Plant Cell Tiss Organ Cult (2007) 88:333–338
DOI 10.1007/s11240-007-9206-7

ORIGINAL PAPER

# Plantlet production from the male inflorescence tips of *Musa acuminata* cultivars from South India

**L. Resmi · Ashalatha S. Nair**

Received: 1 June 2006 / Accepted: 10 January 2007 / Published online: 3 February 2007
© Springer Science+Business Media B.V. 2007

**Abstract** Inflorescence apices are suitable explants for the rapid in vitro propagation of *Musa* spp. However, the diploid and triploid banana cultivars showed different in vitro responses with respect to the hormone combinations in Murashige and Skoog medium. The diploid cultivar (Sannachenkadali, AA) induced a maximum number of multiple shoots in 8.9 μM 6-benzyl adenine (BA) whereas the triploid cultivar (Red banana, AAA) exhibited maximum multiplication in 22.2 μM 6-benzyl adenine. MS medium supplemented with 11.4 μM indole acetic acid and 17.8 μM BA was also suitable for shoot proliferation in triploid cultivar but not in the diploid cultivar. The regenerated shoots were rooted in Murashige and Skoog basal medium within 10–15 days. The rooted plantlets were transferred to vermiculite and maintained at a temperature of 25 ± 2°C for 10 days and then at room temperature (30–32°C) for 2 weeks before transferring to potted soil compost mixture. The plantlets showed 100% survival.

**Keywords** 6-Benzyl adenine · Apical meristem · In vitro regeneration · Multiple shoots · *Musa acuminata* cv Red · *Musa acuminata* cv Sannachenkadali

L. Resmi · A. S. Nair (✉)
Department of Botany, University of Kerala,
Kariavattom, Kerala 695 581, India
e-mail: ashabot2004@yahoo.co.uk

**Abbreviations**

| | |
|---|---|
| BA | 6-benzyladenine |
| IAA | indole-3-acetic acid |
| 2iP | isopentenyl adenine |
| Kin | kinetin |
| MS | Murashige and Skoog |

Bananas and Plantains are monocotyledonous, perennial herbs, cultivated in more than 130 countries in the tropics and subtropics. They are ranked as 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. India, being the largest producer contributes only 17.30% of global production; because a considerable portion of the total production is consumed domestically. The cultivation for local consumption is based on a large number of cultivars adapted to different conditions of production as well as the varied use and taste of local consumers. Many of the cultivars in Kerala, South India are adapted to domestic cultivation and have unique morphological features as well as fruit qualities. It is recorded that the wealth of banana varieties in the coast of Kerala is unequalled by any other banana growing regions in the world (Nayar 1962). However, the classification of these bananas were difficult as a single variety is sometimes known by different names in different

regions and farmers may identify cultivars in local dialect. A study has been conducted in 36 South Indian cultivars for genome classification using IRAP primers (Nair et al. 2005) and observed a few variations from the original classification. Genome characterization of cultivars facilitates the molecular breeding programmes in banana. Differential effects of genome and cytokinins on in vitro propagation were reported in some Indian banana cultivars (Kulkarni et al. 2004). Considering the genotypic influence on in vitro growth, specific protocols are essential for different banana cultivars.

In vitro propagation of bananas employing the shoot tip culture has been practiced in many of the commercial cultivars (Kulkarni et al. 2004, 2006). The present study exploited the capacity of male inflorescences of bananas to regenerate in culture as suggested by Krikorian et al. (1993). Inflorescence apices were collected from two *Musa acuminata* Colla cultivars, namely Sannachenkadali (2n = 2*x* = 22, AA) and Red banana (2n = 3*x* = 33, AAA) from the Banana Nursery, Peringammala, Kerala, India. The diploid cv. Sannachenkadali is characterized by the presence of red to purple coloured hairy peduncle and red to reddish purple fruits in smaller bunches. The lower surface of the leaves is also coloured. The triploid cv. Red banana is also marked by the red colour of plant parts and fruits. It is a tall, long-duration cultivar with heavy bunches and used in commercial plantation.

The inflorescences were collected from the field; the bracts with associated hands of male flowers were removed in a stepwise manner until they became too small to be removed by hand. The remaining portion having an approximate size of 2–3 cm length was immersed in 1% (v/v) labolene (Qualigens, India) for 5 min and kept under running tap water for 20 min. The explants were surface sterilized in 0.1% (w/v) mercuric chloride for 5 min followed by three rinses in autoclaved double-distilled water, 5 min for each rinse. The tiny bracts along with minute hands of male flowers were removed aseptically without damaging the apical dome and the explants of about 1–5 mm were inoculated on MS medium (Murashige and Skoog 1962) supplemented with plant growth regulators, 30 g $l^{-1}$ sucrose, 0.7 g $l^{-1}$ agar and the pH of the media was adjusted to 5.8 before autoclaving. The cultures were maintained at a temperature of 25 ± 2°C with a photoperiod of 16 h/day under 50 µmol $m^{-2}$ $s^{-1}$ light intensity provided by fluorescent lamps. The subcultures were done within an interval of 30 days and the observations were taken at 60 and 120 days. Then individual shoots of 4–5 cm length were separated and transferred to MS basal medium for adequate rooting and elongation of shoots. Rooted plantlets were transferred to plastic cups filled with vermiculite and hardened in the culture room conditions at 25 ± 2°C for 10 days and at 30–32°C for 2 weeks. Then the plantlets were successfully transferred to pots containing soil compost (2:1) mixture.

Experiments were set up in culture jars with seven replications for each treatment. Number and length of shoots were recorded after 60 and 120 days. Statistical analysis was performed with the software SPSS/PC Version 4.0 (SPSS Inc., Chicago, USA). Mean and SE were calculated and differences between means were tested using Scheffe's Multiple Comparisons at the level of $P = 0.05$. Student's *t*-test revealed the significance of genome variation and multiple shoot production in the two cultivars in different hormone concentrations.

All the explants expanded, became green in colour and tiny shoots developed within 40–50 days. After 60 days, the shoot clusters were subdivided into two pieces and subcultured on the same medium for further multiplication. After 120 days, MS medium supplemented with 8.9 µM BA produced an average of 21.57 ± 0.69 shoots in the diploid cv. Sannachenkadali (Table 1). When the concentration of BA was increased to 22.2 µM, the number of shoots reduced to 18.33 ± 0.32/explant. However, in the triploid cv. Red banana, a significant increase (21.60 ± 0.51 shoots/explant) was observed at 22.2 µM BA. Of the different cytokinins used, Kin showed least effect in the induction of multiple shoots in both the cultivars. MS medium supplemented with 2iP alone at 24.6 µM induced 16.33 ± 0.33 shoots/explant in the diploid cultivar and 15.83 ± 0.31 shoots/explant in the triploid cultivar, respectively. Incorporation of different concentrations of IAA in the medium along with BA decreased

**Table 1** Influence of different hormone concentrations and combinations for multiple shoot proliferation from inflorescence tips of Sannachenkadali and Red banana

| Hormone concentration (μM) | | | | Average number of shoots/explant (±SE) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Sannachenkadali (AA) | | Red banana (AAA) | |
| BA | KIN | 2iP | IAA | After 60 days | After 120 days | After 60 days | After 120 days |
| 8.9 | – | – | – | 9.28 ± 0.61[a] | 21.57 ± 0.69[a] | 3.67 ± 0.33[de**] | 6.17 ± 0.31[f**] |
| 13.3 | – | – | – | 8.71 ± 0.42[ab] | 19.20 ± 0.58[ab] | 6.60 ± 0.51[c*] | 10.60 ± 0.51[cde**] |
| 22.2 | – | – | – | 8.57 ± 0.37[ab] | 18.33 ± 0.32[b] | 13.80 ± 0.37[a**] | 21.60 ± 0.51[a**] |
| 8.9 | 4.7 | – | – | 4.43 ± 0.48[def] | 10.17 ± 0.48[d] | 3.40 ± 0.24[de] | 7.60 ± 0.60[ef*] |
| 8.9 | 13.9 | – | – | 5.71 ± 0.42[cde] | 9.17 ± 0.40[def] | 4.40 ± 0.40[cde] | 7.80 ± 0.58[def] |
| 8.9 | – | 4.9 | – | 6.14 ± 0.40[bcd] | 13.50 ± 0.56[c] | 6.17 ± 0.31[cd] | 11.20 ± 0.58[cd*] |
| 13.3 | – | 4.9 | – | 4.86 ± 0.55[cdef] | 9.17 ± 0.31[def] | 6.83 ± 0.48[bc*] | 15.17 ± 0.48[b*] |
| – | – | 24.6 | – | 7.43 ± 0.48[abc] | 16.33 ± 0.33[bc] | 6.80 ± 0.37[bc] | 15.83 ± 0.31[b] |
| – | 23.2 | – | – | 5.29 ± 0.42[cde] | 9.83 ± 0.60[de] | 2.60 ± 0.24[e**] | 6.20 ± 0.37[f**] |
| 8.9 | – | – | 5.7 | 3.71 ± 0.18[def] | 7.40 ± 0.51[def] | 4.67 ± 0.42[cde] | 9.33 ± 0.61[def*] |
| 8.9 | – | – | 11.4 | 3.14 ± 0.26[ef] | 6.80 ± 0.49[efg] | 4.17 ± 0.48[cde] | 9.67 ± 0.67[def**] |
| 17.8 | – | – | 11.4 | 3.86 ± 0.40 [def] | 6.17 ± 0.31[fg] | 13.80 ± 0.37[a**] | 24.60 ± 0.51[a**] |
| 17.8 | – | – | 22.8 | 2.43 ± 0.20[f] | 3.80 ± 0.37[f] | 9.60 ± 0.68[b**] | 13.40 ± 0.51[bc**] |

Means within the column followed by different letters significantly different according to Scheffe's Multiple Comparison Test ($P = 0.05$)

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

Average number of shoots/explant of diploid cultivar after 60 and 120 days has been compared with those of triploid cultivar

* Significant at 5% level; ** Significant at 1% level

the number of shoots in the diploid cultivar. In the triploid cultivar, MS medium supplemented with 17.8 μM BA and 11.4 μM IAA produced an average of 24.60 ± 0.51 shoots per explant with an average of 2.47 ± 0.09 cm length.

BA (8.9 μM) and Kin (4.7 μM) reduced the length of shoots in the diploid cultivar, whereas BA (13.3 μM) and 2iP (4.9 μM) reduced the length of shoots in the triploid cultivar. BA in combination with IAA reduced the length of shoots in both the cultivars (Table 2). Individual plantlets were separated, transferred to MS basal medium and within 10–15 days white, hairy roots developed. Rooted plantlets were transferred to plastic cups filled with vermiculite, covered with transparent plastic bags, maintained at a temperature of 25 ± 2°C for 10 days and then at room temperature (30–32°C) for 2 weeks. The hardened plants showed 100% survival in soil.

Banana is probably the most intensely micropropagated crop commercially (Mendes et al. 1999). However, a large number of banana genotypes still need to be screened for exploring their potential for mass production and genetic improvement. Inflorescence tip explants reduces the contamination rate compared to soil grown suckers. The numerous active meristems of the inflorescence apices will directly be induced to form multiple shoots in culture (Krikorian et al. 1993). These bud primordia produce a vegetative system and give rise to small shoot clusters, which for all practical purposes are identical to those from non-flowering apices. The culture of inflorescence apices offers an opportunity to select in situ, a male bud from a bunch showing elite or highly desirable characteristics (i.e., a greater number of hands and fruits per bunch), thus enabling one to generate a progeny in vitro from the same bud (apex) that would be lost during harvest. Sebastian and Mathew (2004) reported the induction of multiple shoots from the inflorescence tip culture of South Indian triploid cultivars such as Nendran (AAB) and Poovan (AAB). In other Indian triploid cultivars namely Rasthali (AAB), Basrai (AAA), Shreemati (AAA), Lokhandi (AAA) and Trikoni (AAA) normal plant development via somatic embryogenesis was observed from embryogenic cultures derived from male inflorescence apex explants (Ganapathi et al. 1999).

**Table 2** Influence of different hormone concentrations and combinations for length of shoots proliferated from inflorescence tips of Sannachenkadali and Red banana

| Hormone concentration (μM) | | | | Average length of shoots (cm) (±SE) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Sannachenkadali (AA) | | Red banana (AAA) | |
| BA | KIN | 2iP | IAA | After 60 days | After 120 days | After 60 days | After 120 days |
| 8.9 | – | – | – | 5.37 ± 0.36[a] | 8.81 ± 0.12[a] | 6.50 ± 0.36 [a] | 8.68 ± 0.25[ab] |
| 13.3 | – | – | – | 4.91 ± 0.15[ab] | 8.41 ± 0.13[ab] | 5.87 ± 0.15[ab] | 8.60 ± 0.25[ab] |
| 22.2 | – | – | – | 4.32 ± 0.24[ab] | 6.51 ± 0.10[c] | 5.22 ± 0.17[b] | 8.20 ± 0.14[ab] |
| 8.9 | 4.7 | – | – | 1.75 ± 0.13[d] | 3.34 ± 0.20[e] | 6.21 ± 0.15[ab] | 7.84 ± 0.24[b] |
| 8.9 | 13.9 | – | – | 4.61 ± 0.19[ab] | 7.56 ± 0.21[bc] | 6.58 ± 0.12[a] | 8.48 ± 0.18[ab] |
| 8.9 | – | 4.9 | – | 3.85 ± 0.11[bc] | 6.72 ± 0.16[c] | 1.26 ± 0.10[cd] | 4.78 ± 0.13[cd] |
| 13.3 | – | 4.9 | – | 3.14 ± 0.08[c] | 5.24 ± 0.11[d] | 1.04 ± 0.08[cd] | 3.58 ± 0.18[def] |
| – | – | 24.6 | – | 4.30 ± 0.16[ab] | 7.95 ± 0.13[ab] | 6.60 ± 0.20[a] | 9.19 ± 0.14[a] |
| – | 23.2 | – | – | 1.06 ± 0.14[d] | 6.62 ± 0.20[c] | 1.88 ± 0.12[c] | 5.60 ± 0.18[c] |
| 8.9 | – | – | 5.7 | 1.04 ± 0.10[d] | 2.62 ± 0.16[e] | 1.01 ± 0.05[cd] | 3.74 ± 0.12[de] |
| 8.9 | – | – | 11.4 | 0.84 ± 0.11[d] | 2.81 ± 0.11[e] | 0.81 ± 0.08[d] | 2.84 ± 0.21[efg] |
| 17.8 | – | – | 11.4 | 0.81 ± 0.07[d] | 2.41 ± 0.16[e] | 0.63 ± 0.05[d] | 2.47 ± 0.09[gh] |
| 17.8 | – | – | 22.8 | 0.65 ± 0.07[a] | 2.34 ± 0.12[e] | 0.61 ± 0.05 [d] | 2.34 ± 0.07[h] |

Means within the column followed by different letters significantly different according to Scheffe's Multiple Comparison Test ($P = 0.05$)

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

Cytokinins are known to reduce the dominance of apical meristems and induce axillary as well as adventitious shoot formation from meristematic explants (Madhulatha et al. 2004). The high performance of BA over the other cytokinins in inducing multiplication in shoot tip cultures has been reported in different cultivars of banana (Wong 1986; Kulkarni et al. 2004; Kumar 2005). In other plants such as *Centella asiatica*, *Kaempheria galanga* and *Bacopa monerria*, important role of BA for stimulation and proliferation of lateral bud growth were reported (Tiwari et al. 2000, 2001; Shirin et al. 2000). Cronauer and Krikorian (1985) obtained multiple shoot clusters from the terminal floral apices of *Musa acuminata* cv. Dwarf Cavendish (AAA), inoculated on modified MS medium supplemented with 22.2 μM BA and 10% (v/v) coconut water. Shoot apices explants also produced similar results (Cronauer and Krikorian 1984; Jarret et al. 1985). Each banana cultivar has an optimum concentration for maximum response, beyond which the proliferation rate cannot be increased or will even decrease (Vuylsteke 1989). The cv. 'Bwara' (AAA) showed significant increase in shoot proliferation rate when the concentration of BA was increased from 16.8 to 28.8 μM (Arinaitwe et al. 2000). In the present study, in vitro response of diploid and triploid banana cultivars was different with respect to the amount of BA in the medium. Lower concentration of BA produced maximum number of shoots in the diploid cultivar whereas higher concentration of BA produced similar response in the triploid cultivar. Suckers of cv. Chingan (AA), and cv. Robusta (AAA) also showed similar results (unpublished data).

Strosse et al. (2004) reported that the rate of shoot multiplication depends both on the cytokinin concentration and the genotype. In the present study, the correlation occurred only in the case of BA. In *M. acuminata* cv Grandnaine (AAA), histological analysis showed high levels of endogenous hormones in the shoot apices inoculated on MS medium supplemented with 2iP than any other cytokinins. But when BA was supplemented with IAA, the endogenous levels of hormones increased in the explants (Zafari et al. 2000). In the present study also BA (17.8 μM) with IAA (11.4 μM) in the medium induced high shoot multiplication in the triploid cv. Red banana. Banerjee and DeLanghe (1985) reported that in banana cultivars shoot multiplication is noticed with a concomitant suppression

of shoot elongation. The type of cytokinin and their concentration influenced shoot multiplication and elongation in banana shoot cultures. Also, a moderate concentration of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation (Gubbuk and Pekmezci 2004). However, in the present study shoot elongation was suppressed in the medium with BA and IAA in both diploid and triploid cultivars.

During micropropagation of Red banana, occurrence of green somaclonal variants was reported due to the high concentration of BA in the medium (Vidhya and Nair 2002). The synergistic action of cytokinins and auxin in inducing multiple shoot formation was observed in other triploid *Musa* cultivars (Hirimburegama and Gamage 1997). Multiple shoot production from MS medium supplemented with BA and IAA in lower concentration was reported in two diploid cultivars of South India (Mukunthakumar and Seeni 2005). This difference may simply be due to the physiological differences between the explants as in the present study infloresence apices were used as explants. In the early reports in banana cultures, highly variable behaviour has been observed among micropropagated progenies of the same genotype in terms of shoot multiplication (Afza et al. 1996; Israeli et al. 1996; Mendes et al. 1999). Though, these micropropagated progenies were of the same genotype, the differences in growth rate might reflect accumulated mutations in the donor explant.

Micropropagation is the most widely used commercialized global application of plant biotechnology in horticulture. In vitro propagated plants of banana, and many other ornamental crops have become a commercial reality. However, micropropagation of many elite cultivars of local importance is still confined to research laboratories. Our study presents the rationale for the conservation and genetic improvement of the diploid cultivars, as they need special care with reference to disease resistance. Moreover, the unavailability of a large number of suckers of elite cultivars can be met with inflorescence tip culture. Establishment of micropropagation protocols for local cultivars with different genome status will provide a great impetus to the global banana improvement programmes.

**Acknowledgements** The first author is thankful to the University Grants Commission, New Delhi, India for financial assistance in the form of Junior Research Fellowship.

## References

Afza R, Van Duren M, Morpurgo R, Novak FJ (1996) Banana tissue culture and its prospective use in the developing countries. In: Islam AS (ed) Plant tissue culture. Science Publishers, Lebanon, New Hampshire, pp 58–70

Arnaitwe G, Rubaihayo PR, Magambo MJS (2000) Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. Sci Hort 86:13–21

Banerjee N, De Langhe (1985) A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (Banana and Plantain). Plant Cell Rep 4:351–354

Cronauer-Mitra SS, Krikorian AD (1984) Multiplication of *Musa* from excised stem tips. Ann Bot 53:321–328

Cronauer SS, Krikorian AD (1985) Reinitiation of vegetative growth from aseptically cultured terminal floral bud apex of banana. Am J Bot 72:1508–1601

Ganapathi TR, Suprasanna P, Bapat VA, Kulkarni VM, Rao PS (1999) Somatic embryogenesis and plant regeneration from male flower buds in banana. Curr Sci 76:1228–1231

Gubbuk H, Pekmezci M (2004) In vitro propagation of some new banana types (*Musa* spp.). Turk J Agric Forum 28:355–361

Hirimburegama K, Gamage N (1997) Cultivar specificity to in vitro micropropagation of *Musa* spp. (banana and plantain). J Hort Sci 72:205–211

Israeli Y, Ben-Bassat D, Reuveni O (1996) Selection of stable banana clones which do not produce dwarf somaclonal variants during in vitro culture. Sci Hort 67:197–205

Jarret RL, Fisher JB, Litz RE (1985). Organ formation in *Musa* tissue cultures. Plant Physiol 121:123–130

Krikorian AD, Irizarry H, Cronauer-Mitra SS, Rivera E (1993) Clonal fidelity and variation in plantain (*Musa* spp.) regenerated from vegetative stem and floral axis tips in vitro. Ann Bot 71:519–535

Kulkarni VM, Suprasanna P, Ganapathi TR, Bapat VA, Rao PS (2004) Differential effect of genome and cytokinins on shoot-tip cultures of Indian banana cultivars. Physiol Mol Biol Plants 10:75–81

Kulkarni VM, Suprasanna P, Bapat VA (2006) Plant regeneration through multiple shoot formation and somatic embryogenesis in a commercially important and endangered Indian banana cv. Rajeli. Curr Sci 90:842–846

Kumar R, Sinha K, Kumar S (2005) Micropropagation of banana cv Malbhog through meristem tip culture in

consort with thermotherapy. Phytomorphology 55:17–22

Madhulatha P, Anbalagan M, Jayachandran S, Sakthivel N (2004) Influence of liquid pulse treatment with growth regulators on in vitro propagation of banana (*Musa* spp. AAA). Plant Cell Tiss Org Cult 76:189–191

Mendes BMJ, Filippi SB, Demetrio CGB, Rodriguez APM (1999) A statistical approach to study the dynamics of micropropagation rates, using banana (*Musa* spp.) as an example. Plant Cell Rep 18:967–971

Mukunthakumar S, Seeni S (2005) In vitro cloning and homestead cultivation of primitive *Musa* cultivars. Ind J Exp Biol 43:90–95

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plantarum 15:473–497

Nair AS, Teo CH, Schwarzacher T, Harrison PH (2005) Genome classification of banana cultivars from South India using IRAP markers. Euphytica 144:285–290

Nayar TG (1962) Banana in India. The Fact technical society, Udyogamandal, Kerala, India

Sebastian L, Mathew MM (2004) Micropropagation of Nendran and Poovan varieties of *Musa* through inflorescence tip culture. Plant Cell Biotech Mol Biol 5:141–148

Shirin F, Kumar S, Mishra Y (2000) In vitro plantlet production system for *Kaempferia galanga*, a rare Indian medicinal herb. Plant Cell Tiss Org Cult 63:193–197

Strosse H, Van den Houwe I, Panis B (2004) Banana cell and tissue culture—review. In: Jain SM, Swennen R (eds) Banana improvement. Science Publishers, Inc., Plymouth, UK, pp 1–12

Tiwari KN, Sharma NC, Tiwari V, Singh BD (2000) Micropropagation of *Centella asiatica* (L.), a valuable medicinal herb. Plant cell Tiss Org Cult 63:179–185

Tiwari V, Tiwari KN, Singh BD (2001) Comparative studies of cytokinins on in vitro propagation of *Bacopa monniera*. Plant Cell Tiss Org Cult 66:9–16

Vidhya R, Nair AS (2002) Molecular analysis of somaclonal variation in *Musa acuminata* (AAA) cv. Red. Phytomorphology 52:293–300

Vuylsteke DR (1989) Shoot-tip culture for the propagation, conservation and exchange of *Musa* germplasm. International board for plant genetic resources, Rome, pp 20–25

Wong WC (1986) In vitro propagation of banana (*Musa* spp.): initiation, proliferation and development of shoot-tip cultures on defined media. Plant Cell Tiss Org Cult 6:159–166

Zaffari GR, Kerbauy GB, Kraus JE, Romano CE (2000). Hormonal and histological studies related to in vitro banana bud formation. Plant Cell Tiss Org Cult 63:187–192