number of shoots. The data on rooting media was recorded for 6 treatments consisting of 6 replications with 10 shoots per replication. Mean and SE (standard error) were calculated and differences between means were tested using Duncan's Multiple Range Test (DMRT) at the level of p=0.05. Arc sin transformation was used for the data expressed as percentage.

When shoot-tips were cultured on MS medium without plant growth regulators, only 12.50 per cent explants responded towards *in vitro* shoot formation (Table 1). Maximum shoot initiation (87.50%) was recorded with 6 mg L^{-1} BAP + 2 mg L^{-1} IAA which was at par with 6 mg L^{-1} BAP + 1 mg L^{-1} IAA (83.33%). The results indicated that BAP either alone or in combination with IAA significantly increased per cent shoot initiation as compared to control. This may be due to the combined effects of cytokinin and auxin which increased shoot elongation with cell division and enlargement. BAP alone at higher concentration (6 mg L^{-1}) showed reduced per cent shoot induction but when IAA was added to the same concentration of BAP, very high percentage of shoot induction was observed which showed the positive effect of IAA on shoot elongation.

Addition of BAP and IAA either singly or in combination decreased the time required for shoot initiation (Table 1). MS media without growth regulators (control) recorded maximum number of days to shoot initiation (57 days). When MS media is supplemented with BAP and IAA, early shoot induction (31.66 days) was recorded with 6 mg L^{-1} BAP + 2 mg L^{-1} IAA which was at par with 6 mg L^{-1} BAP + 1 mg L^{-1} IAA (32.66 days). The reduction in shoot induction time with higher BAP and IAA levels in the media may be due to the higher endogenous hormonal levels leading to increased rate of cell multiplication and elongation. In conclusion, the best combination for shoot initiation was found to be 6 mg L^{-1} BAP + 1 mg L^{-1} IAA since a lower concentration of growth regulators is always beneficial over higher concentrations to avoid morphological abnormalities in the explants. Hwang *et al.* (1984) and Zaffari *et al*. (2000) also suggested combination of cytokinin and auxin for banana culture initiation. Vuylsteke (1989) suggested use of low level of BAP in shoot induction media for controlling the phenolic secretions which is a common problem in banana micropropagation.

Table 1. Influence of different concentration of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) on *in vitro* shoot initiation per cent and days to shoot initiation of banana cv. Dwarf Cavendish.

Growth regulator concentration (mg L^{-1}) BAP	IAA	Shoot initation (%)	Days to shoot initiation
0	0	12.50 (20.70)^{h}	57.00^{a}
2	0	29.16 (32.68)^{fg}	50.66^{b}
4	0	41.66 (40.20)^{de}	47.00^{c}
6	0	33.33 (35.26)^{ef}	43.33^{de}
0	1	20.83 (27.16)^{gh}	50.66^{b}
2	1	45.83 (42.61)^{cde}	43.66^{cde}
4	1	58.33 (49.80)^{bc}	39.66^{f}
6	1	83.33 (65.91)^{a}	32.66^{i}
0	2	20.83 (27.16)^{gh}	45.33^{cd}
2	2	54.16 (47.39)^{bcd}	37.00^{g}
4	2	66.66 (54.74)^{b}	35.00^{h}
6	2	87.50 (69.30)^{a}	31.66^{i}
Mean		46.17 (42.81)	42.80
SD		15.41	7.69
SE		2.57	1.28
CV (%)		9.94	2.49

Means within the column followed by different letters are significantly different according to Duncan's multiple range test ($p=0.05$).
Values in parentheses are *arc sin* transformation.

Shoot multiplication with the addition of cytokinin is noticed with a concomitant suppression of shoot length. Also, a moderate concentration of BAP increased the shoot proliferation rate, but very high concentrations decreased multiplication and depressed shoot elongation (Fig. 1A, B & C). However, with the addition of auxin in the media, shoot elongation was noticed. The number of shoots per explant increased when MS medium was fortified with BAP either singly or in combination with IAA. Maximum shoot proliferation (6.83 shoots/explant) was recorded at BAP (6 mg L^{-1}) + IAA (1 mg L^{-1}). Thus, a combination of BAP (6 mg L^{-1}) + IAA (1 mg L^{-1}) was found to be optimum for maximum shoot

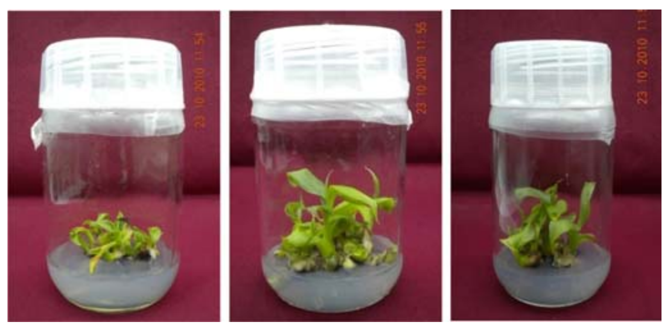

Fig. 1A. Shoot multiplication at 4 mgL^{-1} BAP and different levels of IAA (0, 1, 2 mg L^{-1}).

Fig. 1B. Shoot multiplication at 6 mg L^{-1} BAP and different levels of IAA (0, 1, 2 mg L^{-1}).

Fig. 1C. Shoot multiplication at 8 mg L^{-1} BAP and different levels of IAA (0, 1, 2 mg L^{-1}).

proliferation. Similar proliferation behavior with BAP in banana was observed by Arinaitwe *et al.* (2000) during *in vitro* multiplication of 'Ndiziwemiti' and 'Kibuzi' as the rate of multiplication increased with increase in BAP concentration up to 16 µM (3.6 mg L^{-1}) beyond which it declined suddenly. Madhulatha *et al.* (2004) reported that higher levels of growth regulator in the media cause necrosis and reduction in shoot formation during *in vitro* multiplication of 'Nendran'. Similarly, Muhammad *et al.* (2007) reported the saturation concentration of BAP for micropropagation of 'Basrai' to be 6 mg L^{-1} beyond which, necrotic shoots develop and multiplication rate decreased drastically.

During *in vitro* multiplication, shoot length varied according to different media composition. On the plain MS medium, the average shoot length was 1.91 cm (Table 2). Interaction effects of BAP and IAA resulted in maximum shoot length (5.95 cm) in MS medium fortified with 4 mg L^{-1} BAP + 2 mg L^{-1} IAA followed by 4 mg L^{-1} BAP + 1 mg L^{-1} IAA (5.78 cm). Higher levels of BAP (6-8 mg L^{-1}) showed lower shoot length either alone or in combination with IAA. It was observed that at higher concentration of BAP, number of shoots were more with relatively less shoot length. The reason may be the suppression of apical dominance at higher cytokinin levels. Madhulatha *et al.* (2004) reported similar negative correlation between shoot number and length during *in vitro* propagation of 'Nendran'.

During *in vitro* multiplication, it was observed that a lower cytokinin:auxin ratio favours root formation. Shoots were in cluster when multiplied so they were separated from clusters before being transferred to rooting medium. Significantly higher rooting per cent (100 per cent) was observed in full and half strength MS media at 1 mg L^{-1} IAA (Table 3). The rooting per cent was recorded minimum (15%) when shoots were cultured in full strength MS media while 65 per cent rooting was observed at half strength MS media (Fig. 2). IAA showed significant increase in per cent rooting with increasing concentrations regardless of media strength. Cronauer and Krikorian (1984) and Wong (1986) reported similar findings of increased rooting percentage in banana with addition of a weak auxin like NAA.

Table 2. Influence of different concentration of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) on *in vitro* shoot multiplication and shoot length of banana cv. Dwarf Cavendish.

Growth regulator concentration (mg L^{-1}) BAP	IAA	No. of shoots per explant	Shoot length (cm)
0	0	1.0^{g}	1.91^{k}
2	0	2.50^{f}	3.26^{gh}
4	0	3.33^{e}	3.73^{f}
6	0	4.83^{c}	3.60^{fg}
8	0	2.16^{f}	2.31^{i}
0	1	1.00^{g}	3.61^{fg}
2	1	2.66^{f}	4.80^{e}
4	1	4.33^{d}	5.78^{ab}
6	1	6.83^{a}	4.73^{e}
8	1	2.66^{f}	2.86^{hi}
0	2	1.00^{g}	4.81^{e}
2	2	2.16^{f}	5.36^{bcd}
4	2	3.16^{e}	5.95^{a}
6	2	5.33^{b}	5.43^{bc}
8	2	3.66^{e}	3.16^{hi}
Mean		3.11	4.09
SD		1.679	1.27
SE		0.177	0.13
CV (%)		13.72	8.27

Means within the column followed by different letters are significantly different according to Duncan's multiple range test ($p=0.05$).

The time required for root initiation was minimum (6.5 days) in half strength MS media + 1 mg L^{-1} IAA followed by full strength MS media + 1 mg L^{-1} IAA (8.6

Table 3. Influence of MS media strength and indole-3-acetic acid (IAA) on *in vitro* rooting, days to rooting and number of roots per micro-shoot in banana cv. Dwarf Cavendish.

MS media strength	IAA (mg L^{-1})	Rooting (%)	Days to rooting	No. of roots per micro-shoot
Full	0	15.00 (22.79)^{e}	21.33^{a}	2.16^{d}
Half	0	65.00 (53.73)^{c}	15.83^{b}	4.16^{c}
Full	0.5	56.66 (48.83)^{d}	12.83^{c}	4.50^{c}
Half	0.5	90.00 (71.57)^{b}	10.50^{d}	6.50^{b}
Full	1	100 (90.01)^{a}	8.66^{e}	7.83^{a}
Half	1	100 (90.01)^{a}	6.50^{f}	8.66^{a}
Mean		71.11 (57.49)	12.61	5.63
SD		24.46	5.04	2.39
SE		4.07	0.84	0.39
CV (%)		4.09	7.56	14.05

Means within the column followed by different letters are significantly different according to Duncan's multiple range test ($p=0.05$).
Values in parentheses are *arc sin* transformation.

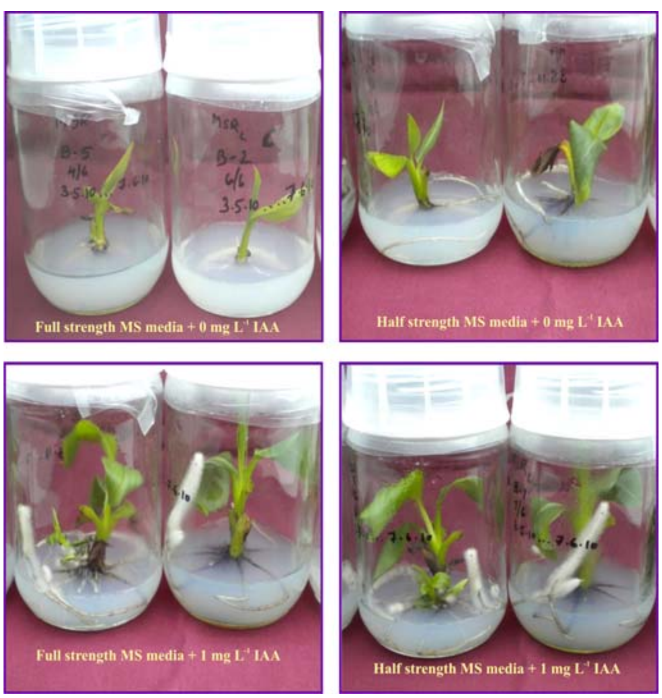

Fig. 2. *In vitro* rooting in banana plantlets.

days). Addition of IAA in the media significantly reduced the days to rooting irrespective of the media strength. Though, maximum number of roots was observed with half as well as full strength MS media + 1 mg L^{-1} IAA (8.66 and 7.83 respectively).

From this study it has been found that there is an optimum concentration of BAP when used alone in the media for shoot multiplication. BAP concentration above optimal level does not increase the shoot proliferation rate and in fact shoot proliferation rate can decrease. But when BAP was supplemented with IAA, the shoot multiplication rate increased on the same optimum concentration of BAP (when used alone in the media) which may be due to increased endogenous levels of hormones in explants. A higher multiplication rate with concomitant suppression of shoot length was also observed in this study. On the basis of the findings of present investigation for culture establishment and multiplication 6 mg L^{-1} BAP + 1 mg L^{-1} IAA was found to be the best combination showing maximum shoot induction as well as multiplication. For rooting, half strength MS media + IAA (1 mg L^{-1}) was found to be effective for maximum rooting, reduction in days to root induction and number of roots per micro-shoot.

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