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In vitro micropropagation of banana *cv.* Poovan (AAB)

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Abstract

In vitro micropagation of banana is nowadays pinned towards development of disease free clones. An efficient protocol has been developed for micropropgation of banana *cv.* Poovan by using shoot tip as explant. The explants were cultured on Murashige and Skoog (MS) medium containing different concentrations of benzyl amino purine (BAP) and thidiazuron (TDZ) for the development of shoots and inodole butyric acid (IBA) for root induction. MS medium supplemented with TDZ was found to be effective for shoot multiplication than MS medium supplemented with BAP. The highest average number of shoots (7.1) for each explant was found in MS medium containing 1.0 mg L^{-1} TDZ, while, the maximum of five shoots were produced per explants in MS medium containing BAP (3 mg L^{-1}). The result of this study showed that the maximum multiplication of shoots (8) was obtained in MS medium containing BAP (3 mg L^{-1}) and TDZ (0.5 mg L^{-1}) with four successive subcultures. Shoot elongation was found to be the best in MS medium containing GA_3 (0.4 mg L^{-1}). The well-developed shoots were transferred to the rooting media after three to four subcultures. More number of roots were produced in the medium having IBA (1.0 mg L^{-1}). Rooted plantlets were successfully transferred to plastic pots containing autoclaved garden soil, farmyard manure and sand (2:1:1) for hardening. Regenerated plantlets successfully established in field and showed morphological characters identical to mother plants with success rate of 90 per cent. These findings suggested that the protocol might be used for commercial production of disease free Poovan clones through micropropagation.

Key words*:* Banana, benzyl amino purine (BAP), indole butyric acid (IBA), Poovan, shoot tip, thidiazuron (TDZ)

Introduction

Banana is one of the important commercial fruit crops grown in India and Tamil Nadu is one of the largest producer and exporter of banana in India. The edible parts of banana are rich in carbohydrates, minerals, potassium, phosphorus and other nutritional elements (Ashok Kumar *et al.,* 2018). Banana and plantains are traditionally propagated through sword suckers. The conventional propagules are not the ideal planting materials because they carry insect pest and disease causing pathogens. Micropropagation is an alternative and viable technology to produce and supply quality planting materials to the banana growing farmers without any pest and diseases.

Among different banana cultivars and varieties, Poovan is an important cultivar of banana grown for fruit purpose in southern states of India especially in the Tamil Nadu state. The fruits are fairly larger in size and are bright glossy yellow in appearance (Rao, 2014). Poovan banana fruits are normally recommended for children and age old peoples for their easy digestion and energy. The plant is highly susceptible to panama wilt and leaf spot diseases (Dita *et al.,* 2018). Therefore, in the present situation, requirement of large numbers of quality planting materials for local banana cultivar, is need of the hour. There are a number of published studies describing successful *in vitro* regeneration protocols for *Musa* spp. by using shoot tip and other explants (Wong *et al.,* 1986; Drew *et al.,* 1989; Ko *et al.,*1991; Pandey *et al.*, 1993; Sudhavani and Reddy, 1997; Kotecha and Kadam, 1998; Nandwani *et al.*, 2000; Malik *et al.*, 2000; Rahman *et al.*, 2002; Martin *et al.*, 2006; Khaldun *et al.*, 2007; Farhani *et al.*, 2008; Srivastava *et al.*, 2012; Rahman *et al.*, 2013; Suman *et al.*, 2013; Ngomuo *et al.*, 2014; Manjula *et al.*, 2014; Ferdous *et al.*, 2015; Lakshmi *et al.*, 2019; Selvakumkar and Parasurama, 2020). However, only a few studies have been reported about micropropagation technique of banana *cv.* Poovan (AAB) (Nair *et al.,* 2017). Hence, the present study aimed to formulate and standardize the micropropagation technique in banana *cv.* Poovan (AAB) for the benefit of farmers.

In addition, the objective of this study was also to test the efficiency of TDZ either as sole cytokinin or in combination with the frequently used cytokinin as BAP for shoot regeneration of banana *cv.,* Poovan.

Materials and methods

Explant collection and surface sterilization: The present investigation was carried out at Plant Tissue Culture Laboratory of Agricultural College and Research Institute, Tamil Nadu Agricultural University, Eachangkottai, Thanjavur, Tamil Nadu during May 2016 to September 2019. Rhizomes were collected from disease free banana field in Karamanithoppu village and the collected suckers were washed with running tap water to remove adhering soil residues. The trimmed suckers were soaked in 1 per cent Bavistin (fungicide) solution for 30 minutes. The older leaves and the outer leaves were trimmed off carefully by unwhorling of leaf sheath and a small portion of rhizome. The trimming was continued till the shoot tip measured 4.0 cm long with a rhizome length of 3.0 cm and width of 2.5 cm. The trimmed rhizome buds were stored for 15 minutes in antioxidant solution containing ascorbic acid 100 mg $L^{-1}$and citric acid 150 mg L^{-1}. The pre-treatment was done to overcome the problems of phenol

exudation and resultant browning. The pre-treated explants were surface sterilized with 70 % ethanol for 30 seconds and 2 % sodium hyphochlorite for seven minutes. The explants were washed several times with sterilized distilled water to remove the traces of sterilants (Fig. 1a). The explants were finally trimmed to remove the outermost whole of tissue exposed to sterilants (Fig. 1b). A vertical cut was given to arrest the apical dominance and to induce adventitious buds (Fig. 1c).

Culture initiation and multiple shoot induction: The sterilized individual explants were inoculated in MS (Murashige and Skoog, 1962) medium containing different concentration of BAP (1, 2, 3, 4 and 5 mg L^{-1}) either alone or in combination with Thidiazuron (0.5, 1.0, 1.5, 2, 2.5 and 3 mg L^{-1}) with 3 % sucrose and 4 g L^{-1} phytagel at 25 ºC under a 16 h photoperiod. The inoculated culture materials were kept in culture room at relative humidity of 80 % (Fig. 1d).The experiment was carried out in three replications with ten explants for each replication. Four to five sub cultures were carried out every 3 weeks interval. Number of shoots was counted after every subculture. The stunted shoots developed from TDZ medium were transferred to shoot elongation medium containing GA_3 at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg L^{-1}) for two weeks. The experiment for each treatment was replicated three times.

Root induction and hardening: Multiple shoots were separated from the cluster and inoculated into MS medium supplemented with different concentration of IBA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}) for two to four weeks. Rooted plantlets were successfully transferred to plastic pot containing autoclaved garden soil, farmyard manure and sand (2:1:1) for primary hardening of one month. After one month primary hardening, well developed plantlets were transferred to polythene bag containing 1:1 ratio of soil and FYM for 45 to 60 days.

Data collection and analysis: Four traits *viz.,* number of days required for shoot initiation, shoot numbers per explant, root number per plantlet and survival rate were recorded after specific interval of time. The collected data was analyzed by using statistical significance two ways analysis of variance (ANOVA). The significant differences ($P \leq 0.05$) between individual treatment means were determined applying Duncan's multiple range test (DMRT).

Results and discussion

Effects of cytokinins for shoot initiation and multiplication of shoots: In the present investigation, explant was inoculated in MS medium containing different concentration of BAP and TDZ for multiple shoot induction. There were five to eight multiple shoots observed from each explant in different media combinations after 6-8 weeks in multiplication medium. The step was repeated 3-5 times to get required number of shoots. Among different concentrations of BAP used for culture initiation and multiplication, the MS medium containing BAP 3 mg L^{-1} favoured for higher multiplication rate (5.1/explant; Table 1). In the recent published report, Selvakumkar and Parasurama, (2020) found the MS medium containing lower concentration (2 mg L^{-1}) of BAP with IAA (1 mg L^{-1}) was the most favourable concentration for shoot regeneration in banana cultivars, Grand Naine and Elakki.

Among different concentrations of TDZ, the MS medium containing TDZ 1.0 mg L^{-1} induced higher multiplication rate (7.1 shoots/explants) than any other concentration tried for micropropagtion. The MS medium containing TDZ (0.5 mg L^{-1}) and BAP (3 mg L^{-1}) favoured higher multiple shoot initiation (8 shoots/explant; Fig.1i) than other combinations tried for micropropgation (Table 1).

Table 1. Effect of BAP and TDZ on shoot regeneration from shoot tip explants

MS medium composition		Number of shoots regenerated from explants
BAP (mg L^{-1})	TDZ (mg L^{-1})	
0	0	0
1	0	1.6±0.23h
2	0	3.5±0.37f
3	0	5.1±0.33d
4	0	4.3±0.23e
5	0	3.5±0.24f
0	0.5	4.1±0.18e
0	1.0	7.1±0.33b
0	1.5	4.0±0.23e
0	2.0	4.0±0.18e
0	2.5	2.6±0.23g
0	3.0	2.0±0.36g
3.0	0.5	8.0±0.48a
3.0	1.0	6.3±0.36c
3.0	1.5	6.3±0.23c
3.0	2.0	5.5±0.24d

Means in the same columns followed by different letters are significantly different ($P \leq 0.05$) with DMRT.

Among two cytokinins tested for multiple shoot induction, TDZ containing medium was found slightly better for the shoot multiplication rate than BAP. In our study, the MS medium containing BAP (3 mg L^{-1}) and TDZ (0.5 mg L^{-1}) favoured for enhancing the multiplication rate in banana *cv.* poovan. The effect of BAP was better than other cytokinins for shoot initiation and multiplication as reported in different banana cultivars (Arinaitwe *et al.*, 2000; Rahman *et al.*, 2004; Roels *et al.*, 2005; Resmi and Nair, 2007; Nair *et al.* 2008; Farahani *et al.*, 2008; Sholi *et al.*, 2009; Buah *et al.*, 2010; Ahmed *et al.* 2014). In contrary, the lower concentration of BA was found to favor for robust growth in Poovan cultivar (Nair *et al.,* 2017). However, Michael *et al.* (2008) found that the higher concentration of BAP lead to some inhibitor effect for multiplication of shoots in banana. The use of TDZ in any concentration might produce abnormal and dwarf shoots in banana (Huetteman and Preece, 1993). This was confirmed by Alvard *et al.* (1993) in different banana cultivars. Similarly, in the present investigation, the plants produced from TDZ medium showed stunted growth. Thus, the plants were transferred to MS medium containing different concentration of GA_3 (Table 2). In contrast to our report, Bhaya and Al-Razzaq Salim (2019) reported the maximum number of shoots produced in lower concentration (0.2 mg L^{-1}) of TDZ in combination with IAA (1.5 mg L^{-1}). Ahmed *et al.* (2014) reported that MS medium supplemented with BAP (4 mg L^{-1}) and IAA (2 mg L^{-1}) would favour for the production of maximum number of multiple shoots in Grand Naine. Similarly, Karule *et al.* (2016) reported that MS based media supplemented with BAP (10 mg L^{-1}) and IAA (1 mg L^{-1}) produced more number of shoots by using shoot tip as explants of Virupakshi banana cultivar.

Sub culturing and shoot elongation: The rhizome buds after 4-6 weeks were subcultured by trimming off the blackened tissues followed by vertically cutting into small pieces. Multiple shoots were elongated on MS medium containing different

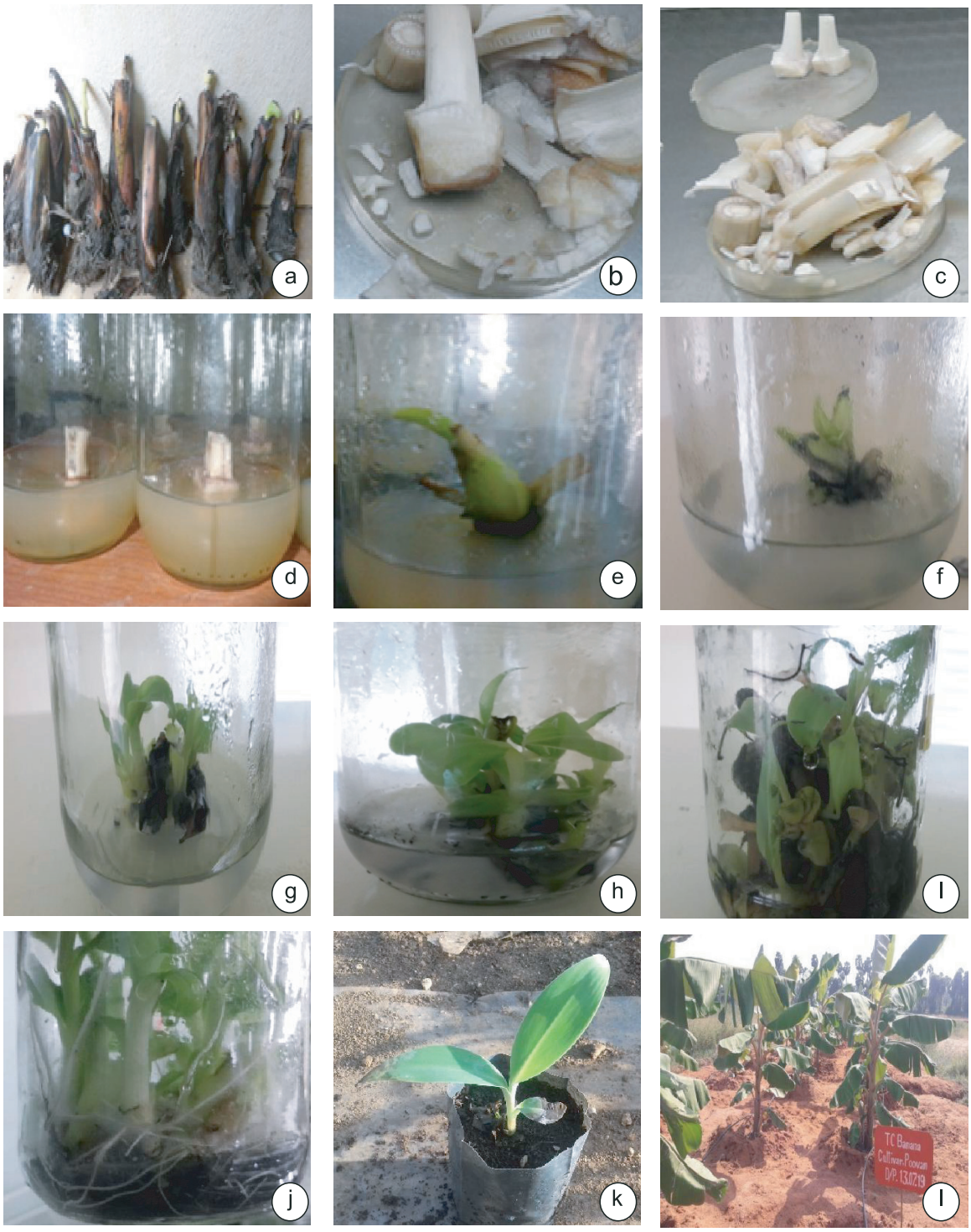

Fig. 1. Stages of *in vitro* micropropagtion of banana *cv.* Poovan. a: Explant, b: Explant preparation, c: Ideal size of explants, d: Culture initiation, e: Greening of culture, f: shoots induction in MS + BAP (3 mg L^{-1}), g: Shoots induction in MS + BAP (3 mg L^{-1}), h: Shoots induction in MS + BAP (3 mg L^{-1}), i: Shoots induction in MS + BAP (3 mg L^{-1}) +TDZ (0.5 mg L^{-1}), j: Rooting in MS+ IBA, k: Primary hardening of plants, l: TC plants grown in field.

concentrations of GA_3. Among different combinations, the MS medium containing GA_3 (0.4 mg L^{-1}) favoured faster shoot elongation (Table 2). In the present investigation, the stunted growth of shoots was observed due to use of TDZ as sole cytokinin and or in combination with BAP. Similarly, Farhani *et al.* (2008) found that the minimal concentration of TDZ favours for shoot induction and simultaneous increase the concentration of TDZ reduced the normal shoot formation and produced abnormal shoots.

***In vitro* rooting:** Root initiation was observed 15 days within the

Table 2. Effect of GA_3 on elongation of shoot length

MS medium composition GA_3 (mg L^{-1})	Shoot length (cm)
0	0
0.2	4.3±0.27c
0.4	5.8±0.27a
0.6	5.2±0.21b
0.8	5.2±0.16b
1.0	4.7±0.27c

Means in the same columns followed by different letters are significantly different ($P \leq 0.05$) on application of Duncan's multiple range test

transfer of shoots onto the MS basal medium. The mean number of 5.8 roots per shoot and root length of 4.6 cm was observed over a period of 4 weeks (Table 3). In the present study, early rooting was observed when the basal media was supplemented with IBA. Good rooting performance was observed in 1.0 mg L^{-1} of IBA (Fig. 1j). Also, De Langhe (1985) used IBA hormone for root induction in different banana cultivars. Nevertheless, Ahmed *et al.* (2014) observed higher rooting in MS medium supplemented with activated charcoal 200 mg L^{-1} and IBA 1.00 mg L^{-1}. In contrast, Selvakumkar and Parasurama (2020) reported that half strength MS medium containing activated charcoal 200 mg L^{-1} and 20 mg L^{-1} adenine sulfate (AS) favoured for rooting in Grand Naine and Elakkia cultivars of banana.

Table 3. Effect of IBA on root regeneration from regenerated shoots

MS medium IBA concentration (mg L^{-1})	Number of roots/ shoot	Root length (cm)
0	0	0
0.5	7.3±0. 16a	5.6± 0.17bc
1.0	7.6 ± 0.28a	6.1± 0.10a
1.5	7.1± 0.17a	5.8.± 0.16ab
2.0	6.5 ± 0.23b	5.6± 0.17bc
2.5	6.7± 0.22b	5.3± 0.16bc
3.0	5.1± 0.22c	5.0 ± 0.16d
Average	5.8	4.6

Means value within same column bearing different letters a, b, c, d are significantly different ($P< 0.05$) on application of Duncan's multiple range test (DMRT)

Hardening and field transfer: Elongated and rooted plantlets were taken out from culture vessels and roots were carefully washed thoroughly with tap water to remove the agar. Rooted plantlets were successfully transferred to plastic pot containing autoclaved garden soil, farmyard manure and sand (2:1:1) for hardening (Fig. 1k). Regenerated plantlets successfully established in field and showed similar morphological traits identical to mother plants with a success rate of 90 per cent (Fig.1l).

The present study showed that maximum multiplication of shoots (8) was observed in MS medium containing BAP (3 mg L^{-1}) and TDZ (0.5 mg L^{-1}). The MS medium containing GA_3 (0.4 mg L^{-1}) and IBA (1 mg L^{-1}) performed excellent shoot elongation and higher number of roots, respectively. MS media with increasing concentration of TDZ had produced increased number of shoots, but significantly reduced elongation of shoots. The present investigation also demonstrated that, the separate use of TDZ or BAP was not sufficient to produce more number of plants in Poovan as compared to combination of higher concentration of BAP and lower concentration of TDZ. Furthermore, 90 percent success rate was observed for establishment of regenerated plantlets in field condition. These findings implied that the protocol is highly helpful for commercial production of Poovan cultivar through micropropagation for the benefit of the farming community.

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