Short Communication

# Induction of multiple shoots through *in vitro* male bud culture in banana *Musa* (AA) cv. 'Kadali'

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**Abstract**

Male buds of banana (*Musa* (AA) cv. 'Kadali') collected 20-25 days after the emergence of bunches were used for *in vitro* shoot regeneration, during 2016-2018. Sterilized male buds were inoculated on Murashige and Skoog basal medium with different combinations of naphthalene acetic acid (NAA) and benzyl adenine (BA). The earliest and highest percentage of culture establishment and multiple shoot induction (15.99) were observed on full strength MS medium with NAA 1.0 $mgL^{-1}$ and BA 4.0 $mgL^{-1}$.

**Key words**: Direct organogenesis, 'Kadali', Male bud, Micropropagation, MS medium, *Musa,* Tissue culture.

'Kadali' is one of the banana varieties with great demand in the state of Kerala, India, as it is used for offering in temples, and the fruits are bestowed with a special flavour besides possessing medicinal properties. Major problem faced by the 'Kadali' growers is the non-availability of good quality planting material. Normally suckers and tissue culture plants (raised through shoot tip culture) are used for planting, but their availability is limited (Sapheera, 2005). Further, contamination is a problem in shoot tip culture. In addition to suckers, male buds can be used as a potential explant for raising tissue culture plants as this part goes unutilized during the harvesting of banana bunches. Therefore, *in vitro* male bud culture forms a good alternative since the male buds are less contaminated and male inflorescences of different sizes from different bract positions can be taken as explants (Darvari et al., 2010; Mahadev et al., 2011). With this in view, the present study was undertaken to explore the potential of male buds for mass multiplication of the diploid banana *Musa* (AA) cv. 'Kadali' under *in vitro* conditions. The work was carried out in the Plant Tissue Culture Laboratory at Banana Research Station, Kannara during 2016-2018.

Male flower buds from field grown healthy plants were collected 20-25 days after the emergence of bunches. According to the size of male bud, up to 35-45 bracts covering the hands of male bud were removed in a stepwise manner, without causing any injury until it became too small to be separated. Male buds (4-5cm in length) were washed four times thoroughly in tap water. The explants were first surface sterilized with 0.1 per cent (w/v) mercuric chloride for four minutes and then rinsed thrice with autoclaved double distilled water in the laminar air flow cabinet. About 45-50 protective bracts covering the male flowers were removed under sterile conditions using sterilized blade and forceps, and then male flowers having a size of 0.5-1.0 cm (Fig. 1. A) were taken out and inoculated in full MS/ half MS (Murashige and Skoog) medium

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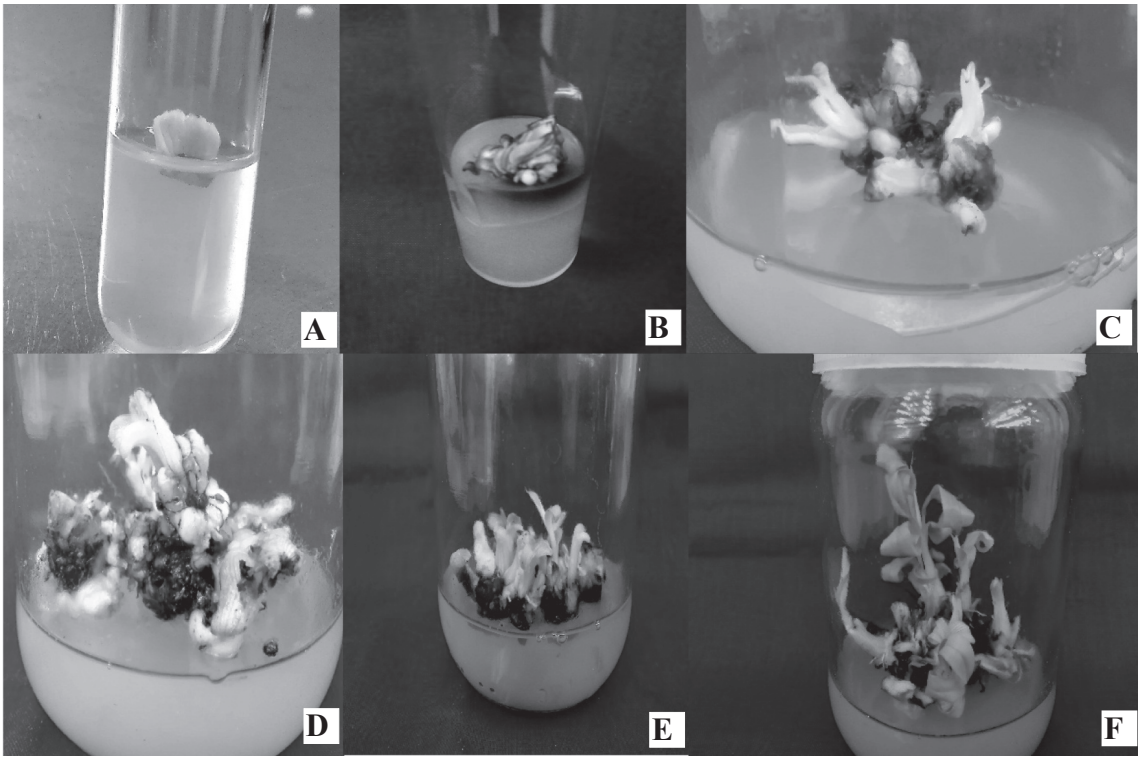


*Figure 1.* (A) A group of male flowers (hands) used to establish the culture (B) Male flowers turning green after 2 weeks. (C and D) Proliferation of cultures within 4-5 weeks (E) Multiple shoot induction (F) Shoots and young leaves developed after 90 days of culture.

(Murashige and Skoog, 1962). The experiment was laid out in CRD. Each treatment was replicated thrice with six cultures for each replication. Cultures were incubated at 25 ± 2°C with 16 hour photoperiod of light intensity 2000 lux supplied by cool white fluorescent tubes. After 30 days of culturing on full MS/ half MS medium, the cultures were transferred to fresh media at three weeks interval until the formation of shoot buds. Axillary shoots of 1.0-1.5 cm length from established cultures were used for shoot multiplication in full MS/half MS media containing different combinations of naphthalene acetic acid (NAA) and benzyl adenine (BA). The multiple shoots induced were separated into clumps of two to three shoots and sub-cultured at intervals of 30 days. Three subculturings were done to induce more number of shoots in multiple shoot induction stage. After 90 days, the numbers of shoots and leaves produced per culture were recorded.

A differential response was observed in both full MS and half MS media supplemented with different concentrations of NAA and BA, on number of days taken for culture establishment. Among the combinations, earliest culture establishment (26.99 days) was in a combination of full MS medium containing NAA 1.0 $mgL^{-1}$ and BA 4.0 $mgL^{-1}$ which was significantly superior to all the other treatments (Table 1). Full MS media containing BA 4.0 $mgL^{-1}$ and NAA 0.5 $mgL^{-1}$, and also BA 4.0 $mgL^{-1}$ and NAA 1.0 $mgL^{-1}$ recorded cent per cent culture establishment and were statistically on par. The cultures got established without any contamination. The quantity of salts present in half MS medium was insufficient to nourish the explants which in turn delayed the culture establishment. Choice of correct growth regulator and its use in optimum concentration gave the best result in *in vitro* propagation of *Musa* species (Krikorian, 1982). In earlier studies also combinations of NAA and BA

*Table 1*. Effect of growth regulators on *in vitro* establishment of male bud cultures

| Treatments | Days taken for culture establishment | | Cultures established (%) | | Contamination (%) | |
|---|---|---|---|---|---|---|
| | Half MS | Full MS | Half MS | Full MS | Half MS | Full MS |
| NAA 0+ BA1.0 | 30.33 | 30.33 | 72.21 | 16.66 | 0 | 0 |
| NAA 0+ BA2.0 | 29.66 | 29.33 | 77.77 | 72.21 | 0 | 0 |
| NAA 0+ BA3.0 | 29.66 | 29.49 | 66.66 | 77.77 | 0 | 0 |
| NAA 0+ BA4.0 | 29.33 | 29.16 | 88.88 | 94.44 | 0 | 0 |
| NAA0.5+ BA1.0 | 30.66 | 30.33 | 38.88 | 44.44 | 0 | 0 |
| NAA0.5+ BA2.0 | 29.66 | 29.83 | 61.10 | 72.21 | 0 | 0 |
| NAA0.5+ BA3.0 | 29.83 | 28.83 | 77.76 | 83.33 | 0 | 0 |
| NAA0.5+ BA4.0 | 29.50 | 27.66 | 88.88 | 100.00 | 0 | 0 |
| NAA1.0+ BA1.0 | 30.66 | 30.16 | 44.44 | 27.77 | 0 | 0 |
| NAA1.0+ BA2.0 | 30.00 | 29.83 | 88.88 | 77.77 | 0 | 0 |
| NAA1.0+ BA3.0 | 29.83 | 29.33 | 83.33 | 88.88 | 0 | 0 |
| NAA1.0+ BA4.0 | 29.33 | 26.99 | 94.44 | 100.00 | 0 | 0 |
| NAA1.5+ BA1.0 | 30.16 | 29.99 | 22.21 | 22.21 | 0 | 0 |
| NAA1.5+ BA2.0 | 29.49 | 29.49 | 77.77 | 61.10 | 0 | 0 |
| NAA1.5+ BA3.0 | 29.83 | 29.33 | 72.21 | 88.88 | 0 | 0 |
| NAA1.5+ BA4.0 | 29.49 | 27.66 | 83.33 | 94.44 | 0 | 0 |
| SEm ± | 0.14 | 7.67 | | | | |
| CD (0.05) | 0.41 | | NS | | | |

*Data were collected after 30 days of culture. Values represent mean of three replications, with six explants per replication.

were found best for culture establishment (Jarret et al., 1985; Bhaskar et al.,1993). The male inflorescence cultures of the cultivars Matti (AA), Sannachenkadali (AA), Njalipoovan (AB) and Chingan (AB) turned completely green around 8-10 days after inoculation (Smitha et al., 2014) while in Berangan (AAA), Rastali (AAB), Nangka (AAB) and Abu (ABB) the explants were found to expand and they became green within 10-15 days after their inoculation (Darvari et al., 2010). Successful *in vitro* multiplication of male inflorescence of banana cultivars, namely, Sannachenkadali, Red Banana, Njalipoovan, Virupakshi and Sirumalai belonging to South Indian tracts have been reported previously. The studies revealed that contamination rate of male flowers is very less as compared to shoot tip (Resmi and Nair, 2007; Mahadev et al., 2011; Smitha et al., 2014). In the present study, explants showed greenish colouration and proliferation of cultures within 2 weeks (Fig. 1. B) on full MS/ half MS medium supplemented with different concentrations of NAA and BA.

The proliferation of cultures was observed within 4-5 weeks of sub culturing (Fig. 1. C and D). Through further subculturing, a dense clump of multiple shoots were found to develop from the base (Fig. 1. E) after 60 days. After 90 days, shoots attained 4-5 cm length and young leaves were found to develop (Fig. 1. F). By the application of NAA, rooting of shoots was also achieved in multiple shoot induction media (Fig. 1. F).

Significant response was observed in MS media containing different levels of NAA and BA with respect to number of shoots and number of leaves. Maximum number of shoots per explant (15.99) was

*Table 2*. Effect of growth regulators on *in vitro* induction of multiple shoots

| Treatments | Number of shoots per culture | | Number of leaves | |
|---|---|---|---|---|
| | Half MS | Full MS | Half MS | Full MS |
| NAA0.5 + BA 2.0 | 8.66 | 11.00 | 13.00 | 14.83 |
| NAA0.5 + BA 4.0 | 10.49 | 11.83 | 11.58 | 15.83 |
| NAA0.5 + BA 6.0 | 11.66 | 13.66 | 12.17 | 15.33 |
| NAA0.5 + BA 8.0 | 13.33 | 12.83 | 14.33 | 14.66 |
| NAA0.5 + BA10.0 | 10.16 | 10.33 | 12.66 | 10.00 |
| NAA1.0 + BA 2.0 | 9.99 | 9.66 | 11.33 | 11.66 |
| NAA1.0 + BA 4.0 | 9.99 | 15.99 | 9.66 | 19.50 |
| NAA1.0 + BA 6.0 | 10.49 | 14.66 | 12.50 | 19.83 |
| NAA1.0 + BA 8.0 | 9.49 | 9.16 | 8.00 | 12.50 |
| NAA1.0 + BA10.0 | 9.33 | 9.33 | 8.16 | 8.33 |
| SEm ± | 0.27 | 2.37 | | |
| CD(0.05) | 0.78 | 6.77 | | |

observed in full MS medium supplemented with NAA 1.0 $mgL^{-1}$ and BA 4.0 $mgL^{-1}$ and was superior to all other treatment combinations. Highest number of leaves (19.83) were recorded in full MS medium containing NAA 1.0 $mgL^{-1}$ and BA 6.0 $mgL^{-1}$ and was on par with full MS medium supplied with NAA 1.0 $mgL^{-1}$ and BA 4.0 $mgL^{-1}$ (19.50). Earlier studies indicated that BA along with NAA gave better results for banana shoot multiplication (Bhaskar et al., 1993; Rahman et al., 2004). The number of shoots was directly correlated to the number of leaves. Addition of BA favoured the induction of multiple shoots from male buds. The rate of shoot multiplication mainly depended upon the concentration of cytokinins (Strosse et al., 2004). The better performance of BAP over other cytokinins in the multiplication of shoot tips has been reported in different cultivars of banana (Wong, 1986; Zaffari et al., 2000). The use of cytokinins is reported to reduce the apical dominance, leading to more axillary shoots (Madhulatha et al., 2004). Induction of more number of shoots with optimum concentration of BA in male bud culture has been reported by Mahadev et al. (2011) in Virupakshi and Sirumalai, where the best multiplication was in medium containing BAP 5.0 $mgL^{-1}$.

A cost effective method for *in vitro* shoot multiplication through direct organogenesis from male buds of 'Kadali' has been developed. This method has an added advantage of the absence of latent contamination, a problem often faced during tissue culture protocols which are currently in use for commercial production of bananas (Harirah and Khalid, 2006). One male bud was sufficient to provide an average of 10 explants and each explant under *in vitro* conditions produced an average of 16 shoots within a period of three months. Thus, within 90 days, around 160 shoots could be induced from a single male bud. Hence, male bud culture is recommended as a more efficient strategy to produce large number of planting material in cv. 'Kadali'.

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