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RESEARCH ARTICLE

PACLOBUTRAZOL MEDIATED ENHANCED MULTIPLICATION OF *MUSA PARADISIACA* LINN. CV. POOVAN (AAB).

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Abstract

An efficient micropropagation system using shoot tip has been developed for *Musa paradisiaca* cv. Poovan. Among the growth regulators tested, N⁶-Benzyl adenine (BA) was more effective for inducing multiple shoots. Basal medium supplemented with 5mg L⁻¹ BA showed 7shoots/explant with an average shoot growth of 4.1 cm in 20 days. Effect of paclobutrazol was investigated to study its influence on shoot growth and multiplication in 'Poovan'. Paclobutrazol (3 mg L⁻¹) along with BA (5 mg L⁻¹) reduced maximum longitudinal growth of shoots to average 1.62 cm. However, 5 mg L⁻¹ BA and 1 mg L⁻¹ paclobutrazol treated cultures achieved substantial growth reduction (2.14 cm) and enhanced the rate of multiplication to 8.2 shoots/ explant in 20 days. The shoots gained normal growth when subcultured onto paclobutrazol free medium; hence it is a temporary phenomenon. The shoots rooted in ½ MS + 150 mg L⁻¹ activated charcoal + 30 g L⁻¹ sucrose + 0.7 mg L⁻¹ IBA and they established successfully in the greenhouse condition with 96 % survival. The enhanced production as well as longitudinal growth reduction with the help of PBZ is beneficial for cost effective micropropagation.

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Introduction:-

Musa paradisiaca Linn. monocotyledons perennial herb is considered as the first fruit commodity in the world in terms of production and trade value (FAO STAT database, 2010). Triploid cultivars are the most widely cultivated clones of commerce due to more vigorous growth and higher yield than diploids (Ray, 2002). There are many varieties of banana popular to Kerala; South India and 'Poovan' (Silk-AAB) is one among them. The variety is locally known as 'Poovan' in Kerala, 'Rasthali' in Tamil Nadu and 'Rasabale' in Karnataka. The plant has moderately vigorous and robust growth with crop duration of 15 to 16 months. The fruit is very sweet with pleasant apple flavour. The plant is highly susceptible to infection by *Fusarium oxysporum* (Rao, 2005) hence cultivation of the variety is shrinking in Kerala. The major constraints in the 'Poovan' banana production system are the non-availability of disease free planting material. Micropropagated plants are uniform, free from pests and disease and obtainable in large quantities (Chadha, 2003). Shoot tip explant has been widely employed for mass multiplication

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of banana cultivars such as 'Dwarf Cavendish' (Srivastava, *et al.*, 2012), 'Ambalacadali' (Mukundakumar *et al.*, 2011), 'Nendran' (Radhika *et al.*, 2016).

A common problem of banana tissue culture is the excessive pseudostem growth, which can reach up to the bottle cap in short period, demanding more space inside the culture vessel. Hence frequent subcultures are required. Moreover, aseptic handling of overgrown culture is very difficult. A growth retardant has been used along with cytokinins to boost bud development and to reduce shoot growth *in vitro* (Albany *et al.*, 2005; Daquinta *et al.*, 2001). Enhancing the shoot production and reducing the culture growth are of great importance in mass multiplication of banana. In the present paper we report the influence of various plant growth regulators (PGRs) and Paclobutrazol (PBZ) on *in vitro* multiplication of the banana Poovan.

Materials and Methods:-

Infection free and healthy sword suckers of *Musa cv.* Poovan were collected from Vakayar, Pathanamthitta, Kerala, and brought to lab for experimental purpose. They were washed thoroughly with running tap water for about 10 minutes to remove adhering soil. Extraneous sucker tissues and pseudostem were chopped off so as to get shoot tip with 5 cm above and 3 cm below. These shoot tip blocks were dipped in 5% bleach (sodium hypochlorite solution - NICE). Then the shoot tip explants were trimmed to about 5 cm and soaked in 0.4% labolene (Qualigens - India) and 5% bleach for 45 min. They were further trimmed to 3-4 cm before treating with a mixture of antibiotics containing streptomycin (Ambistryn) 600 mg L⁻¹ and cefotaxime sodium (Taxim) 500 mg L⁻¹ for three hours. Such shoot apices were sterilized with 15% bleach for 15 min followed by 0.1% mercuric chloride for 7 min and washed thrice in sterile distilled water. Finally, thin layers (1 mm) of brown tissues were removed from all sides and the shoot apices were inoculated for *in vitro* studies. Shoots regenerated from these cultures were subcultured periodically as mentioned elsewhere for increasing the bud populations.

The MS medium (Murashige and Skoog, 1962) was used as basal medium (BM) for the study. BM was enriched with 3% sucrose, 10% coconut water and different concentrations and combinations of plant growth regulators (PGRs) such as N⁶-Benzyl adenine (BA), Kinetin (KN), Indole-3-Acetic acid (IAA) and α - Naphthalene Acetic Acid (NAA) for multiplication studies (Table-1). However, culture establishment was achieved with the help of BA alone. Effect of PBZ was also tested. All media were adjusted to pH of 5.7 before adding 0.7% agar and autoclaved for 20 min. at 121°C. All cultures were incubated at 25 ± 2°C and 16 hour light period under 2000 lux provided by cool white fluorescent lamps.

Elongated shoots were separated and transferred to ½ MS + activated charcoal (150 mg L⁻¹) medium supplemented with different concentrations of NAA or Indole-3-Butyric Acid (IBA) for inducing rhizogenesis. After 15-20 days rooted plantlets were carefully removed and washed with tap water without damaging the roots. These plantlets were treated with 3% commercial fungicide (Indofil M-45) for 5 min. before planting in disposable tea cup containing garden soil and treated coir pith (3:1) for *in vivo* establishment. New plantlets were kept in semi-shade and high humid greenhouse for initial establishment and transplanted to poly bags containing a mixture of river sand and garden soil (3:1) after 30 days.

Culture responses were observed and recorded periodically. The number of shoots, shoot length, number of roots and root length were recorded and analyzed by single factor analysis of variance and the means were compared using Duncan's Multiple Range Test (DMRT) at p=0.05.

Results:-

Shoot tip explants of 'Poovan' were initially established with 70-80 % survival. Shoot tip explants isolated from suckers were initially cultured on MS basal medium supplemented with BA 6 mg L⁻¹. Explants enlarged and turned to light green at the pseudostem region and brownish at the basal region in 10-15 days. After 15 days, the shoot tip explants were cut longitudinally into two halves to encourage axillary bud proliferation. Each part showed proliferation of existing meristem in 15-20 days. Further dissection into smaller pieces consisting of 1-2 buds resulted in multiple shoot development when treated with the above culture establishment medium. These shoot buds were used as explants for further studies.

Table1:-Effect of growth regulators on shoot multiplication from shoot tip explants of 'Poovan' banana.

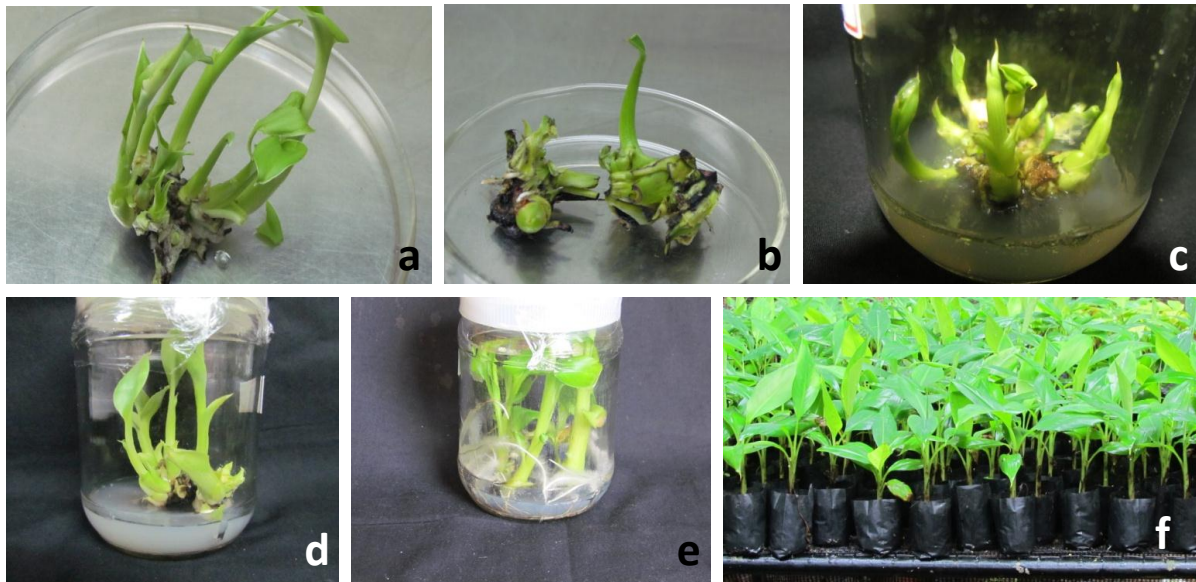
Treatment (mg L ⁻¹)*				Average Shoots per Explant **	Shoot Length (cm)**
BA	KN	IAA	NAA		
3.0				4.1 ± 0.18 ^f	6.7 ± 0.3 ^l
4.0				5.7 ± 0.15 ^{hijkl}	5.9 ± 0.48 ^{kl}
5.0				7.0 ± 0.25 ⁿ	4.1 ± 0.23 ^h
6.0				6.0 ± 0.21 ^{klm}	2.0 ± 0.21 ^a
7.0				5.3 ± 0.21 ^{ghij}	1.9 ± 0.23 ^a
	3.0			0.6 ± 0.16 ^a	1.6 ± 0.45 ^a
	4.0			0.7 ± 0.21 ^a	1.3 ± 0.39 ^a
	5.0			1.4 ± 0.22 ^b	2.2 ± 0.32 ^a
	6.0			1.4 ± 0.22 ^b	1.7 ± 0.30 ^a
4			0.1	3.7 ± 0.21 ^{def}	5.8 ± 0.46 ^g
4			0.5	3.4 ± 0.16 ^{cde}	6.1 ± 0.45 ^{kl}
4			1.0	3.0 ± 0.14 ^c	5.1 ± 0.48 ^{hijk}
4		0.1		4.0 ± 0.25 ^{cf}	5.3 ± 0.47 ^{hijk}
4		0.5		3.8 ± 0.24 ^{def}	5.4 ± 0.61 ^{ijk}
4		1.0		3.3 ± 0.15 ^{cd}	5.0 ± 0.36 ^{hijk}
5			0.1	6.0 ± 0.25 ^{klm}	4.3 ± 0.36 ^{hi}
5			0.5	5.4 ± 0.16 ^{ghijk}	4.7 ± 0.44 ^{hij}
5			1.0	5.2 ± 0.20 ^{ghi}	4.2 ± 0.41 ^{hi}
5		0.1		6.3 ± 0.30 ^{lm}	4.3 ± 0.66 ^{hi}
5		0.5		6.1 ± 0.23 ^{klm}	4.2 ± 0.32 ^{hi}
5		1.0		5.8 ± 0.20 ^{hijklm}	4.1 ± 0.48 ^h
6			0.1	6.2 ± 0.20 ^{lm}	2.3 ± 0.30 ^g
6			0.5	5.1 ± 0.23 ^{gh}	2.6 ± 0.26 ^g
6			1.0	4.9 ± 0.27 ^g	2.4 ± 0.22 ^g
6		0.1		6.5 ± 0.16 ^{mn}	2.1 ± 0.23 ^a
6		0.5		5.9 ± 0.23 ^{klmi}	2.4 ± 0.16 ^g
6		1.0		5.7 ± 0.30 ^{hijkl}	2.2 ± 0.24 ^a

*Basal medium: MS +30 g L⁻¹ sucrose + 10% coconut water + 7g L⁻¹ agar and pH 5.7. Data were collected after 20 days of culture. **Average values of 10 replicates and data were expressed as mean ± SE. Means within column having different letters are significantly different according to DMRT at the 0.05 level of probability.

Shoot bud explants of 'Poovan' showed organogenic responses in all the PGR treatments irrespective of its concentration or combination in 10-14 days. Out of the two cytokinins tested, BA was most efficient for *in vitro* shoot production (Table-1). The existing buds of the explants were started growing initially while new buds sprouted from their basal portion.

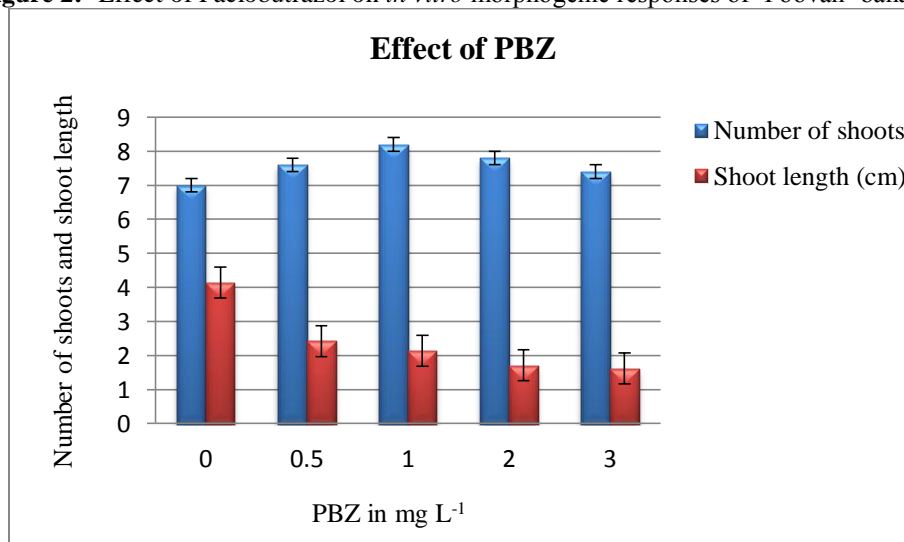
Out of 5 concentrations of BA tested, 5 mg L⁻¹ yielded the best multiplication of average 7.0 shoots per explants (Fig.1a). Treatments above and below this level were less effective in terms of shoot production. Leaf development was substantial and noticed 1-3 leaves in most of the BA supplemented media. The rate of multiplication was 5.3 and 4.1 shoots/explant when cultured on 7 mg L⁻¹ and 3 mg L⁻¹ BA respectively (Table-1). Compared to BA the shoot production was less in KN supplemented medium. So that maximum 1.4 shoots/ explant was obtained at 5-6 mg L⁻¹ concentrations (Fig.1b). Moreover, elongation of all the shoots were either delayed or not achieved. For examining further enhancement of multiplication, auxins (IAA and NAA) were tested in combination with BA. Though multiple shoots were developed in presence of different combination of auxins with BA, their presence did not improve the rate of multiplication. Nevertheless, inclusion of auxins

Figure 1 (a-f): *In vitro* propagation of *Musa paradisiaca* cv. Poovan using shoot tip explant (a) Shoot multiplication in MS + BA 5 mg L⁻¹ after 3 weeks, (b) Shoot development in MS + 5 mg L⁻¹ KN, (c) Culture showing enhanced shoot production with reduced shoot size on MS + 5 mg L⁻¹ BA + 1 mg L⁻¹ PBZ, (d) Culture regaining elongation when transferred from PBZ medium to PBZ free medium, (e) Shoots developing root on ½ MS + 0.7 mg L⁻¹ IBA, (f) Micro plants of 'Poovan' banana establishing in greenhouse conditions after 45 days.



Resulted reduced multiplication rate (Table-1). Treatments of IAA + BA combinations yielded more buds compared to NAA + BA. In the present study growth of *in vitro* shoots was also varied according to different PGRs tested. Maximum stimulatory effect on shoot elongation has been observed in BA at 3 mg L⁻¹ and attained an average length of 6.7 cm in 20 days. Shoot elongation is inversely proportional to the concentration of BA tested. Shoot length was significantly less in the presence of KN where shoots attained a maximum growth of average 2.2 cm. Combination of IAA or NAA along with BA did not improve shoot elongation. Growth responses were better in BA and NAA combinations than in BA and IAA and obtained average 6.1 cm and 5.4 cm long shoots respectively.

Figure 2:- Effect of Paclobutrazol on *in vitro* morphogenic responses of 'Poovan' banana after 20 days.



Basal medium: MS + 5 mg L⁻¹ BA + 30 g L⁻¹ sucrose + 10% coconut water + 7g L⁻¹ agar and pH 5.7. Vertical bars are mean ± SE.

It was also observed that best multiplication media simultaneously supported significant shoot growth and leaf development which hamper speedy aseptic transfer during subsequent subculture. Hence, the effect of PBZ on shoot growth has been investigated. The addition of PBZ (0.5-3 mg L⁻¹) to the multiplication medium (BA 5 mg L⁻¹) resulted in reduced shoot elongation invariably in all the media tested and gave rise to compact shoots compared to the PBZ free medium. The study demonstrated that *in vitro* shoot elongation in 'Poovan' was inversely proportional to the concentration of PBZ tested (Fig. 2), thus, suitable for multiplication programme. PBZ decreased the shoot growth substantially to 2.42 and 1.62 cm when treated with 0.5 mg L⁻¹ and 3 mg L⁻¹ respectively. It has also been

observed that PBZ enriched medium always enhanced shoot production and achieved the best rate of production of 8.2 shoots/explants when the multiplication medium was supplemented with 1 mg L⁻¹ PBZ (Fig. 1c). The shoots from these cultures regained normal growth upon subculture onto PBZ free multiplication medium (Fig. 1d).

Table2:- Effect of growth regulators on root induction from shoots of 'Poovan' banana.

Treatment (mg L ⁻¹)*		Average No. of Roots (± SE)**	Root Length (in cm)**
IBA	NAA		
0.3		5.6 ± 0.40 ^a	5.6 ± 0.50 ^{ab}
0.5		6.6 ± 1.02 ^{ab}	5.8 ± 0.58 ^b
0.7		8.4 ± 1.07 ^b	6.2 ± 0.58 ^b
1.0		6.6 ± 0.24 ^{ab}	5.4 ± 1.02 ^{ab}
	0.3	5.2 ± 0.48 ^a	3.8 ± 0.37 ^a
	0.5	5.4 ± 0.81 ^a	4.8 ± 0.37 ^{ab}
	0.7	5.6 ± 0.50 ^a	4.6 ± 0.40 ^{ab}
	1.0	6.2 ± 0.37 ^a	5.0 ± 0.31 ^{ab}

*Basal medium: ½ MS + 30 g L⁻¹ sucrose + 150 mg L⁻¹ activated charcoal + 7g L⁻¹ agar and pH 5.7 **Data collected after 15 days and the values are mean ± SE., Means within column having different letters are significantly different according to DMRT at the 0.05 level of probability.

Healthy shoots of 4-5 cm size with 2-3 leaves were isolated individually from the bunch and transferred to rooting media containing IBA or NAA (Table-2). All the shoots were found rooted in the above treatments, irrespective of PGRs and concentration tested. Out of the treatments, IBA 0.7 mg L⁻¹ was found to be the best for root induction followed by 0.5 and 1 mg L⁻¹. Rhizogenic response of NAA was not promising in 'Poovan'. The best rooting responses of average of 8.4 roots and a root growth of 6.2 cm were recorded in half strength MS medium supplemented with 0.7 mg L⁻¹ IBA + 3% sucrose and 150 mg L⁻¹ activated charcoal (Fig. 1e) in 15 days. Rooted plants were carefully removed from the bottle and were washed thoroughly in running tap water to remove traces of agar. Then each shoots were planted separately in small perforated disposable tea cups containing river sand and kept in a high humid (70-85% RH) semi-shade greenhouse. After the emergence of new leaf, these plantlets were repotted in small polythene bags for further hardening. About 96 % of the plantlets survived and established into 15-20 cm size in 35-45 days (Fig. 1f).

Discussion:-

The present study revealed that the regenerative potential of shoot tip of *Musa* cv. Poovan has been well expressed as all the explants proliferated in most of the media tested. Shoot tip explant has been identified as the preferred material for micropropagation for a wide range of banana cultivars such as 'Mehersagar' (Rahman *et al.*, 2006), 'Basrai' (Muhammad *et al.*, 2007), 'Virupakshi' (Karule *et al.*, 2016). However, immature inflorescence tip has also been employed for micropropagation in different cultivars (Resmi and Nair, 2007; Mahadevet *et al.*, 2011). Selective plant growth regulators are used to enhance *in vitro* shoot formation and they can successfully change the growth pattern of plant cultures too (George and Sherrington, 1984). BA and KN are known to suppress the apical dominance and induce both axillary and adventitious shoot formation from meristematic explants in banana (Madhulatha, *et al.*, 2004). The current study showed that 5 mg L⁻¹ BA was optimum for shoot multiplication. Similar hormonal requirement has been found suitable for better multiplication in many banana cultivars such as, 'Philippine Lacatan', 'Grande Naine', 'Saba', 'Pelipita' (Cronauer and Krikorian, 1984), 'BARI-I' (Rahman *et al.*, 2004), 'Shrimanti' (Bhosale *et al.*, 2011) 'Nendran' (Radhika *et al.*, 2016), etc. However, the requirement of growth regulator concentration for optimum shoot multiplication varies according to cultivars. In 'Ardhapuri' and 'Basrai' 7 mg L⁻¹ BA was found optimal (Bhosale *et al.*, 2011) while Muhammad *et al.* (2007) noted 4 mg L⁻¹ for better results. The current multiplication response indicated that BA performed better than KN in 'Poovan'. Similarly potential effect of BA over KN has been reported in other cultivars (Wong, 1986; Rahman *et al.*, 2006; Mukunthakumar *et al.*, 2010). The observation in Poovan revealed that combinations of BA with auxins declined the multiplication and shoot growth. Contrary to the results, combinations of BA + NAA (Gebeyehu, 2013; Al-Amin *et al.*, 2009) and BA + IAA (Gubbuk and Pekmezci, 2004; Iqbal *et al.*, 2013) have been reported beneficial for enhanced production in different *Musa* cultivars. The present investigation demonstrated a superior effect of BA for *in vitro* multiplication of *Musa* cv. Poovan and addition of other PGRs such as KN, IAA and NAA have negative effect on culture performance.

Since best multiplication medium has shown significant culture growth within a culture period of 20 days, aseptic handling became difficult during subsequent subcultures. In order to achieve fast aseptic operations, cultures with reduced shoot size are preferred. Influence of PBZ, a plant growth retardant (Snir, 1988) reduced shoot growth of cv. Poovan *in vitro*. PBZ is a widely used growth retardant for field and potted plants. It can cause a kind of dwarfism (PGRSA, 2007) due to considerable depletion in the active GAs (Gibberellins) levels throughout the plant (Rademacher, 2000). It helped the development of dark green leaves (Jiachuan *et al.*, 1999). Ribeiro *et al.*, (2011) reported that growth retarding activity of PBZ is stable even after autoclaving. In the present study, significant decrease of shoot size in PBZ supplemented media was helpful to avoid overcrowding of shoots inside the culture vessels. Twenty days old cultures exhibited about 60% drop in shoot elongation in 3 mg L⁻¹ PBZ supplemented multiplication medium. Moreover, the study also revealed that optimum usage of this growth retardant (1 mg L⁻¹) slightly enhanced the shoot production along with considerable reduction of shoot growth to 50%. Similar findings were reported previously, where PBZ application reduced longitudinal growth of shoots in 'Grand Nain' (Albany *et al.*, 2005), 'Williams' (Jiachuan *et al.*, 1999). PBZ also supported enhanced bud production and here we report 17% increase in the rate of production. Similar to our observation, enhanced shoot multiplication and reduced shoot growth have been reported in banana 'FHIA-18' in presence of PBZ and BA (Daquinta *et al.*, 2001). Identical observations were reported in Cavendish Banana, but in PBZ and Thidiazuron supplemented medium (Lee, 2005). The report is beneficial for mass production by reducing culture duration and improving aseptic transfer rate. IBA was found to be more useful for *in vitro* rooting of 'Poovan' as reported by Haridasanand Caldas, (1989); Rahman *et al.*, (2006); Dore-swamy *et al.*, (1983); Molla *et al.*, (2004) and Muhammad *et al.*, (2007). This is also evident in the present study. On the other hand, Waman *et al.*, (2016) observed best rooting on 2 mg L⁻¹NAA, while IAA performed good rooting in the cultivar 'Williams' (Iqbal *et al.*, 2013).

Conclusion:-

The study showed that BA alone was effective for better multiple shoot development than addition of PBZ which enhanced the multiplication further and reduced the longitudinal shoot growth significantly in *Musa* cv. Poovan. The enhanced shoot multiplication achieved with the help of PBZ is an added benefit that the total culture duration can be reduced while longitudinal growth reduction helps the operators to improve the transfer rate, which keeps the production cost less. It also facilitates to reduce the rate of contamination during transfer operations.

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