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Effect of TDZ on Direct Shoot Regeneration from Whole Male Inflorescence of Four Diploid Banana Cultivars from South India

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Abstract

Male inflorescence has great potential to be used as explants for rapid micropropagation of *Musa* species. In this work whole inflorescence was used as the explant for the propagation. The male flower of four diploid banana cultivars namely *Musa acuminata* cv. Matti and *M. acuminata* cv. Sannachenkadali, *M. acuminata* cv. Chingan and *Musa acuminata* cv.Njalipoovan (2n= 22) belonging to diploid genome types (AA and AB) were selected for the present study. These diploid tolerant cultivars grown in south Indian states are in great demand because of their fruit quality and consumer preference. Direct shoot regeneration was achieved from whole male inflorescence which is inoculated in MS medium supplemented with varying concentrations of BA, TDZ and KIN. All the four cultivars produced the maximum number of shoots in the presence of varying concentrations of TDZ (0.45µM -13.5 µM) irrespective of their genotypes. Male inflorescence reduces the rate of contamination compared to suckers and produces a more number of shoots in culture conditions.

Keywords: diploid banana, TDZ, male inflorescence

1. Introduction

Bananas and plantains (*Musa* spp.) are among the most important fruit crops of the world and are the staple food for millions across the globe (FAO, 2010). Conventional breeding is difficult in edible *Musa* species due to differing levels of ploidy and sterility (Stover & Simmonds, 1987). A large number of banana genotypes need to be screened for commercial micropropagation and genetic improvement. In bananas and plantains different types of explants have been used to regenerate plants. These include shoot tip (Kulkarni, Suprasanna, Ganapathi, Bapat, & Rao, 2004; Kulkarni, Suprasanna, & Bapat, 2006), zygotic embryos (Cronauer-Mitra & Krikorian, 1988; Escalant & Teisson, 1989; Marroquin, Paduscheck, Escalant, & Teisson, 1993; Navarro, Escobedo, & Mayo, 1997), proliferating meristems and scalps (Cronauer & Krikorian, 1983), female flowers (Grapin, Schwendiman, & Teisson, 1996) and male flowers (Resmi & Nair, 2007). Of these explants immature male flowers appear to be the most responsive starting material for initiating direct shoot regeneration. The main advantage of using male inflorescence is that it specifically reduces the rate of contamination during micro propagation as compared to suckers. Moreover, *in vitro* culture of inflorescence apices offers an opportunity to select a male bud with desirable characteristics such as a greater number of hands and fruit per bunch in situ (Resmi & Nair, 2007). Therefore the male inflorescence culture can help to increase the efficiency of

micropropagation as well as produce plantlets, parts which could be lost during harvesting. The present study assesses the effect of genotypes and plant growth regulators, interaction between these two factors on the direct shoot regeneration from whole male inflorescence under *in vitro* conditions during successive sub-culture of four diploid banana cultivars.

2. Materials and Methods

Male flower bunches were obtained from adult field grown banana 25-30 days after inflorescence emission. Inflorescences were collected from 10 month old *Musa acuminata* cultivars namely Matti, Sannachenkadali, Chingan and Njalipoovan from the Banana Nursery, Peringammala, Kerala, India. Matti is medium sized diploid cultivar having AA genome, and is characterized by the presence of brown coloured patches on the pseudostem and green fruit. Each finger is small in size with curved tip. Sannachenkadali also having AA genome, characterized by the presence of red to purple coloured hairy peduncle and red to reddish purple fruit, and each finger is curved at its tip. The plant is medium size. Chingan having AB genetic constitution and the plant is tall and pseudostem possess brown patches and entire peduncle is brown in colour and the fruit also possess brown spots. Njalipoovan (AB) is a popular shade loving tolerant variety, having superior fruit quality. It is a small growing cultivar with relatively small bunches and fingers but the fruits are very sweet with thin peel. The bracts with associated hands of male flowers were removed in a step wise manner until it became too small to be removed by hand. The remaining portion having an approximate size of 6 - 7 cm length was immersed in 1% (v/v) labolene (Qualigens, India) for 6 min and kept under running tap water for 30 min. The explants were surface sterilized in 0.1% (w/v) mercuric chloride for 4 min followed by three rinses in autoclaved double - distilled water, (~5 min for each rinse). Two or three outer protective bracts and corresponding group of male flowers were sequentially removed. Explant extraction continued along the rachis towards the bunch tip. The last excised hand correspond to a size of approximately 5 cm length, 2-3cm width were inoculated on MS medium (Murashige & Skoog, 1962) supplemented with TDZ (0.45-13.5 µM), BA (2.2 - 22.00 µM) and KIN (2.3 - 23.00 µM) for shoot multiplication. The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 18 min. The primary explants were transferred to fresh media every 3 weeks until the formation of shoot buds. The cultures were maintained at a temperature of 25± 2ºC with a photoperiod of 16h/day under 50µ mol m-2s-1 light intensity provided by fluorescent lamps. The experiment was repeated thrice using different concentrations of TDZ (0.45-13.5 µM), BA (2.2 - 22.00 µM) and KIN (2.3 -23.00 µM) to determine the optimum concentration for plant regeneration. The individual shoots of 2cm length were separated and transferred to MS basal medium and kept three weeks for rooting and elongation of shoots. After three weeks rooted plantlets of ~ 5cm were transferred to plastic cups filled with vermiculite and hardened in the culture room conditions at 25±2°C for three weeks and then the plantlets were successfully transferred to pots containing soli compost (2:1) mixture and kept in green house condition for 3- 4 weeks at 30- 32°C. Then the plantlets were transferred to pits in the filed. The regenerated plants show 80% survival rate. The experiments were set up in culture jars. The data were collected weekly based on the number of induced shoot buds. Statistical analysis was performed with the software SPSS/PC Version 7.0 (SPSS Inc., Chicago, USA). Both the mean values and standard errors are shown based on the one way ANOVA of eight replicates per treatment. Mean and SE were calculated and differences between means were tested using Duncan's Multiple range test at the level of ($p<0.05$). Cryotome (Leica CM, 1100) sections (12µm thickness) of male flower with newly induced shoot buds were taken in order to understand the region of shoot bud induction and were stained with 1% safranin. The photographs were taken with the help of image analyzer (Olympus BX51).

3. Results

In bananas and plantains different types of explants such as shoot tip, zygotic embryos, bract of male inflorescence were generally used for the regeneration of plantlets. However, this is the first report that reports the efficiency of whole male inflorescence to regenerate plantlets. The basal portion of all the explants was expanded and they became green around 8-10 days after inoculation. The basal bract meristem of male inflorescence responded to plant growth hormones through the induction of small white shoot buds which became visible after 4 weeks in two diploid cultivars having AA genome (cv.'Matti', (Figure.1), cv.'Sannachenkadali') and 6- 8weeks in the remaining two cultivars having a AB genome (cv.Njalipoovan and cv.'Chingan').The emerging shoot buds were pale yellow or off white in colour (Figure.2). Histological analysis of the shoot bud induced explant also showed the evidence of number of small shoot buds arising from the basal region of explant (Figure.3). The explants which were cultured on the basal MS medium without cytokinin did not show any positive response or development of shoot buds while basal region of the explants turned pale yellow or green colour. After 40 days MS medium supplemented with 3.6 µM TDZ produced average of (37.75±0.45) shoot buds from the cv. Matti (Figure.4) where as 4.5 µM TDZ produced an average of (29.25±0.52), (22.12±0.47) shoot buds in cv. Sannachenkadali, cv. Chingan respectively. In the case of cv. Njalipoovan 0.45 µM TDZ produced an average of (15.00±0.37) shoot buds. The results of shoot bud induction response to different equimolar concentration of BA, TDZ and KIN were shown in Table1. Among the three different cytokinins used, TDZ had the highest effects in inducing the shoot buds in all four diploid cultivars irrespective of their genotypes. The shoot buds developed in the optimum concentration of TDZ in all the cultivars showed an increasing rate of multiplication after subculture in the same hormonal concentrations (Figure. 5). It was also observed that the explants with 4-5cm length exhibited good response (Table 2). The maximum response (80.55 %) was recorded in the medium supplemented with 4.5 µM TDZ with an explant size of 5cm in cv. Sannachenkadali. After the subculture the shoot bud approximately 2 cm height were used for micropropagation and placed in basal MS medium for rooting. Healthy roots were produced from these shoot buds within one week in basal MS medium. These plantlets were kept in same medium for three weeks. After attaining a height of 5cm they were transferred to vermiculite (Figure.6) and kept in culture room condition for three weeks. These plantlets were then transferred to greenhouse condition for three to four weeks and then transplanted to field conditions (Figure.7) with 80% survival rate. The regenerated plants showed no somaclonal variation both *in vitro* and field conditions.

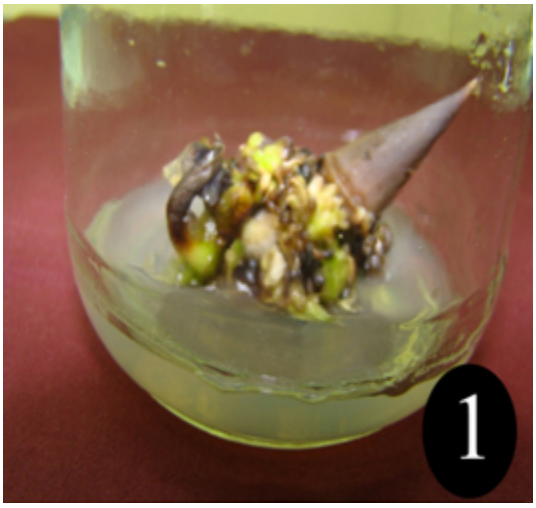

Figure 1. Direct shoot regeneration from male inflorescence of cv.Matti

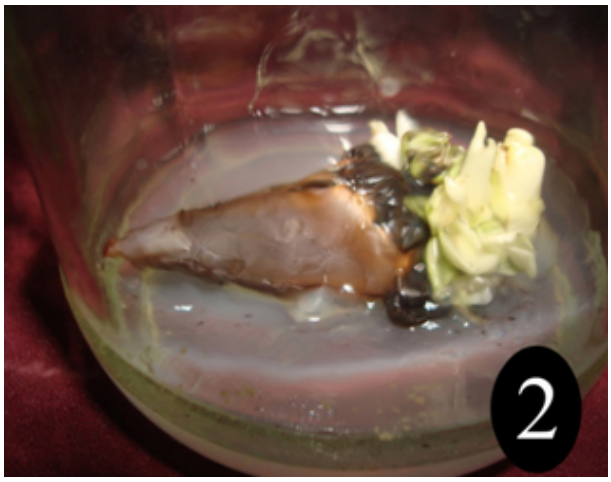

Figure 2. Colour of induced shoot bud turned off white in colour

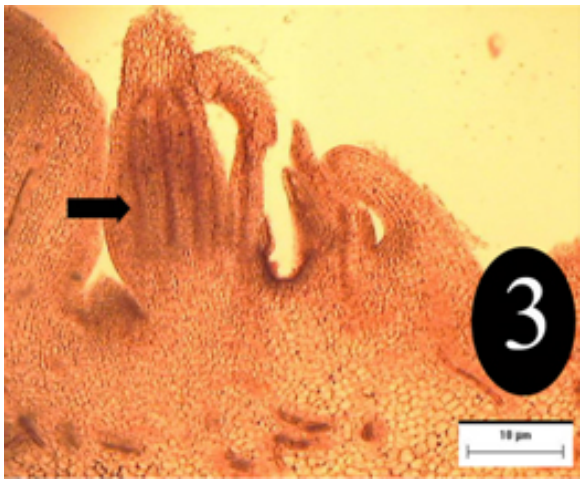

Figure 3. Histology of shoot buds induced from basal bract meristem of explant

Figure 4. After 40 days MS medium supplemented with 3.6 µM TDZ produced maximum number of shoot bud from the cv. Matti

Figure 5. Shoot bud formation after first subculture (Matti)

Figure 5. Shoot bud formation after first subculture (Matti)

Figure 6. Hardened plants in vermiculite (Matti),Fig 7: Plant grown in field condition (Matti)

Table I. Influence of different hormone concentration for multiple shoot proliferation from inflorescence of four diploid banana cultivars

Hormone concentration (μM)			Average number of shoot/ explants after 40 days			
KIN	TDZ	BA	MA	SK	CH	NJ
2.3	-	-	15.63±0.59^{f}	9.63±0.53^{ghi}	10.25±0.45^{gh}	5.62±0.74^{ij}
4.6	-	-	13.00±0.53^{hi}	10.25±0.59^{gh}	12.00±0.75^{efg}	5.12±0.78^{k}
9.2	-	-	15.25±0.64^{fg}	12.38±0.37^{ef}	10.75±0.83^{gh}	5.50±0.50^{ij}
13.8	-	-	15.25±0.41^{fg}	16.63±0.59^{ed}	12.75±0.64^{ef}	7.00 ±0.37^{hi}
18.4	-	-	14.00±0.53^{gh}	13.63±0.53^{e}	16.00±0.65^{bc}	10.75±0.36^{bc}
23.00	-	-	10.63±0.75^{j}	8.00±0.18^{f}	13.25±0.36^{def}	8.50±0.50^{efgh}
-	0.45	-	21.00±0.32^{d}	17.75±1.50^{c}	12.00±0.00^{efg}	15.00±0.37^{a}
-	2.25	-	25.00±0.53^{c}	22.50±0.73^{b}	11.50±0.32^{fgh}	11.25±0.36b^{c}
-	3.6	-	37.75±0.45^{a}	21.00±0.37^{b}	9.75±0.45^{hi}	9.87±0.54b^{c}
-	4.5	-	27.25±0.64^{b}	29.25±0.52^{a}	22.12±0.47^{a}	10.25±1.06b^{c}
-	9.00	-	11.63±0.26^{ij}	15.75±0.59^{hi}	17.00±0.37^{b}	9.37±0.65^{cdefg}
-	13.5	-	14.75±0.49^{fgh}	9.38±0.59^{hi}	13.75±0.59^{de}	8.37.±0.37^{c}
-	-	2.2	14.29±0.52^{gh}	12.71±0.35^{ef}	8.50±0.32^{i}	8.00±0.49^{c}
-	-	4.4	17.78±0.70^{e}	10.78±0.46^{gh}	11.50±0.32^{fgh}	10.25±1.06^{bcd}
-	-	8.8	16.50±0.62^{ef}	17.25±0.36^{ed}	12.00±0.53^{efg}	11.50±0.90^{b}
-	-	13.2	16.50±0.62^{ef}	16.63±0.98^{ed}	13.50±0.37^{de}	9.50±0.73^{c}
-	-	17.6	14.00±0.53^{gh}	11.50±0.48^{fg}	14.75±0.52^{cd}	9.37±0.49^{c}
-	-	22.0	10.75±0.81^{h}	9.63±0.53^{ghi}	9.75±1.03^{hi}	7.50±0.32g^{h}

Note: Significance was determined by ANOVA: significant at P<0.05

SE=Standard Error; Data represent the mean of eight replications

MA-Matti, **SK**-Sannachenkadali, **CH**-Chingan and **NJ**-Njalipoovan

Table 2. Effect of explant size on shoot initiation in diploid *Musa acuminata* cultivars on MS medium supplemented with TDZ

Musa acuminata Cultivar	Explant length (cm)	TDZ (μM)	Percentage of response after 8 weeks
Matti	2	0.45	56.50 ± 2.55^{fghi}
	3	2.25	48.55 ± 5.15^{jkl}
	4	3.6	52.94 ± 3.28^{hij}
	5	4.5	72.88 ± 1.72^{bc}
	6	9.00	49.00 ± 2.43^{jkl}
	7	13.5	62.55 ± 1.72^{ef}
Sannachenkadali	2	0.45	41.50 ± 1.56^{m}
	3	2.25	$56.72\pm2.58f^{ghi}$
	4	3.6	69.83 ± 1.08^{cd}
	5	4.5	80.55 ± 1.49^{a}
	6	9.00	60.33 ± 2.16^{efg}
	7	13.5	43.88 ± 0.49^{lm}
Chingan	2	0.45	44.38 ± 0.69^{klm}
	3	2.25	64.66 ± 2.18^{de}
	4	3.6	77.50 ± 0.78^{ab}
	5	4.5	73.38 ± 1.33^{bc}
	6	9.00	50.77 ± 1.34^{ijk}
	7	13.5	50.50 ± 2.19^{ijk}
Njalipoovan	2	0.45	54.44 ± 0.99^{ghij}
	3	2.25	59.61 ± 1.36^{efg}
	4	3.6	76.22 ± 0.96^{ab}
	5	4.5	76.44 ± 0.90^{ab}
	6	9.00	58.38 ± 2.18^{efgh}
	7	13.5	44.88 ± 2.81^{klm}
	Treatment Df (n-1) = 36		33.534**
	Cultivar Df (n-1) =3		17.03**
	Explant length Df (n-1) = 5		339.33**
	Concentration of TDZ Df (n-1) =5		2.04
	CXEXC Df (n-1) = 143		24.28**

Note: Means with in a column followed by the same letters are not significantly different as determined by DNMRT ($P<0.05$) ** significant at $P<0.01$ and $P<0.05$ level

4. Discussion

To the best of our knowledge it was the first report that addresses the direct shoot regeneration from the whole male inflorescence. The experimental results indicated that the types of cytokinin and their concentrations significantly influenced shoot multiplication. Several studies reported that in banana, shoot multiplication depended on the genotypes of cultivars. Male flowers of banana and plantain have shown their potential as suitable explant for direct regeneration. It was generally accepted that the successful culture of plant material *in vitro* was influenced greatly by the developmental age of the tissue or organ that are used as the source of explants (George, Hall, & De klerk, 2008). Differences in the rate of multiplication of different *Musa* spp. genotypes have been reported earlier (Khatri, Khan, Siddiqui, Ahmed, & Siddiqui, 1997; Vuylsteke 1998; Resmi & Nair, 2007). These previous studies reported the regeneration of plantlets from the bract with associated male inflorescence while in the present study the whole male inflorescence of about 3-5 cm was taken as explant and it was observed that the rate of multiplication was different among the explants of same and different genotypes. The explants with minimal length (4-5cm) exhibited a more progressive response. The explant size which is greater than 5cm showed much lower response compared to the minimal length (Unpublished data). The size of the male inflorescences influenced shoot regeneration response in banana as reported by Darvari, Sariah, Puad, and Maziah (2010). Differences in the mean number of shoot in the same genotype may be due to physiological responses to different explants. The important factors affecting the efficiency of micropropagation system is the rate of multiplication. It has been observed that the banana multiplication rate is genotype dependent as well as variable behavior has been observed among cultures initiated from same genotype (Israeli, Lahav, & Reuveni, 1995; Mendes, Mendes, Neto, Demetrio & Puske, 1996). Apart from the influence of genotypes, shoot proliferation and elongation were affected by cytokinins and their concentration (Strosse, Van den Houwe, & Panis, 2004). Similar to these results, the present study also indicated that the cytokinin and their concentrations significantly influenced the rate of shoot multiplication. TDZ showed maximum shoot initiation in these diploid cultivars. Cytokinins generally reduced the dominance of apical meristems and induced axillary as well as adventitious shoot formation from meristemmatic explants (Madhulatha, Anbalagan, Jayachandran, & Sakthivel, 2004). In the present study among different cytokinins used TDZ showed the greatest effect inducing shoot buds in all the four cultivars. Pérez-Hernández and Rosell-García (2008) obtained the highest multiplication rate of the male inflorescence of Cavendish (AAA) on the medium containing 1mg/L TDZ. In the present study 3.6 µM TDZ was found to be optimum for the cv. ‘ Matti’ and the remaining two cultivars, cv. ‘Chingan’ and cv.’Sannachenkadali’ having AB and AA genome showed optimum shoot multiplication in the presence of 4.5 µM TDZ. Maximum shoot multiplication in the cultivar ‘Njalipoovan’ having AB genome was observed in 0.45 µM TDZ. According to Vuylsteke (1989) each banana cultivar has an optimum concentration for maximum response to proliferation. Arinaitwe, Rubaihayo and Magambo (2000) reported that TDZ was effective against recalcitrant and increased proliferation rates in non-recalcitrant banana cultivars and also stated that shoot proliferation was cultivar dependent. From previous studies and also from the present study it was evident that concentration of TDZ plays a major role in shoot multiplication and elongation in banana shoot cultures. The present investigation also revealed that TDZ was more effective in producing more healthy shoots in *in vitro* propagation than other two cytokinins. Numerous active meristems were directly induced from the inflorescence to form multiple shoots in the presence of cytokinins. Histological analysis of the explants showed direct emergence of shoot buds from the basal portion of the male inflorescence. These bud primordia produced a vegetative regenerative system which developed into small shoot clusters which ultimately develop into plantlets. Thus *in vitro* culture of whole

male inflorescence and can open up new vistas for large scale production of quality planting material.

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