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Genetics and Plant Breeding

Morphological Abnormality among Hardened Shoots of Banana cv. Rajapuri (AAB) after *in vitro* Multiplication with TDZ and BAP from Excised Shoot Tips

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Abstract

To compare the effect of 6-benzylaminopurine (BAP) and thidiazuron (TDZ) on morphological abnormal plants or per cent variant plantlets during secondary hardening period. Shoot tips of Musa spp. were cultured on MS medium supplemented with different concentrations (2.0 and 5.0 mg/L) of BAP and (0.2 and 0.3 mg/L) of TDZ and NAA 0.2 mg/L. Wherever, TDZ is present in the medium some morphological dwarf plants were observed. The maximum plant height (16.17 cm) and number of leaves (4.20) was in T_2. Where, the maximum shoot diameter (4.97 mm), number of primary roots (4.53) and length of longest root (11.17 cm) was in T_1. TDZ at 0.2 mg/L it increases the morphological abnormal plants like dwarf plants (20.00%). In conclusion BAP at 5.0 mg/L, TDZ at 0.2 mg/L and NAA at 0.2 mg/L and BAP at 2.0 mg/L, TDZ at 0.2 mg/L and NAA at 0.2 mg/L were assumed to be the most suitable for commercial micropropagation system with low frequency of abnormal shoot production for local banana cultivars.

Highlights

- BAP is the best for healthy, thinner and lengthy shoots.
- Dwarfness is the major abnormality during hardening where ever TDZ is used.
- Rooting (90-95%) was successfully obtained in NAA medium and survival of plantlets (70-75%) during hardening.

Keywords: Morphological abnormal plants, cytokinins, micropropagation, *Musa spp.*, shoot tip.

Banana (Musa spp.) is the most important fruit as a staple food source for about 400 million people in developing countries and it is the fourth most important food crop in the world as well as in India (Ganapathi *et al.,* 1999). The area under banana is increasing rapidly owing to free from insect pests and diseases and its adaptability to wide range of soil and climatic conditions. Banana ranks first in production and second in area among the fruit crops grown in India with production of 29,780 thousand MT annually from an area of 830 thousand hectares. In Karnataka, it is grown in an area of 111.8 thousand hectares with the production of 2,281.6 thousand MT. The productivity (65.8 t/ha) is highest in Tamil Nadu (Anon, 2011).

In vitro regeneration in banana can be achieved through shoot tip culture as a direct organogenesis (Kulkarni *et al.,* 2007). Different kinds of cytokinins have been used for micropropagation of banana cultivars (Arinaitwe *et al.,* 2000; Roels *et al.,* 2005) and shoot proliferation rate is significantly affected by cytokinin types, their concentrations and type of banana cultivars (Arinaitwe *et al.,* 2000; Gubbuk and Pekmezci, 2004; Roels *et al.,* 2005). However, Huetteman and Preece (1993) stated that thidiazuron (TDZ) may inhibit shoot elongation. The main undesirable side effect of TDZ is abnormal shoot production and dwarf plants (Huetteman and Preece, 1993). Michael *et al.,* (2008) reported that BAP at higher concentration was an inhibitor based on the abnormality index recorded in banana. Farahani *et al.,* (2008) reported that with high concentrations of TDZ the number of normal shoots were reduced and abnormal shoots were observed. The objective of the present investigation was to study about the effect of cytokinins on plant growth and morphologically abnormal plants in local banana cultivar during hardening and through determining suitable concentration of both cytokinins (BAP and TDZ) for production of normal plants with high shoot proliferation.

Materials and Methods

Planting Material: Sword suckers from the elite mother plant, cut from the pseudostem 15 cm above the base level, weighing 500-1500 g and 3-4 months old suckers were used as starting material for micropropagation.

Explant Disinfection: Sword suckers were thoroughly washed in running tap water and rinsed in soap water solution. Older leaves and extraneous sucker tissues were carefully chopped off with a stainless steel knife. From such trimmed suckers, soaked in 1 per cent Bavistin (fungicide) solution for 30 minutes. Again wash the suckers and trimmed and soaked in 1 per cent Bavistin (fungicide) and 0.05 per cent streptocycline (bactericide) solution for 8 hours, shoot tips, containing several sheathing leaf bases and enclosing the axillary buds with underlying sucker tissue, measuring 2.5-3.5 cm in length were isolated. These shoot tips were soaked in 0.05 per cent cetrimide (bactericide) solution for 20-30 minutes, surface-sterilized with mercuric chloride at 0.01 per cent, in an aseptic laminar air flow chamber for further 10 minutes and finally traces of chlorine were removed by repeated washings with sterile distilled water.

Explants Preparation: From the sterilized shoot tips, explants were prepared using sterilized stainless steel scalpels. The explants measuring 0.5-1.0 cm were aseptically inoculated into a sterile MS basal liquid medium in test tubes and preserved in a clean growth room optimized for 250C, 2000 lux light and 60 per cent RH.

Induction of Growth: It was induced in 4 weeks at 25±20C, with 16 hours photo-period and 40-60 per cent RH in growth room, illuminated by cool, white fluorescent lamps (4', 40 Watts, emitting 30-50 µ E.m2sec-1 light intensity), when explants swelled up, turned green, showing intense morphogenetic activity for sub-culturing in the multiplication medium.

Multiple shoot proliferation and Rooting: The multiplication medium comprised of BAP (2.0 and 5.0 mg/L), TDZ (0.2 and 0.3 mg/L) and NAA (0.2 mg/L), sucrose (30 g/L) agar-agar (4.0 g/L). In 4-8 weeks of sub culturing, the explants enhanced axillary bud proliferation, the appearance of tiny, creamy, white protuberances, which were progenitors of multiple shoots and three sub cultures were taken at monthly interval. At the end of third sub culture of multiple shoot generation cycles, individual shoot lets were carefully excised and transferred to rooting medium containing indole 3-butyric acid (IBA) (2 mg/L) and charcoal (4.0 g/L) for inducing rooting within a two weeks. Thus, fully differentiated in vitro plantlets were ready for primary hardening.

Primary Hardening: The plantlets, placed in pro-trays containing coco peat mix as a growth matrix, were allowed to harden in poly tunnel under shade house for 30 days.

Secondary Hardening: The plantlets from primary hardening, placed in 18 × 15 cm black polythene bags containing 1:1 ratio of soil and FYM as a growth matrix, were allowed to harden in shade house for 30-45 days.

Table 1. Effect of cytokinins and their combinations on growth parameters after secondary hardening.

Treatments	Plant height (cm)	No of leaves/ plant	Shoot diameter (mm)
T_1 -	15.53	4.47	4.97
T_2 -	16.17	4.80	4.79
T_3 -	14.07	3.73	4.64
T_4 -	13.67	4.20	4.19
T_5 -	13.17	4.07	3.77
T_6 -	14.03	3.93	4.01
T_7 -	15.57	4.27	4.12
T_8 -	13.57	4.07	4.21
T_9 -	13.50	4.53	4.16
T10 -	13.00	3.93	3.85
Mean	14.23	4.20	4.27
SEm±	0.50	0.08	0.11
CD(P=0.01)	2.04	0.34	0.46

Table 2. Effect of cytokinins and their combinations on root parameters after secondary hardening.

Treatments	No. of primary roots	No. of secondary roots	Length of longest root (cm)	Diameter of root (mm)
T_1 -	4.53	23.20	11.17	1.27
T_2 -	4.40	22.73	11.08	1.24
T_3 -	4.27	22.00	9.88	1.23
T_4 -	4.33	22.60	8.57	1.24
T_5 -	4.00	23.40	10.69	1.24
T_6 -	3.87	22.93	8.19	1.24
T_7 -	3.93	23.67	10.67	1.29
T_8 -	4.13	23.13	8.57	1.26
T_9 -	3.53	21.80	9.29	1.18
T10 -	3.73	21.33	8.31	1.22
Mean	4.07	22.67	9.64	1.24
SEm±	0.14	0.82	0.29	0.02
CD(P=0.01)	0.58	NS	1.19	NS

NS- Non significant

Table 3. Effect of cytokinins and their combinations on per cent variant plantlets after secondary hardening.

Treatments	Per cent of variant plantlets/Abnormal plants		
	Dwarfness (%)	**Leaf colour (%)**	**Pseudostem pigmentation (%)**
T_1 -	0.00 (0.00)*	-	-
T_2 -	0.00 (0.00)	-	-
T_3 -	6.67 (14.96)	-	-
T_4 -	11.11 (19.47)	-	-
T_5 -	6.67 (14.96)	-	-
T_6 -	6.67 (14.96)	-	-
T_7 -	6.67 (14.96)	-	-
T_8 -	6.67 (14.96)	-	-
T_9 -	20.00 (26.56)	-	-
T10 -	13.33 (21.42)	-	-
Mean	7.78	-	-
SEm±	0.35	-	-
CD(P=0.01)	1.41	-	-

*The values given in parenthesis are arc sine transformed values (Sin-1√X/100)

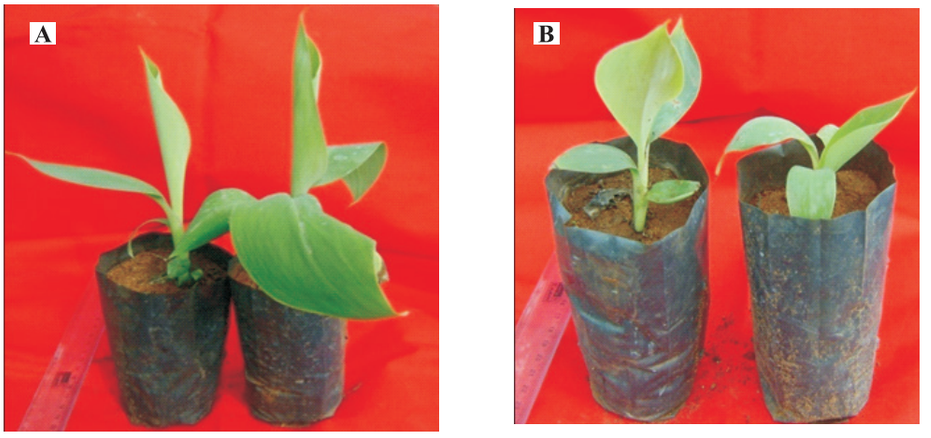

Fig. 1. Morphological abnormal plants caused by TDZ
(A) Normal plants after secondary hardening.
(B) Morphologically dwarf plants.

Identification of off types or morphological abnormal plants: The plantlets which appeared varying from normal were segregated at the end of growth phase during secondary hardening.

Treatment details

T_1-	BAP 2.0 mg/L + NAA 0.2 mg/L
T_2-	BAP 5.0 mg/L + NAA 0.2 mg/L
T_3-	TDZ 0.2 mg/L + NAA 0.2 mg/L
T_4-	TDZ 0.3 mg/L + NAA 0.2 mg/L
T_5-	BAP 2.0 mg/L + TDZ 0.2 mg/L + NAA 0.2 mg/L
T_6-	BAP 2.0 mg/L + TDZ 0.3 mg/L + NAA 0.2 mg/L
T_7-	BAP 5.0 mg/L + TDZ 0.2 mg/L + NAA 0.2 mg/L
T_8-	BAP 5.0 mg/L + TDZ 0.3 mg/L + NAA 0.2 mg/L
T_9-	TDZ 0.2 mg/L
T_{10}-	TDZ 0.3 mg/L

Results and Discussion

Growth and root parameters: The maximum plant height (16.17 cm) was recorded in T_2 which was statistically on par with the treatment T_7 and T_1 (15.57 and 15.53 cm). The significantly maximum number of leaves were recorded in T_2 (4.80) and this was on par with the treatment T9 (4.53) and T_1 (4.47). The highest mean shoot diameter (4.97 mm) was recorded in T_1 and this was on par with T_2 (4.79 mm) and T_3 (4.64 mm) in Table 1. It may be due to the higher competition for reserved food for shoot length development and also leads to formation of thinner shoots. (Gubbuk and Pekmezci, 2004; Youmbi *et al.,* 2006; Rahaman *et al.,* 2004 and Lee, 2005) who observed decreased shoot thickness with increase in concentration of TDZ.

The maximum number of primary roots (4.53) and the significant highest mean length of longest root (11.17 cm) were recorded in T_1 and there were no significant differences between number of secondary roots and root diameter (Table 2). This is because of the plant height in the treatments T_1. It may be due to the presence of the BAP in the treatment it increases the number of primary roots as well as root length.

Per cent variant or morphologically abnormal plants: The types of cytokinins and their combinations significantly influenced on morphological abnormal plants. Critical observations were made in each treatment for traits like dwarfness, deviation or change in leaf colour and pseudostem pigmentation.

The highest per cent variant or abnormal plants showing dwarfness was recorded in the plantlets obtained from the media supplemented with TDZ 0.2 mg/L (20.00%) followed by TDZ 0.3 mg/L (13.33%) and TDZ 0.3 mg/L + NAA 0.2 mg/L (11.11%) (Fig 1 A & B). There were no plantlets showing variations with respect to leaf colour and pigmentation irrespective of treatment in the entire experiment (Table 3). In banana, off-types can be visually detected during acclimatization in the green house before transplanting to the field (Rodrigues *et al.,* 1998; Lee, 2005; Roels *et al.,* 2005 and Michael *et al.,* 2006).

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