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Micropropagation and Field Performance of 'Malbhog' (*Musa paradisiaca*, AAB group): A Popular Banana Cultivar with High Keeping Quality of North East India

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ABSTRACT

A method has been developed to multiply 'Malbhog' (*Musa paradisiaca* AAB group) *in vitro* and to transfer it to the field. It is a very popular banana cultivar in the North Eastern part of India. Meristematic tips were cultured on agar-solidified Murashige and Skoog (MS) medium supplemented with 3% sucrose and fortified with various cytokinins [6-benzyl aminopurine (1-7.5 mg/l), kinetin (1-7.5 mg/l), thidiazuron (TDZ) (0.05-0.44 mg/l)] alone, or in combination with an auxin [indole-3-acetic acid, α-naphthalene acetic acid or IBA (indole-3-butyric acid)], each at 0.5-2.0 mg/l to optimize the suitable concentrations of plant growth regulators. Among the combinations tested, MS + 0.11 mg/l TDZ produced the maximum number of shoots (45 shoots/explant) 8 weeks after inoculation. Subsequently, plantlets rooted *in vitro* with MS supplemented with 1 mg/l IBA (6 roots/explants) within 2 weeks, hardened in the greenhouse and transferred to the field with 95% survival, 5 months after inoculation. Even though micropropagated plants produced more leaves, grew faster and had more suckers than their vegetative counterparts, there was no significant variation in physio-chemical analyses and fruit morphology of the ripened fruits between the two plant types. Micropropagated plants were genetically uniform to donor plants, as indicated by random amplified polymorphic DNA analysis.

Keywords: field performance, genetic fidelity, *in vitro* regeneration, micropropagation

INTRODUCTION

Banana (*Musa* spp.) is the fourth most important food crop in the world as well as in India (Ganapathi *et al.* 1999). It is a staple food and export commodity. It contributes to the food security of millions of people in the developing world and, when traded in local markets, provides income and employment to rural populations. India is a leading country in the world from the perspective of banana production. However, India cannot export a substantial amount of bananas mainly due to postharvest problems. More specifically, due to short shelf-life after ripening, it is almost impossible to export fresh bananas (Anonymous 2001). The North-East of India, including North Bengal, is one of the centres of origin and hence a wide range of diversity exists (Uma *et al*. 2005) in this region. Several banana ecotypes have also received the status of cultivar. For example, 'Malbhog' (AAB group), which is very popular and with high commercial value, has a high demand on markets due to its sweet aroma, taste and higher postharvest life. However, shortage of planting material and synchronisation of fruit ripening are two major bottlenecks that cause unavoidable trouble to local banana growers. There is thus a need to establish a micropropagation protocol for this banana cultivar.

A review of the literature revealed that micropropagation of banana has been dealt with to a great extent. For example, many researchers (Doreswamy *et al*. 1983; Cronauer and Krikorien 1984; Vuylsteke and De Langhe 1985; Arimative *et al*. 2000; Gubbuk and Pekmezci 2004) investigated the role of plant growth regulators (PGRs) on the performance of banana micropropagation. Some groups (Doreswamy and Sahijram 1989; Harirah and Khalid 2006; Kulkarni *et al*. 2006; Bakry *et al*. 2009) studied how media formulation and explant choice control the organogenic response. Madhulatha *et al*. (2004) and others (Kodym and Zapata-Arias 1999; Roels *et al*. 2005) evaluated the effects of variation of some physical parameters such as liquid pulse treatment, natural light, temporary immersion system, etc. on the rate of multiplication. Sheela and Nair (2001) examined field performance of micropropagated plants and subsequently compared with that of sucker-derived plants. However, except for one preliminary report (Acharjee *et al*. 2004) without much detail, no systematic study on this cultivar has been reported. Moreover, it is possible to obtain disease-free (Vuylsteke 1989; Haq and Dahot 2007) as well as synchronous plantlets in banana through *in vitro* propagation.

In addition, it is also well-documented that the response of banana tissues *in vitro* is highly genotype specific. For instance, the response of AAB type banana is much poorer than that of AAA (Wong 1986; Bhagyalakshmi and Sing 1995; Hirimburegama and Gamage 1997; Roels *et al*. 2005). In AAB, the growth of auxiliary buds *in vivo* is inhibited by a high degree of apical dominance. Conversely, many well-developed suckers are often observed in AAA. Ortiz and Vuylsteke (1994) suggested that apical dominance in AAB banana is controlled by a major recessive gene (*ad*), while the dominant allele (*Ad*) is probably fixed in bananas. The *Ad* gene controls the production of GA_3 which is antagonistic to the inhibition factor (apical dominance).

Keeping the above in view, the main objectives of the present work were to develop a rapid method for the *in vitro* propagation of banana cv. 'Malbhog' for large-scale produc-

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tion and to evaluate plantlet performance under field conditions.

MATERIALS AND METHODS

Explant preparation and culture conditions

Healthy meristematic shoot tips from sword suckers of field-grown plants were used for the establishment of an initial culture. The explants (mainly suckers) were collected from the Banana Germplasm Collection Centre of Uttar Banga Krishi Viswavidyalaya (UBKV), Pundibari, Cooch Behar, India. Subsequently, the suckers were brought into the laboratory and washed thoroughly in running tap water for about 20 min then dried in the sun for 2-3 days. After that, leaves and extraneous rhizomes were removed with a knife and trimmed suckers were again washed with Tween-80 (Himedia Laboratories Pvt. Ltd., India). Traces of detergent were removed by repeated washes with running tap water. Leaves and extraneous rhizomes were further shortened with a knife then treated with a fungicide Bavistin (1 mg/l) (BASF India Pvt Ltd., India) and tetracycline (0.05%) (Himedia) for 3 h and for 1 h in ascorbic acid (5%) (Himedia) to reduce phenolic exudation. Thereafter, explants (tips) were soaked in rifampicin (Himedia) for 1 h followed by repeated rinses (4-6 times) with distilled water, after which they were surface sterilized with 0.2% mercuric chloride ($HgCl_2$) (Himedia) in the presence of 1-2 drops of Tween-80 for 15 min inside a laminar air hood. To remove traces of $HgCl_2$, explants were further washed several times with autoclaved sterile distilled water. Finally, explants were cut into 1-1.5 cm containing at least 3-4 leaves enclosing one or more axillary buds. The surface-sterilized explants were carefully inoculated into Murashige and Skoog basal medium (1962) fortified with various concentrations of PGRs (all Himedia): BA (6-benzylaminopurine; 1.0-7.5 mg/l), Kn (kinetin; 1.0-7.5 mg/l), IAA (indole-3-acetic acid; 0.5-2.0 mg/l), IBA (indole-3-butyric acid; 0.5-2.0 mg/l), NAA (α-naphthaleneacetic acid; 0.5-2.0 mg/l). TDZ (thidiazuron; 0.05-0.44 mg/l) (Sigma-Aldrich) was also added to the medium alone or in combination with IAA (0.5 mg/l). The single dose of IAA was selected based upon the available literature (Gubbuk and Pekmeci 2004). All other PGR trials constituted first-time experiments for 'Malbhog'. All media were solidified with agar (0.7%, w/v; Himedia) and sucrose (3%, w/v) after adjusting the pH to 5.8. The cultures were then incubated at 25 ± 1°C under a 12-h photoperiod at 2000 lux and 55% relative humidity. The PGRs were added to the medium before autoclaving at 121°C at 15 psi (1.06 kg/cm^2) for 15 min. Explants were sub-cultured into fresh medium every 3 weeks. After each subculture, the number of shoots per explant and shoot length were determined. The experiment was repeated three times with at least three replicates for each treatment.

For liquid culture (20 ml), the experiment was conducted using the best PGR combination established on the agar-based system i.e., MS + 5 mg/l BA + 0.11 mg/l TDZ minus agar. For the dark treatment, the experiment was executed exactly as above, except that cultures were covered with a dark, thick cloth for 21 days.

In vitro rooting and hardening

For rooting, individual shoots (3-4 cm) with 5-6 leaves were transferred to solid MS medium with various concentrations of IBA for 4 weeks. Only shoots which derived from the solid media treatments were used for root induction. Well-developed plants were removed carefully from 250-ml jam bottles containing 50 ml of media, capped with polypropylene lids and washed carefully with running tap water to remove agar from plantlet roots. Plantlets were planted in root trainers containing autoclaved hardening mixture consisting of organic manure, vermiculite and soil (1: 1: 1) for 6-8 weeks and maintained in controlled humidity (70%) and temperature (25°C) conditions in a greenhouse, after which they were ready for transplantation to the field.

Field performance and biochemical analysis of ripened fruits

To evaluate field performance, one-year-old well-hardened plants 12-14 cm in height were planted in a 2 m × 2 m spacing (plant-to-plant and row-to-row distance) along with vegetatively propagated 'Malbhog' plants of the same age at the Experimental Farm of Uttar Banga Krishi Viswavidyalaya following a randomised block design. Cultivation practices such as fertilizer application and irrigation followed conventional protocols (Chattopadhyay *et al*. 2001). Morphological parameters such as plant height, leaf number, stem diameter and reproductive characters such as flower duration and number of fruits/bunch were recorded. Mature ripe banana fruits around 12 cm long from micropropagated as well as vegetatively propagated 'Malbhog' plants were harvested and used for all chemical analysis. The whole experiment was repeated in triplicate with 20 samples per replication.

Physical determination

1. Texture analysis

To analyze the texture (TA-XT plus, Stable Microsystem, UK), the whole fruit (ripe and with peel; n = 20) was cut with a blade set with knife at a speed of 1.00 mm/s, test speed of 2.00 mm/s and post-test speed of 10 mm/s. The trigger force was 20 × *g* with a travelling distance of 10 mm. The experiment was performed in triplicate to minimize the error.

2. Estimation of pH

The pH was determined by a glass electrode pH meter (Cyberscan 510, EUTECH Instruments Singapore) using pH 4.0 and 7.0 buffers for calibration. Ripe banana fruits (n = 20) from micropropagated as well as sucker-derived plants were tested. The whole examination was repeated in triplicate.

3. Estimation of titrable acidity

Titrable acidity (TA) was determined as described by Ranganna (1977). Ripe fruits (n = 20) from both micropropagated and sucker-derived plants were used for the estimation. Briefly, the TA of banana was estimated by titrating the sample extract in water against 0.1 N NaOH. To do so, 5 g of ripe banana pulp was turned into a paste with distilled water by a mortar and pestle. The solution was then filtered through filter paper (Whatman No. 4). Subsequently, the volume was made up to 100 ml with sterile distilled water. A 20 ml sample solution was poured in a conical flask and a few drops of phenolopthelin (Merck, India) were added. The solution was titrated against 0.1 N NaOH. The end of titration was indicated by a light pink colour. The experiment was repeated in triplicate to minimize experimental error.

Chemical determination

For all the chemical analysis 20 ripe banana fruits of both micropropagated and sucker-derived plants were used and the whole experiments were repeated three times.

1. Determination of sugars

Total and reducing sugars were determined according to the method of Ranganna (1977). Briefly, to determine the reducing sugars, 5 g of banana pulp (after removing the outer-skin) was turned into a paste with distilled water in a mortar and pestle. This paste was filtered (Whatman No. 4) and an equal volume of Fehling's solutions (A and B) was poured into a conical flask with 15 ml of distilled water. After heating the solution until the mixture began to boil (around 100°C), two drops of methylene blue indicator were added. The sample was placed in a burette and titrated against this warm Fehling's solution. The end point was indicated by the appearance of a brick-red colour. To estimate total sugar, a banana sample was placed in a conical flask with 1 ml of HCl (1: 1) and kept overnight. On the next day, two drops of phenolopthelin indicator were added, neutralized with 0.1 N NaOH and titrated as described above for reducing sugar.

2. Determination of soluble solids

Total soluble solid (TSS) content of banana pulp was determined by a digital hand refractometer (Henan Hi-Tech Instruments Co. Ltd., China) on a scale of 0-45 °Brix at 25°C. The experiments were repeated in triplicate.

3. Estimation of β-carotene

β-Carotene was determined by a colorimetric method (Ranganna 1977). Around 5 g of ripe whole banana fruits was crushed with 10 ml of acetone in a mortar and pestle. All supernatants were placed in a separating funnel. Then 15 ml of petroleum ether was added to the funnel and mixed thoroughly. After some time, the solution naturally divided into two layers. The lower layer was discarded. The upper layer was collected into a volumetric flask and the volume was made up to 100 ml with petroleum ether and optical density was recorded at 452 nm with a spectrophotometer (Hitachi, Japan). The whole experiment was repeated in triplicate with 20 samples/ replicate.

4. Estimation of protein

Protein was determined following the protocol of Lowry *et al.* (1951). Around 0.5 g of banana pulp was ground in water with a pestle and mortar. After centrifugation, the supernatant was collected and absorbance was recorded at 660 nm by a spectrophotometer (Hitachi Co. Ltd.). The whole experiment was repeated in triplicate with 20 samples/ replicate.

PCR-compatible DNA isolation and DNA fingerprinting

Genomic DNA was isolated from the young leaves of randomly selected micropropagated shoots to test for genetic fidelity following a CTAB method (Rogers and Bendich 1985). The purity of DNA was checked on a 0.8% agarose gel and also from the values obtained by the 260/280 nm UV absorbance ratio. Isolated DNA from *in vivo* donor and micropropagated plants were subjected to PCR to generate fingerprinting patterns using a total of 40 random decamer primers (20 OPA and 20 OPE) obtained from Operon Technologies (Alameda, CA). DNA was amplified in a thermal cycler (iCycler, Biorad, USA). The 25 µl reaction mixture contained 10X PCR buffer, 2 mM $MgCl_2$, 100 mM dNTP (Bangalore Genei Ltd., India), 200 mM decamer random primers, 25 ng of template DNA and 0.5 U *Taq* DNA polymerase (Bangalore Genei). The PCR reaction conditions were: One initial cycle of 5 min at 94°C, followed by 35 amplification cycles of 1 min at 94°C, 1 min at 35°C and 2 min at 72°C, then a final extension step of 7 min at 72°C. A 15-µl sample of each reaction mixture was then subjected to 1.4% agarose gel electrophoresis and stained with ethidium bromide (10 mg/ml). A 1-kb ladder (Bangalore Genei) was used as the DNA marker and the amplified fragments were visualized under UV light and documented by a gel documentation system (BioRad, Hercules, CA). PCR analyses were repeated at least twice to check reproducibility.

Statistical analysis

The experiments were set up in a randomized block design. Data were analyzed using analysis of variance (ANOVA) to detect significant differences among the means (Sokal and Rohlf 1987), expressed as the mean ± standard error (SE). Means differing significantly were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$ with STATISTICA software ver. 5.0 (StatSoft Inc. 1995).

RESULTS AND DISCUSSION

Initiation and multiplication

Since the initial explants were collected from adult field-grown plants, establishment of an aseptic culture was a big challenge due to high contamination and phenolic exudation. We also found that the rate of contamination during September to October was as high as 50% and lowest (20%) during January to February (data not shown). Even so, the initial aseptic culture turned black due to phenolic exudation which forced us to treat it subsequently with an antioxidant, ascorbic acid (5%), for 1 h before inoculation, which markedly reduced phenolic exudation.

For multiplication, sprouting shoot tips were initially cultured on MS basal medium supplemented with various PGRs. When Kn, either alone or in combination with various auxins such as NAA, IAA, IBA was used, a maximum of 5.1 shoots/explant formed with 5 mg/l Kn and 1 mg/l NAA after 12 weeks (**Table 1**). An increase or decrease in the concentration of either PGR did not improve the multiplication rate. Rather, prolonged sub-culture resulted in the formation of very hard woody-like callus at the shoot base; separating this callus often resulted in death of the culture. To increase the multiplication rate, BA with various concentrations of auxin such as NAA, IAA and IBA was evaluated. BA at 5 mg/l + 1 mg/l IAA resulted a maximum of 8.23 shoots/explant. Moreover, the multiplication rate did not increase that much when higher or lower concentrations of these two PGRs were used. These conventional PGRs have been used for micropropagation of banana by several other groups. For example, Cronauer and Krikorian (1984) carried out micropropagation of banana with the cultivar 'Philippine Lactum' (AAA), 'Grande Naine' (AAA), 'Saba' (ABB), 'Pelipita' (ABB) and found that 5 mg/l BA was the best for shoot multiplication. Similarly, Mendes *et al.* (1996) observed multiplication of shoot tips of cv. 'Naincao' on MS media supplemented with 4.5 mg/l BA. In a separate study, Abeyaratne and Lathiff (2002) achieved an average of 10 shoots/ explant on banana 'Rathambala' when cultured on 10 mg/l BA + 0.75 mg/l IAA. Kalimuthu *et al.* (2007) reported that 3 mg/l BA + 0.2 mg/l NAA were most suitable for micropropagation of 'Cavendish Dwarf' (7-8 shoots/explant). Karim *et al.* (2009) noticed highest (> 6 shoots/expant) shoot proliferation in 'BARI-1' on 7.5 mg/l BA + 0.5 mg/l NAA. Further, to improve the multiplication rate, we decided to check the effect of a cytokinin analogue such as TDZ alone or with IAA. While the control (i.e., without TDZ) showed the lowest multiplication rate (0.73 shoots/explant), shoot proliferation was highest (45 shoots/explant) with 0.11 mg/l TDZ after 12 weeks of subculturing (**Table 2**). Additionally, TDZ + IAA did not yield a better multiplication rate compared to TDZ alone. The positive effect of TDZ on micropropagation of banana has also been shown by several other groups (Arinaitwe *et al.* 2000; Gubbuk and Pekmezci 2004). However, to the best of our knowledge, none of them reported such a high multiplication rate (45 shoots/explant), especially for the genotype AAB. Arinaitwe *et al.* (2000) compared various traditional cytokinins (16.8-28.8 µM) and lower concentrations (0.045-9.1 µM) of TDZ for *in vitro* multiplication of three cultivars ('Kibuzi' (AAA), 'Ndiziwemiti' (ABB) and 'Bwara' (AAA)) and found that TDZ as better for multiplication than other cytokinins. Shoot multiplication of 'Ndiziwemiti' progressively increased as the concentration of TDZ increased (9.5 shoots/ explant) whereas the proliferation rate of 'Bwara' and 'Kibuzi' decreased (2-5.4 shoots/ explant) with increasing concentration of TDZ. Gubbuk and Pekmezci (2004) used 0.4-3 µM TDZ, either alone or with 1 µM IAA, to obtain a high proliferation rate of three cultivars ('Alanya 5', 'Anamur 10' and 'Bozyazi 14') for micropropagation of banana. On average, > 7 shoots/explant were obtained in 2 µM TDZ alone or in combination with 1 µM IAA. Similarly, for the multiplication of 'Cavendish Dwarf' and 'Valery', there was a positive association between 0.15 mg/l TDZ and 2 mg/l IAA; 25 shoots/ explant were produced within 120 days of sub-culture (Farahani *et al.* 2008). Gubbuk and Pekmezci (2004) and Shirani *et al.* (2009) both reported that the response of multiplication of banana is influenced by different PGR concentrations and genotypes. Youmbi *et al.* (2006) also reported that the concentration of TDZ to achieve best multiplication is not fixed but is rather genotype dependent. TDZ is stable and more

Table 2 Effect of different concentration of TDZ along with IAA and TDZ alone on micropropagation of banana.

Treatment (µM)	No of shoots/explant	Shoot length (cm)
MS 0	0.73 h	0.56 h
0.05 TDZ + 0.5 IAA	16.83 d	3.36 de
0.11 TDZ + 0.5 IAA	23.66 c	3.96 c
0.22 TDZ + 0.5 IAA	11.03 e	2.50 f
0.33 TDZ + 0.5 IAA	7.40 f	2.63 f
0.44 TDZ + 0.5 IAA	3.93 g	1.70 g
0.05 TDZ	26.33 b	4.30 b
0.11 TDZ	45.00 a	4.80 a
0.22 TDZ	17.26 d	3.60 d
0.33 TDZ	8.43 f	3.10 e
0.44 TDZ	4.33 g	2.50 f

Data (mean ± SE) pooled from three independent experiments. Means followed by the same letters within a column do not differ significantly according to DMRT.

active at lower concentrations than adenine-based cytokinins (Mok *et al*. 1987; Hutteman and Preece 1993).

Further, to improve the multiplication rate, we compared static liquid vs. solid medium using the best media formulation. However, solid medium was better than liquid (data not shown). Although liquid media is better for micropropagation of a number of plants (Escalona *et al*. 1999) and banana in particular (Alvard *et al*. 1993), poor performance of liquid culture in our experiment may be attributed to high phenolic exudates. In addition, we observed the formation of hyperhydric structures, which is quite common in liquid culture of several plant species.

In vitro rooting and hardening

Well-developed shoots (3-4 cm) were transferred to rooting medium consisting of MS + 1.0 mg/l IBA for 4 weeks. Around 97% (data not shown) of shoots produced an average of 6 well-developed roots with each round of sub-culture (within 4 weeks). These were ~8 cm long and coiled inside the culture vessel. These findings are in accordance with the observations made by others (Mante and Tepper 1983; Cronauer and Krikorian 1984) where IBA was used for rooting. Mante and Tepper (1983) found that MS media with either NAA (0.1-1 mg/l) or IBA (2-10 mg/l) was best for root formation in *Musa textilis*. Similarly, Cronauer and Krikorian (1984) used IAA (1 mg/l), NAA (1 mg/l) or IBA (1 mg/l) for *in vitro* rooting of 'Philippine Lactum', 'Grand Naine', 'Saba' and 'Pelipta' banana cultivars. Two other auxins, 1 mg/l IBA (Nandwani *et al*. 2000) or 1 mg/l NAA (Kulkarni *et al*. 2006) was also used for *in vitro* rooting of banana. Plantlets with distinct roots and shoots were transferred to plastic pots (**Fig. 1I**) where 92% of plantlets survived (data not shown). After 24 weeks, they were finally transferred to the field (**Fig. 1J**) and after 5 months the survival rate of plants was ~95%.

Analysis of genetic fidelity by RAPD marker

RAPD markers have been applied to many plant species, including banana (Damasco *et al*. 1996; Teixeira da Silva 2005; Teixeira da Silva *et al*. 2005; Venkatachalam *et al*. 2007) to evaluate clonal fidelity and genetic stability among micropropagated plants and its donor plant. Venkatachalam *et al*. (2007) analysed dessert banana cv. 'Nanjanagudu

Table 1 Effect of different PGRs on micropropagation of banana.

Treatments		Characters		Treatments		Characters	
		No. of shoots/explant	Shoot length (cm)			No. of shoots/explant	Shoot length (cm)
MSO (Control)		0.73 n	0.66 m	MSO (Control)		0.70 p	0.45 m
1.0 Kn	0.5 IAA	1.06 n	1.20 l	1.0 BA	0.5 IAA	2.50 jkm	1.76 ik
	1.0 IAA	1.40 n	1.56 l		1.0 IAA	2.66 ij	1.96 hik
	2.0 IAA	1.23 n	1.20 l		2.0 IAA	2.00 mn	1.70 ik
	0.5 IBA	1.00 n	1.40 l		0.5 IBA	1.30 o	1.30 l
	1.0 IBA	1.56 m	1.57 k		1.0 IBA	1.86 n	1.63 l
	2.0 IBA	1.16 n	1.70 ijk		2.0 IBA	1.86 n	1.40 l
	0.5 NAA	1.26 n	1.40 l		0.5 NAA	1.60 o	1.16 l
	1.0 NAA	2.21 ij	2.16 h		1.0 NAA	2.23 lm	1.56 l
	2.0 NAA	2.00 jk	1.70 ijk		2.0 NAA	2.16 m	1.53 l
2.5 Kn	0.5 IAA	1.53 m	1.63 jk	2.5 BA	0.5 IAA	3.90 f	2.50 fg
	1.0 IAA	3.06 cd	2.16 gh		1.0 IAA	4.26 e	2.83 def
	2.0 IAA	1.80 lm	2.03 hi		2.0 IAA	3.90 f	1.96 hik
	0.5 IBA	1.63 m	1.36 l		0.5 IBA	2.20 m	1.70 k
	1.0 IBA	2.23 ij	2.10 h		1.0 IBA	2.86 ij	2.16 gh
	2.0 IBA	2.20 ij	2.33 fgh		2.0 IBA	2.53 j	2.00 hik
	0.5 NAA	2.48 hi	1.96 hij		0.5 NAA	2.03 mn	1.60 l
	1.0 NAA	2.65 fg	2.56 efg		1.0 NAA	2.60 ij	3.26 bc
	2.0 NAA	3.00 defg	2.80 cde		2.0 NAA	2.16 m	2.03 hik
5.0 Kn	0.5 IAA	2.16 ij	3.00 bcd	5.0 BA	0.5 IAA	6.43 c	3.33 b
	1.0 IAA	3.43 c	3.30 b		1.0 IAA	8.23 a	4.20 a
	2.0 IAA	2.83 defg	3.03 bcd		2.0 IAA	6.90 b	2.63 ef
	0.5 IBA	2.70 fg	2.16 h		0.5 IBA	3.36 h	2.90 de
	1.0 IBA	4.10 b	3.13 bc		1.0 IBA	4.36 e	2.86 de
	2.0 IBA	2.76 fg	2.33 fgh		2.0 IBA	3.66 fgh	2.23 gh
	0.5 NAA	3.00 defg	3.10 bc		0.5 NAA	4.36 e	3.13 bcd
	1.0 NAA	5.10 a	4.16 a		1.0 NAA	4.73 d	3.33 b
	2.0 NAA	3.06 de	3.03 bcd		2.0 NAA	3.90 f	2.76 ef
Kn 7.5	0.5 IAA	1.20 n	1.46 l	7.5 BA	0.5 IAA	3.43 gh	2.06 ghi
	1.0 IAA	2.23 ij	1.70 ijk		1.0 IAA	4.23 e	2.96 de
	2.0 IAA	1.63 m	1.56 k		2.0 IAA	3.60 gh	2.80 def
	0.5 IBA	2.16 ij	1.53 l		0.5 IBA	1.73 o	2.20 gh
	1.0 IBA	1.60 m	2.20 fgh		1.0 IBA	1.60 o	1.73 ik
	2.0 IBA	1.36 n	1.56 k		2.0 IBA	1.63 o	1.70 k
	0.5 NAA	3.13 cd	3.20 b		0.5 NAA	1.83 o	1.53 l
	1.0 NAA	2.30 ij	3.30 b		1.0 NAA	1.86 o	1.93 hik
	2.0 NAA	2.96 defg	2.70 def		2.0 NAA	1.43 o	1.76 ik

Data (mean ± SE) pooled from three independent experiments. Means followed by the same letters within a column do not differ significantly according to DMRT

Fig. 1 Regeneration of cv. 'Malbhog' through multiple shoot formation. (**A**) Meristematic shoot tips. (**B**) Initiation of meristematic shoot tips. (**C-E**) Different stages of multiplication after 8 weeks. (**F-G**) Elongation of multiplied shoots. (**H**) Rooting of *in vitro* shoot. (**I**) Hardening of *in vitro* rooted plants in greenhouse. (**J**) Acclimatized plant in the field after 15 days of transplantation. (**K**) Fruiting of *in vitro* plants.

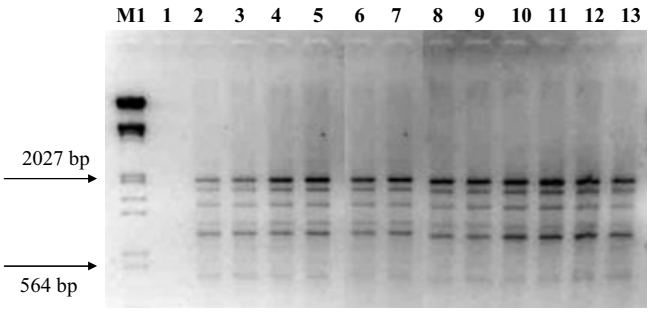

Fig. 2 RAPD profile of micropropagated banana cv. 'Malbhog' plants with OPE 04. Lanes: M1 = 1 kb ladder; No. 1 = positive control; No. 2 = donor plant; No. 3–13 = micropropagated plants.

Rasabale' to study genetic stability of regenerated and micropropagated plantlets by using RAPDs and inter simple sequence repeats (ISSR) markers. They did not observe genetically unstable plants grown *in vitro*. However, in micropropagation of banana, a wide range of somaclonal variation was detected by RAPDs especially in 'Williams' (AAA) (Damasco *et al*. 1996), 'Robusta' (AAA) and 'Giant Governor' (AAA) (Ray *et al*. 2006). In a separate study, clonal fidelity of *in vitro* grown banana cv. 'Berangan' derived from male-inflorescence was checked by RAPDs (Harirah and Khalid 2006), although no somaclonal variation was detected.

In our study, fingerprinting profiles of both micropropagated and donor plants were generated by 40 random 10-mer primers. Monomorphic RAPD profiles yielded a total of 125 alleles (**Fig. 2**), with an average of 3.01 fragments, ranging from 2 to 5 per primer, indicating homogeneity among the micropropagated plants and genetic uniformity with that of the donor (mother) plants. Characterization of micropropagated plants at the genetic level indicates that the protocol followed in our studies is capable of generating large numbers of clonal 'Malbhog' plants throughout the year, which is a prerequisite for conservation of *Musa* germplasm. The RAPD-based DNA fingerprinting profiles generated in micropropagated banana plants may be used to identify true-to-type clones, which is necessary for commercial trade.

Morphological and field performance

The success of micropropagation depends upon the performance of micropropagated plants in the field. In our experiments, not many significant differences were observed between donor and micropropagated plants (**Table 3**). During vegetative growth, micropropagated plants produced 44 leaves/plant whereas vegetative sucker-derived plants of same age i.e., one-year-old formed only 37 leaves/plant. The average height and girth of the pseudostem of micropropagated plants were 243 and 44 cm, while for sucker-derived plants these values were 240 and 39 cm, respectively. Importantly, flowering and harvesting occurred at least one month earlier in micropropagated plants than in conventional ones. Other parameters such as bunch weight, number of fingers, etc. did not vary much between the two plant sources. The observation recorded in this experiment agrees with the findings of several other groups who found that micropropagated banana grew faster and more vigorously than its conventionally propagated counter part (Drew

Table 3 Field performance of micropropagated and conventionally propagated plants.

Planting material	No of leaves	Height of pseudostem (cm)	Girth of pseudostem (cm)	Weight of bunch (kg)	Number of hands	Number of fingers	Number of fingers in / hand	Length of finger (cm)	Diameter of Fruits (cm)	Months taking for fruiting
Tissue culture plant	44.46 a	242.93 a	43.79 a	10.32 a	7.98 a	101.2 a	10.48 a	15.16 a	34.30 a	11.46 b
Vegetative suckers	37.13 b	240.0 b	38.79 b	8.50 b	6.88 b	94.35 b	9.09 b	14.81 b	33.75 b	12.24 a

Data was recorded after 12 months; Data (mean ± SE) pooled from three independent experiments. Means followed by the same letters within a column do not differ significantly according to DMRT.

Table 4 Comparative biochemical analysis of ripened banana between micropropagated and vegetatively propagated plants.

Planting material	Parameters					
	Total soluble solid (%)	Reducing sugar (%)	Total sugar (%)	Titratable acidity (%)	Total protein (%)	β-carotene (µg/100 g)
Micropropagated plants	26.88 a	7.83 a	20.78 a	0.50 a	1.1 a	0.07 a
Vegetatively propagated plants	25.60 b	6.70 b	19.50 b	0.45 a	0.96 b	0.03 a

Data (mean ± SE) pooled from three independent experiments. Means followed by the same letters within a column do not differ significantly according to DMRT.

and Smith 1990; Robinson *et al*. 1993). Drew and Smith (1990) reported that the superior performance of micropropagated bananas was due to the active root and shoot system of plants which exists at the time of planting. Liu *et al*. (1989) compared the performance of micropropagated versus conventional propagated banana plants and found that micropropagated plants grew vigorously and had significantly higher yield than conventionally propagated plants. Similarly, Vulysteke and Ortiz (1996) noticed healthier and taller micropropagated plants but they did not observe any higher yield of *in vitro* plants than conventionally propagated one. Zamora *et al*. (1989) reported that the yield of micropropagated banana was significantly higher than sucker-derived plants in 'Lakatan' but not in 'Bungulan'. Faster growth and higher yield were not always observed in *in vitro* propagated banana but pseudostem circumference and height of the plants were always significantly higher than sucker-derived plants (Kwa and Ganry 1990; Robinson and Anderson 1991).

Physical determination

All the measurements regarding physical properties of banana were carried out at the same stage of ripeness characterized by peel colour (fully yellow). The measured peel puncture strength of micropropagated banana was 1.36 kg whereas for vegetatively propagated banana, it was 1.04 kg indicating the same range for these two types of banana. Similarly, the estimated pH values of micropropagated and vegetatively propagated fruits were 4.5 and 4.2, respectively. Therefore, they were acidic in nature. The pH of 'Berangan' and 'Mas' cultivars were 4.54 and 4.84, respectively (Yousaf *et al*. 2006). In another study, the pH of three cultivars ('Spanish Enana', 'Spanish Gran Enana' and 'Latin-American Enana') ranged between 4.7 and 4.8 (Cano *et al*. 1997), which is in agreement with our result. Corresponding TA of these two types were 0.45 and 0.5%, respectively (**Table 4**). These values were similar with the TA values of 'Berangan' (0.51%) and 'Mas' (0.35%) (Yousaf *et al*. 2006).

Chemical analysis

In general, sugar is one of the main constituents of soluble solids in fruit and therefore TSS can be used as an estimate of sugar content. Moreover, organic acids, amino acids, and soluble pectin also contribute to soluble solids. The percentage of TSS of micropropagated and conventionally propagated banana was 26.88 and 25.6%, respectively (**Table 4**). Cano *et al.* (1997) found 24.56, 21.36 and 16.30% TSS in 'Spanish Enana', 'Spanish Gran Enana' and 'Latin-American Enana', respectively whereas Yousaf *et al.* (2006) reported 22.48 and 26.30% TSS in 'Berangan' and 'Mas', respectively. Sugars are an important source of carbohydrate in banana. The percentage of reducing sugar of micropropagated and vegetative banana was 7.83 and 6.7%, respectively while the percentage of total sugar was 20.78 and 19.5%, respectively (**Table 4**). Total protein was 1.1% in banana fruit derived from micropropagated plants but 0.96% from its vegetative counterpart (**Table 4**). Similarly, β-carotene content was 0.07 µg/100 g for the former and 0.03 µg/100 g for the latter (**Table 4**).

CONCLUSION

'Malbhog', a native banana cultivar of North Eastern India, is a popular cultivar to rural banana growers due to its higher keeping quality, better taste and aroma. For this reason there is an increasing demand of this cultivar which can be supported by rapid micrparopagation. The rate of multiplication was high (45 shoots/ explant) and the resulting clones showed no somaclonal variation following RAPD analysis. Subsequent evaluation of field performance demonstrated that the micropropagated plants flowered early with better vegetative growth. However, biochemical analysis of ripening fruit did not indicate much significant difference to fruits derived from conventionally propagated plants.

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