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High Frequency Regeneration of Plantlets from Immature Male Floral Explants of *Musa paradisica* cv. Puttabale - AB Genome

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Abstract

High frequency *in vitro* regeneration for mass multiplication from immature male floral explants of *Musa paradisica* cv. Puttabale on MS supplemented with adenine sulfate (160 mg/l), tyrosine (100 mg/l), sucrose (40 g/l) and gelled with 0.8 g/l agar was attempted. For callus induction the combinations of 2, 4-D and BAP were tested at 1.0 - 10.0 mg/l and 0.5 - 5.0 mg/l, respectively. For shoot bud formation combinations of BAP and TDZ were also tested at 1.0 - 5.0 mg/l and 0.1 - 0.5 mg/l, respectively. Luxuriant proliferation and high frequency induction (97.0%) of callus was noticed from the accessory floral part of the explant at 7.0 mg/l 2, 4-D and 1.0 mg/l BAP, later it preceded towards the gynoecium. Interaction of BAP (2.0 – 5.0 mg/l) and TDZ (0.2 - 0.5 mg/l) would provoke high frequency shoot bud differentiation from the floral calli and a mean of 29.40 ± 6.10 shootlets per callus was obtained at 4 mg/l BAP and 0.4 mg/l TDZ. Rooting of the microshoots was achieved on MS containing 0.6 mg/l NAA and 0.2% activated charcoal.

Introduction

Banana is one of the important fruit crop grown in Karnataka, cultivated in 52,613 ha, with a production of 1.3 million tones and productivity of 24.6 tones/ha. *Musa paradisica* cv. Puttabale is a one of the popular cultivars in Malnad region of Karnataka, India which is known for its delicious taste and flavour. But due to the infection of *Fusarium oxysporum* and *Mycosphaerella fijiensis* leads to the losses of crop production (Sasson 1997) and *in vitro* propagation of banana is interfered by endogenous bacterial contamination is a great problem. Although initially surface sterilization works, later on microbial contamination at the base of the explant is observed within 7 - 15 days after inoculation and also observed

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around the explants in the culture media. Huge number of explants is destroyed in the culture due to endogenous bacteria (Uddin et al. 2007, Hadiuzzaman et al. 2001). Hence farmer community is facing the problem of nonaviability of healthy suckers.

Different explants have been used for banana and plantain propagations. Although, *in vitro* propagation of bananas using shoot tips has been reported for many commercial cultivars (Kulkarni et al. 2004, 2006), male inflorescence can also be applied as a potential regenerable explant. Specifically, male inflorescence reduces contamination rate during micropropagation as compared to soil grown 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 fruits per bunch *in situ* (Resmi and Nair 2007). In this aspect, utilization of immature floral bud as explants material can favour minimal contamination rates compared to other tissues. This method of *in vitro* propagation demonstrates that hundreds of clones can be obtained successfully from a single flower of inflorescence (Loutfi and Chlyah 1998, Loutfi et al. 1997). Around 20 cultivars *viz.,* Dwarf Cavendish, Robusta, Monthan, Poovan, Nendran, Red banana, Safed Velchi, Basrai, Ardhapuri, Rasthali, Karpurvalli, Karthali and Grande Naine etc., are commercially cultivated. Although several commercial and elite clones have been induced to embryogenesis, a large number of banana genotypes still need to be explored for embryogenic potential for use in propagation and genetic improvement. The protocol for mass multiplication of this indigenous banana cv. Puttabale has not yet been standardized so far. In this regards, *in vitro* protocol has been standardised for high frequency regeneration through flower derived callus.

Materials and Methods

Immature male inflorescences of banana cv. Puttabale was collected from farmyard of Shimoga district, Karnataka as an explant material. The male bud was shortened to 6 - 8 cm in length by removing the enveloping bracts and these explants were sterilized in 2% $NaCl_2$ solution for about 5 min and rinsed three - five times with sterile distilled water. The immature young flowers of 2 cm in length were cultured on callus induction medium.

The callogenic media consisted of MS with 40 g/l sucrose, 8 g/l agar, 160 mg/l adenine sulfate, 100 mg/l tyrosine. The growth regulators 2, 4-D and BAP were tested at the range of 1.0 – 10.0 mg/l and BAP 0.5 – 5.0 mg/l, respectively and pH was adjusted to 5.8 before autoclaving at 121ºC for 20 min. The cultures were incubated at 25 ± 2ºC with 12 hrs photoperiod and the callogenic frequency was evaluated after 40 days with 10 replications for each plant growth regulators.

The calli were subcultured on to the shoot differentiating media containing MS with 160 mg/l adenine sulfate, 100 mg/l tyrosine. The combined effect of BAP and TDZ were tested. BAP 1 - 5 mg/l and TDZ 0.1 - 0.5 mg/l, respectively. The mean number of multiple shoots differentiated per callus mass was evaluated after four weeks of inoculation. The individual microshoots were harvested from the clump and transferred aseptically to maturation medium augmented with 0.2 to 1 mg/l NAA, 0.2% activated charcoal, 4% sucrose and 0.8% agar and incubated at 25 ± 2ºC, 12 hrs photoperiod, provided by cool white fluorescent tubes (Philips, India, 1000 Lux) and 65 to 70% relative humidity. The number and length of shoots per explants were statistically evaluated after four weeks of culture using one way ANOVA (0.98 version) and significance was determined at 5% level (Duncan 1955). The well rooted plantlets were hardened in coco pits containing equal proportion of garden-soil, sand and cattle dung manure in polythene house for a period of 2 - 3 weeks. Then the plantlets were subjected to secondary hardening in polythene bags in the green house.

Results and Discussion

Immature floral buds (*Musa paradisica* cv. Puttabale) were isolated aseptically and cultured on callus induction medium with different concentrations of 2,4-D and BAP for initiating callus formation.

When immature floral bud placed in medium, they became enlarged into ten-folds of its normal size, gradually initiation of callus was noticed from the excised margin within 30 - 35 days and upon subculturing on to the same media. Callogenesis was proceeded all over the surface of the explant and appeared in the form of whitish transparent flashy juicy and compact undifferentiated mass. The different concentrations of 2,4-D and BAP were used (Table 1). Among the best result of callus formation was obtained (97.5%) at 7.0 mg/l 2,4-D and 1 mg/l BAP. Callus formation was found to be good, however, subculturing on same medium these aggregates became brownish and no embryo like growth or further differentiation took place. For further development callus was transferred to shoot differentiation medium.

The interaction of exogenous BAP at higher levels (2.0 – 5.0 mg/l) and TDZ at lower levels (0.1 - 0.5 mg/l) provoked multiple shoot differentiation from the calli. The mean number of shoots organized per callus mass and mean length of shoots per callus at each combination of BAP and TDZ is shown in the Table 2. Tongdee and Boon-Long (1973) induced callus in fruit explants from different clones of *Musa* without regeneration of shoot buds and callus induction from discs of the inflorescence axis was also achieved by Rao et al. (1982) but they were able to regenerate only roots from callus without any shoot bud. In the present investi- gation BAP alone at 2 - 5 mg/l did not induce the shoot differentiating potency of the flower calli. On the contrary, interaction with the

TDZ at 0.1 - 0.5 mg/l would provoke multiple shoot differentiation from the flower calli. The shoot multiplication was optimized at 4 mg/l BAP and 0.4 mg/l TDZ with a mean of 29.40 ± 6.10 shoots per callus. On differentiating media, the whitish nodules formed on callus. On further incubation on same medium formation of photosynthetic loci appeared from the tip of the nodules and these photosynthetic loci were transformed in to shoot buds. In a 25-day-old culture the shoot buds grew up (Fig. 1) well with 2 - 3 leaf primordia and mean of 10.28 ± 0.75 length of shoots/explants was observed.

Table 1. Effect of 2,4-D and BAP on the frequency of callus formation from the immature male flower of *M. paradisica* cv. Puttabale.

Growth regulators		Callus formation (%)
2,4-D (mg/l)	BAP (mg/l)	
5	0.5	17.5
5	1.0	27.5
5	1.5	22.5
5	2.0	12.5
6	0.5	57.5
6	1.0	62.5
6	1.5	32.5
6	2.0	82.5
7	**0.5**	**97.5**
7	1.0	72.5
7	1.5	57.5
7	2.0	52.5
8	0.5	62.5
8	1.0	57.5
8	1.5	57,5
8	2.0	22.5
9	0.5	52.5
9	1.0	45.0
9	1.5	15.0
9	2.0	7.5

The value of each combination consisted of percentage of callus induction from the three leaf discs of 4 × 10 replicates.

The induction of roots depends on the composition of mineral nutrients and growth regulators. Shoot buds were transferred to MS with 0.6 mg/l NAA, 0.2% activated charcoal. Roots were initiated from the base of microshoots after three weeks of culture. Similar result obtained by Berg and Bustamante (1974) with 1.0 mg/l NAA in the culture medium shows better rooting than IAA or IBA. The

well rooted plantlets were hardened primarily in coco pits containing equal proportion of garden soil, sand, and cattle dung manure in polythene house for a period of two weeks and then subjected to secondary hardening in perforated polythene bags in green house.

Table 2. Effect of BAP and TDZ on shoot bud differentiation from the immature male flower calli of *M. paradisica* cv. Puttabale.

Growth regulators		Mean number of shoot/explants	Mean length of shoots formed/explants (cm)
BAP (mg/l)	TDZ (mg/l)	(Mean ± SD)	(Mean ± SD)
2	0.2	3.90 ± 1.20	1.08 ± 0.23
2	0.3	4.30 ± 1.34	1.37 ± 0.47
2	0.4	6.10 ± 1.97	1.33 ± 0.26
2	0.5	5.20 ± 1.75	1.24 ± 0.30
3	0.2	8.50 ± 1.58	2.68 ± 0.36
3	0.3	9.30 ± 2.21	3.01 ± 0.19
3	0.4	10.20 ± 2.25	3.67 ± 0.33
3	0.5	6.30 ± 1.89	2.45 ± 0.36
4	0.2	11.70 ± 1.77	6.36 ± 0.53
4	0.3	19.20 ± 4.92	7.33 ± 0.45
4	**0.4**	**29.40 ± 6.10**	**10.28 ± 0.75**
4	0.5	22.50 ± 4.17	8.71 ± 0.45
5	0.2	3.40 ± 3.03	3.77 ± 0.33
5	0.3	4.60 ± 2.37	3.75 ± 0.58
5	0.4	4.10 ± 1.10	3.41 ± 0.17
5	0.5	2.50 ± 1.65	2.16 ± 0.21
F - value		76.0	483.0

The value of each combination consisted of ± S.D. of 10 replicates. The F value is significantly different when $p < 0.05\%$.

Many researchers standardized the protocol of different banana cultivars and commercialized for mass multiplication through clonal propagation. In present study high frequency of plantlets regeneration through immature floral bud callus was developed and further work on genomic analysis of *in vitro* grown regenerants and induction of somatic embryogenesis is in progress.

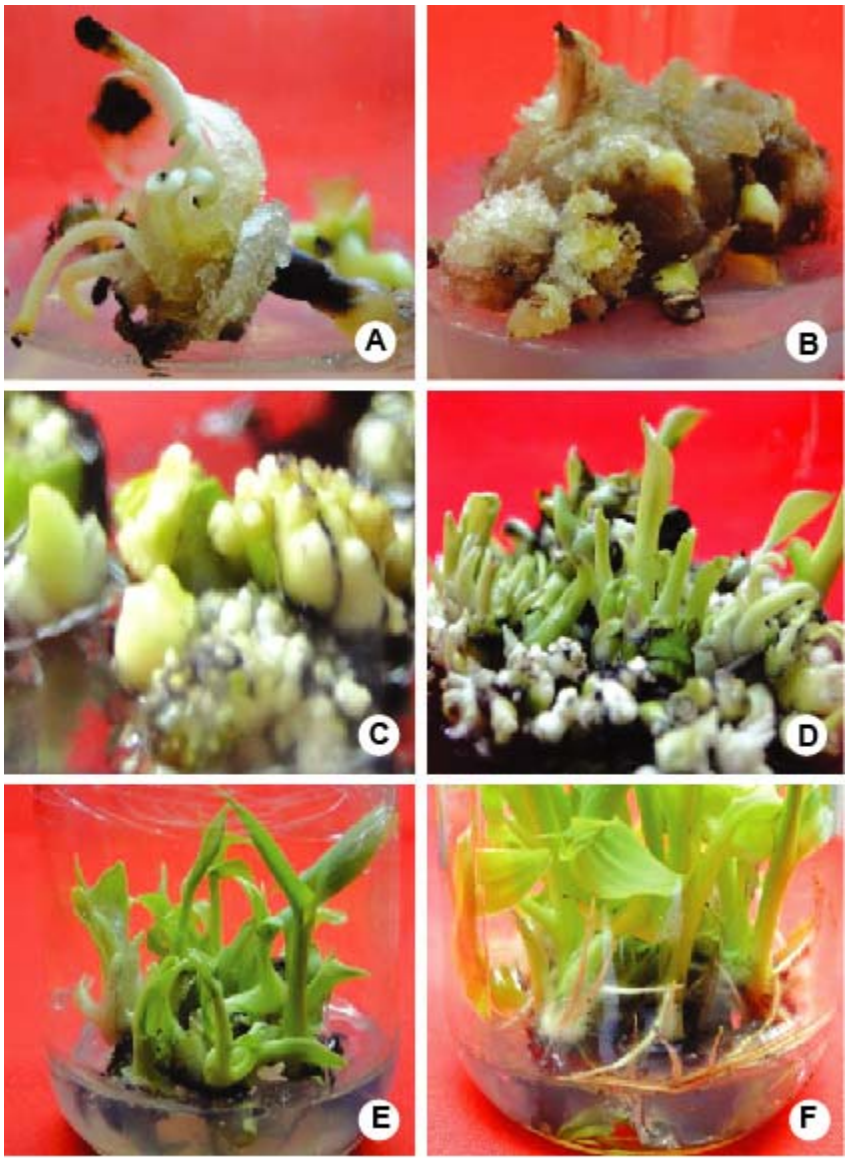

Fig. 1. High frequency regeneration of plantlets from immature male floral explants of *Musa paradisica* cv. Puttabale. A. Callus initiation from the male floral inoculum MS + 7 mg/l 2, 4-D + 0.5 mg/l BAP. B. Callogenesis was preceded all over the surface of the explants on same medium. C. Initiation of shoot bud on from the flower callus on MS + 4 mg/l BAP +0.4 mg/l TDZ. D. Sprouting of multiple shoot buds. E. Growth of multiple shoots on differentiation media. F. Root induction and complete plantlet development on MS + 0.6 mg/l NAA + 0.2% activated charcoal.

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