

Rapid Propagation of Two Seedless Diploid (AB) Land Races of *Musa* through *in Vitro* Culture of Shoot and Inflorescence Apices

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ABSTRACT

In vitro propagation of two diploid landraces of *Musa* cv. 'Poonkadali' and 'Rasakadali' was achieved through organogenesis by culturing shoot primordia (~35 × 18 cm) and inflorescence apices (~15 × 10 cm) dissected out from healthy flowering plants. Murashige and Skoog (MS) medium fortified with 1.5 mg l⁻¹ 6-benzyl amino purine (BAP) induced initiation of healthy shootlets from shoot primordial meristems of 'Rasakadali' whereas a combination of 2.5 mg l⁻¹ BAP and 0.5 mg l⁻¹ indole-3-acetic acid (IAA) is required for shoot induction from non-dominant inflorescence meristems of 'Rasakadali'. In cultivar 'Poonkadali' 3.0 mg l⁻¹ BAP and 0.5 mg l⁻¹ IAA was obligatory for optimal shoot initiation from shoot apices while inflorescence apices responded maximum at concentrations of 4 mg l⁻¹ BAP and 1.5 mg l⁻¹ IAA. Transfer of shoots (4-5 cm) to MS basal medium containing 0.1% activated charcoal favored augmented growth of healthy shootlets and profusion of roots within 4 weeks. Multiple shoot proliferation was achieved through five subcultures of the isolated shoots without any decline. Enhanced multiplication rate achieved from floral meristems of these diploid cultivars can have unswerving impact in popularization of banana cultivation particularly in rural farmer lands.

Keywords: activated charcoal, cytokinin, mass propagation protocol, meristem apices, *Musa* cultivars, Southern Western Ghats

INTRODUCTION

Bananas and plantains are one of the world's most important yet poorly studied crops. In terms of gross value of production, they rank fourth important global food crop and first as fruits (Frison *et al.* 1997). They are of major importance to food security as well as providing a valued source of income through local and international trade. As a staple food, banana in particular, contributes to the food security of millions in the developing world, and locally to employment generation for rural communities (Uma *et al.* 2005; FAO 2008). Usually bananas are propagated vegetatively by means of suckers. However, this method is seriously limited by its low rate of multiplication. As the traditional clonal propagation methods are unable to cope with the demand for planting materials, attention has been focused on non-conventional production of propagules using *in vitro* techniques (Berg and Bustamente 1974; Cronauer and Krikorian 1986). Advances in banana improvement made in recent years indicate that a high return may now be expected on investment in *Musa* research.

The fruits of the classic diploid *Musa* cultivars 'Poonkadali' and 'Rasakadali' are said to have some good effects on curing of intestinal ulcers (Uma *et al.* 2005). These cultivars take almost 10-12 months to produce bright yellow, smooth skinned fruits having hard white pulp. The fruits are of high quality, sweet flavor and moderate shelf life. The present study is aimed at developing a mass propagation methodology through *in vitro* culture of shoot tip as well as immature male floral apices of both the cultivars, thereby enhancing the population of these sought after *Musa* cultivars in the rural hamlets of the south Western Ghats region.

MATERIALS AND METHODS

Young suckers (approx size 35 × 18 cm) and inflorescence (15 × 10 cm) of two native cultivars 'Poonkadali' and 'Rasakadali' (Fig. 1A, 1B) were separated from the flowering plants of the germ-

plasm collection organized in the nursery area of Tropical Botanic Garden and Research Institute.

Culture initiation in shoot primordia

The outer sheaths were removed and sliced upto a block having 10 cm length × 5 cm diameter. This meristematic block was washed in distilled water thoroughly. The blocks were then washed well in 2% Teepol [Reckit India] detergent, surface sterilized by immersion for 20 min. in 4% w/v sodium hypochlorite solution (Merck, India) along with 5 ml of 70% ethanol and rinsed three to four times in sterile distilled water (Banerjee *et al.* 1985). The surplus hard basal tissues of the block were trimmed off further to 2 × 2 cm. size and plunged in Murashige and Skoog (1962) basal liquid medium. After incubating in the dark for a week, the exudation free meristematic apices were vertically dissected into 8 equal segments and implanted in MS basal agar nutrient medium containing full complement of macro and micronutrients, vitamins and 30 g/l sucrose. The pH of the medium was adjusted to 5.8 before adding 0.6% agar (SRL, Mumbai). The media were sterilized by autoclaving at 121°C and 104 k Pa pressure for 18 min (Mukunthakumar and Seeni 2005). All the cultures were incubated for 45 days in the culture room at 24 ± 2°C under a 16 hr photoperiod at an illumination of 30-50 μM⁻² provided by cool white fluorescent lamps (Philips India Ltd., Mumbai).

The shootlets proliferated upon the shoot apices under suitable incubation conditions were separated as independent clumps having 2-3 shootlets were subcultured in different media containing combinations of BAP (6-benzylamino purine; 1.0-5.0 mg l⁻¹) and IAA (indole-3-acetic acid; 0.5-2.0 mg l⁻¹) in single and cumulative dosages (Pullman and Gupta 1991). Four replications were maintained under each treat. Observations on shoot proliferation were made after 90 days and the data on shoot multiplication were collected for variant analysis and student's *t*-test of significance using Microsoft Excel software – ANOVA two factors without replication (Microsoft Corporation, California, USA).

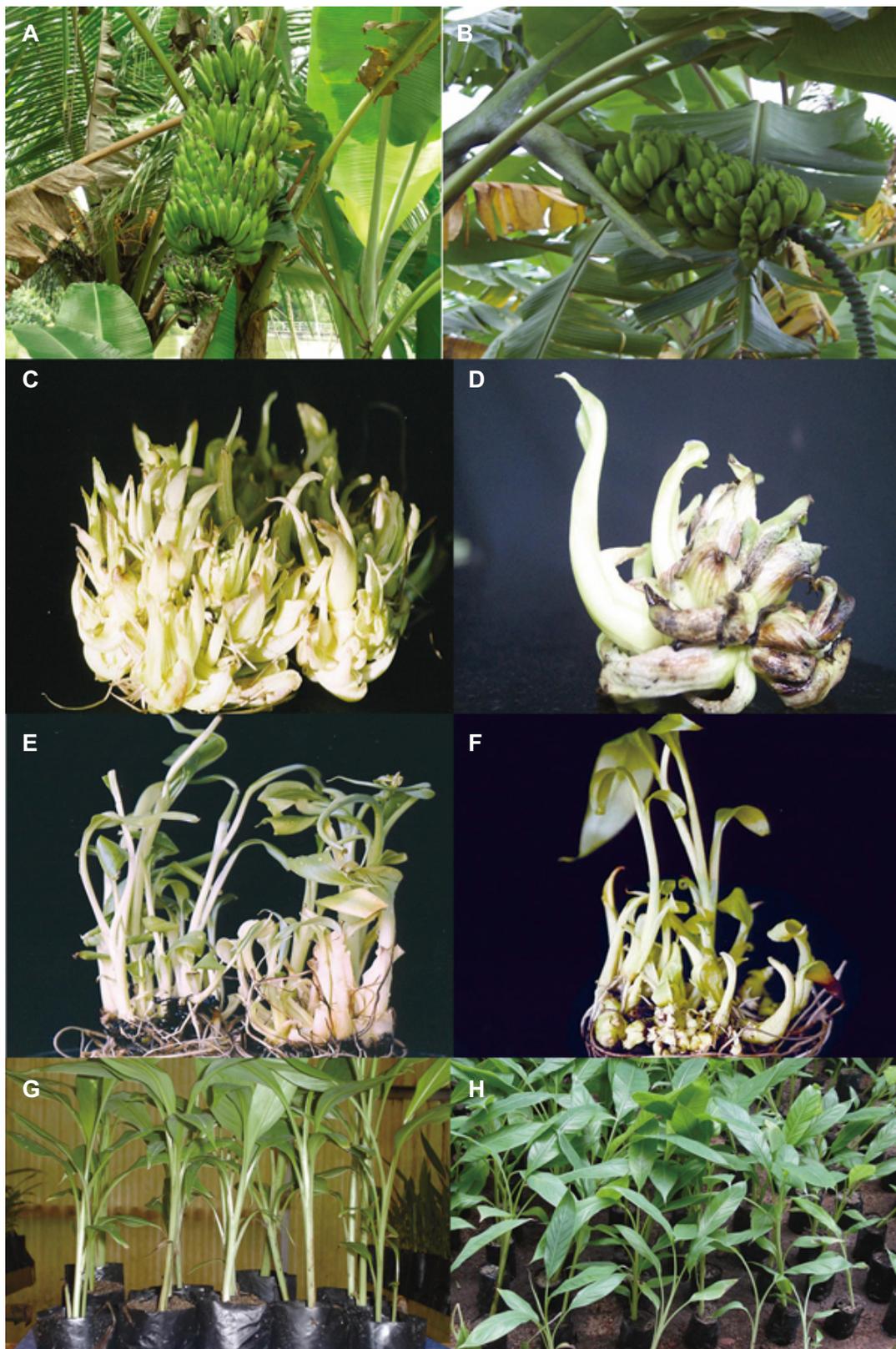


Fig. 1 *Musa* cultivars 'Poonkadali' (A) and 'Rasakadali' (B) in their natural habitat. Profuse shoot clusters developed from inflorescence meristem of 'Poonkadali' (C). Proliferation of shoot meristem of 'Rasakadali' (D) under *in vitro* conditions. Shootlets of 'Poonkadali' (E) and 'Rasakadali' (F) after rooting. Hardened plants of 'Poonkadali' (G) and 'Rasakadali' (H).

Culture initiation in inflorescence tips

Male inflorescence apices collected from healthy grown mother plants of both the cultivars peeled off the exterior bracts and interior meristematic floral cluster of 5 cm length was subjected to surface sterilization as in the case of shoot apex. The explants were then washed for 15-18 min with 5 changes of sterile distilled water. The outer bracts as well as the floral hands under each bract

were trimmed off using sterile surgical blade to reduce the size to about 2 cm each. The meristematic apices were then transferred into MS liquid media for a period of 7 days and then to semi solid agar (0.6%) medium. The cultures were incubated at $24 \pm 2^\circ\text{C}$ in the dark or under a 16 h photoperiod provided by cool white fluorescent lamps (Philips India Ltd., Mumbai). Observations on culture initiation were made at two-week intervals. After 30 days, each floral hands having 4-5 fringed clumps were detached along

with their bracts and taken for further proliferation studies under single and combined dosages of BAP ($1-4 \text{ mg l}^{-1}$), and IAA ($0.5-2 \text{ mg l}^{-1}$) by keeping four duplicates of fringed clumps under each combination. The plantlets proliferated in presence 0.1% activated charcoal (Pullman and Gupta 1991) were healthy with profuse rooting and hence for further sub culturing, this inorganic supplement was included in proliferation media. The results were taken after the time interval of three weeks. Data on *in vitro* proliferation were collected after 90 days of culture incubation and subsequent proliferation and were subjected for variant analysis and student's *t*-test of significance using Microsoft Excel software – ANOVA two factors without replication (Microsoft Corp., California, USA).

Healthy plantlets regenerated from both types of meristematic tissues were separated, washed in running tap water and transplanted in $20 \times 13 \text{ cm}$ polythene bags filled with potting mixture having river sand, farmyard manure and top soil (1: 1: 1). The plants were hardened in a mist chamber maintained at 70-80% RH for 3-4 weeks and subsequently transferred to the nursery for 4 weeks before distribution to interested individuals for homestead cultivation in Thiruvananthapuram district of Kerala for assessing performance in multi-location trials. After 12-15 months, data on field performance including yield were collected from the individuals.

RESULTS AND DISCUSSION

Enlargement of meristematic blocks of shoot primordia and in florescence tips and initiation of shoot buds were observed after 45 days of incubation in the dark. However, the rate of shoot growth and the number of multiple shoots produced varied from one nutrient medium to another. In media containing higher concentration of cytokinin, the explants showed calloid growth and such cultures were discarded from further proliferation studies for multiple shoot induction.

It is not uncommon that banana tissues in cultures get browned and release exudates that are toxic to the cultures. In the present study the deleterious effects of oxidized phenolic products was largely overcome by immersing the explants in liquid medium, which diluted the toxic exudates. Transfer of the explants after one week to agar medium greatly helped to rescue of the explants that responded adequately. The contamination free meristematic blocks were chopped vertically into eight segments and were implanted into MS agar medium containing full strength micro-macro nutrients, vitamins and sugar. Rapid bud proliferation was however reported to be dependent on exogenous supply and hasty absorbability of mineral nutrients (Amiri and Arzani 2006). The use of BAP (1.5 mg l^{-1}) alone and combination of 3 mg l^{-1} BAP and 0.5 mg l^{-1} IAA were projected in the present study as growth supplements. Optimal shoot proliferation in 'Poonkadali' and 'Rasakadali' (Fig. 1C, 1D) respectively indicated the physiological differences existing between same genotypic variants. The increased number of shoot initiation from the non-dominant inflorescence meristems of 'Rasakadali' (42) compared to 'Poonkadali' (31) further highlighted differences in responses of these two diploid cultivars. Similar differences in proliferation exhibited by the *Musa* cultivars of same ploidy constitution have already been reported (Mukunthakumar and Seenii 2005). By separating individual clusters having 3 to 4 shootlets and culturing in the same media for 90 days, (Figs. 2, 3) up to 14-17 shoots were harvested from each explant. The intensity of browning and release of exudates in male inflorescence apices were less than of the shoot tips. After two weeks of incubation in the dark the contamination free meristematic block was chopped vertically into eight segments and were used for culture initiation under the influence of different concentrations of growth regulators ($0-4 \text{ mg l}^{-1}$ of BAP and $0.5-2 \text{ mg l}^{-1}$ of IAA) as suggested by Jhambal *et al.* (2001). Maximum shootlet proliferation in 'Poonkadali' (Fig. 1C) was obtained in media containing cumulative dosage of 4.0 mg l^{-1} BAP and 1.5 mg l^{-1} IAA while as in 'Rasakadali', inflorescence responded the most

at 2.5 mg l^{-1} BAP and 0.5 mg l^{-1} IAA. The requirement of a combination of both cytokinin and auxin for further proliferation indicated possible differences in indigenous interactions of growth regulators between the explant types, at least in 'Poonkadali'. The minimal shootlets were obtained in the media supplemented with independent dosages of IAA that enhanced only the apical dominance. From the data obtained from this study (Figs. 2, 3), it is evident that plant regeneration capacity of the meristematic zones present in the male inflorescence is more than that shoot primordial meristems.

Tissue culture multiplication using shoot tip cultures and axillary meristems is a most popular biotechnological application of in agriculture (Dale 1990) and forestry (Bonga and Durzan 1987) as it helps in production of true to type plants. This technique is currently used for the mass production of many plants including banana. But current trends are there to enhance the *in-vitro* multiplication rate by culturing inflorescence meristems (Resmi and Nair 2007; Wirakarnain *et al.* 2008). In the present study also it is demonstrated the usage of apical and lateral meristems present in the explants of suckers as well as the inflorescence apices for inducing healthy shoot clumps in the presence of ideal growth stimulating hormones. The use of BAP in this study for induction of shoot bud differentiation was based on the earlier publications (Dore Swami *et al.* 1983; Vuylsteke and De Langhe 1985; Uma *et al.* 2005) and its strong cytokinin activity compared to 6-furfuryl aminopurine and isopentynyl adenine (data not shown). Beside the reported non-dominant meristematic zone multiplication in presence of cytokinin (Wong 1986) was evident as more than a few shoots were initiated perhaps from both dominant apical buds and non-dominant lateral meristems particularly in the explants of the suckers.

In the case of shoot buds, the single action of cytokinin has been found more effective for both multiple shoot induction and its subsequent proliferation. In the latter one using inflorescence apices also, the best result was observed in single treatments in cytokinin rather than in auxin-cytokinin combinations (Fig. 2). From the above observations it is concluded that single dosage of cytokinin is effective in this particular cultivar of banana irrespective of the nature of the explant used. Here, the relevance is also there in lowering of cytokinin concentration as better multiplication rate was achieved by performing so. But optimal concentrations required under different multiplication stages differ for both types of explants, which can be attributed to the difference in arrangement of meristematic tissues in shoot and inflorescence apices. However, through this work MS media fortified with 2.5 mg l^{-1} BAP and 0.5 mg l^{-1} IAA is ideal to obtain mass proliferation from inflorescence meristems of 'Rasakadali' and MS media with 4.0 mg l^{-1} BAP and 1.5 mg l^{-1} IAA for Inflorescence apex cultures of 'Poonkadali'. This clearly shows the influence of the type of explant used over the requirements of hormone concentrations and combinations. In both the explant types; optimum concentration required for the successive steps was different. It was of importance to note that when different organic compounds were tried for the formulation of ideal proliferating media, better results were recorded with the media containing 0.1% activated charcoal that promotes the development of robust plants (Fig. 1E, 1F). The mode of action for AC generally relate to the adsorptive properties of AC in tissue culture medium (Pullman and Gupta 1991; Ebert *et al.* 1993; Toldi *et al.* 1996; Van Winkle and Pullman 2003). Yet another important observation was that the shoot cultures with higher number of shootlets resulted in comparatively weaker plants whereas the cultures with the least number of shootlets gave healthier plants. In addition to this, the plantlets produced from shoot tips showed healthier nature than the plants grown from male inflorescence apices, even though both were in the similar cultural conditions. However, the plantlets of 'Rasakadali' having arisen from inflorescence apices produced fruit bunches weighing an average of about 18 to 22 kg with 6-7 hands having about 13

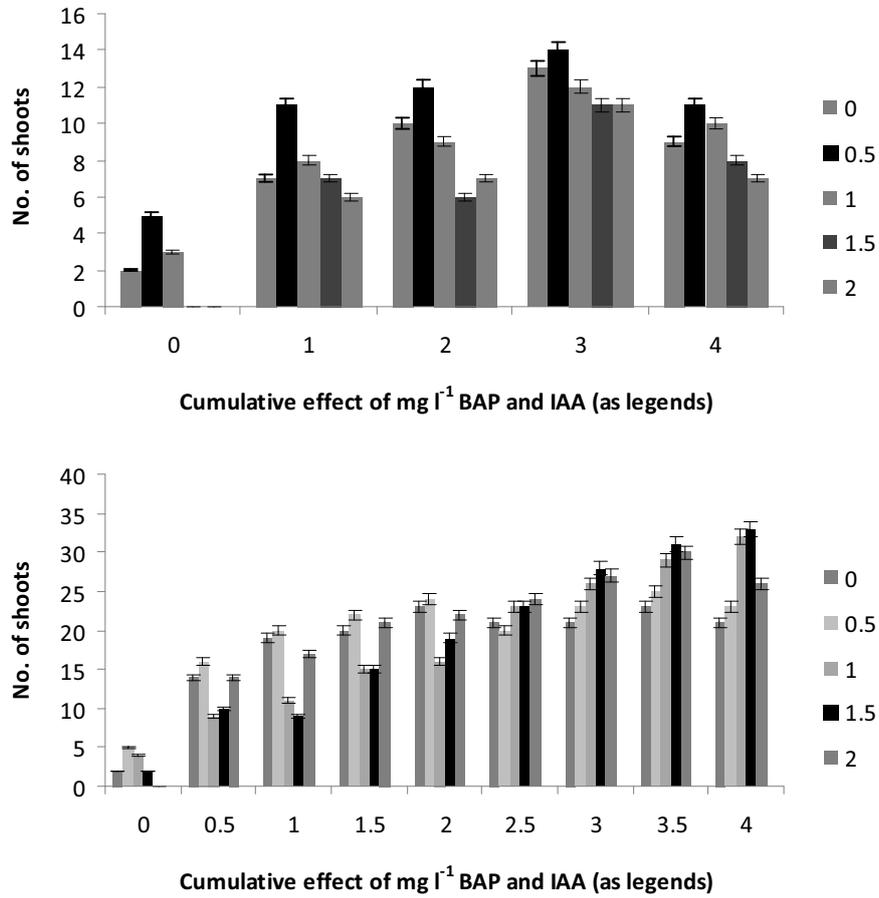


Fig. 2 Shootlets proliferated after 90 days of culture incubation of shoot (top graph) and inflorescence (bottom graph) primodial meristems of 'Poonkadali' (values = mean number of shootlets with a standard error of ± 0.14 ; * = P values of 0.015 and 0.019, respectively).

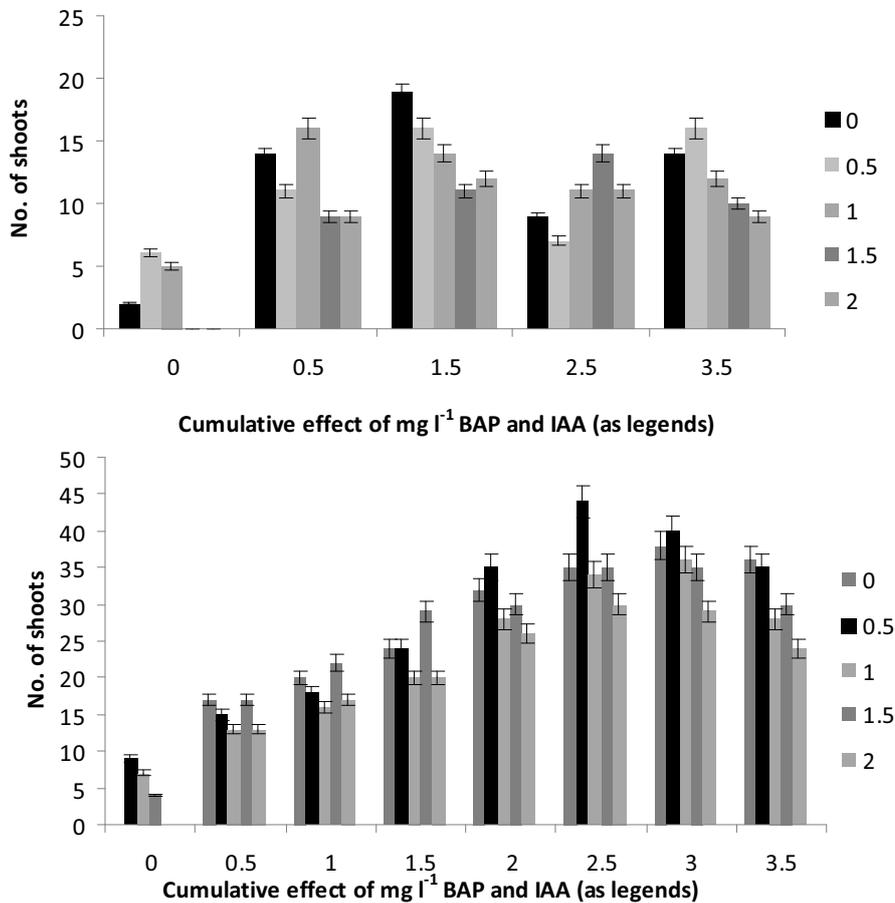


Fig. 3 Shootlets proliferated after 90 days of culture incubation of shoot (top graph) and inflorescence (bottom graph) primodial meristems of 'Rasakadali' (values = mean number of shootlets with a standard error of ± 0.19 ; * = P values of 0.028 and 0.031, respectively).

fruits per hand. In combination with high-density planting and drip irrigation, this is becoming a highly successful cultivar among the homesteads of Thiruvananthapuram district.

Formation of calloids was also observed in media including 1 and 1.5 mg l⁻¹ IAA. The same effect was reported in other varieties of *Musa*, when IAA was added to the media (Ziv and Gadaski 1986; Arias 1992; Pan and van Staden 1998). The inclusion of AC in the culture media for obtaining robust rooted plants and variable relationship between the number of shoots proliferated and its healthy nature appeared to be critical for the *in vitro* multiplication of these two *Musa* cultivars. Such plants showed better rate of establishment (Fig. 1G, 1H) (98% for shoot tip derived cultures and 94% for inflorescence derived cultures) and hence suggested as a requirement for the generation of healthy plantlets having enhanced field establishment potential.

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