



RESEARCH ARTICLE

An efficient micropropagation protocol for the banana cultivar 'Malbhog' (*Musa paradisiaca* L., AAB group) for sustainable production

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Abstract

An efficient and reproducible protocol was developed for *in vitro* micropropagation of the commercially important banana cultivar 'Malbhog' (*Musa paradisiaca* L., AAB group) using swollen basal node explants. Explants were washed, pretreated, surface sterilized, and cultured aseptically on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP). Shoots elongated within 12–14 days of culture. The highest frequency of multiple shoot induction (78.66%) was obtained on MS medium containing 0.5 mg L⁻¹ IAA and 2.5 mg L⁻¹ BAP after the second subculture. The highest average number of 7.56 shoots per explant were produced with a mean shoot length of 5.36 cm after second subculture. Regenerated shoots rooted successfully on half-strength MS medium supplemented with 1.0 mg L⁻¹ α -naphthaleneacetic acid (NAA). After 15 days the rooted plantlets were acclimatized in sterilized soil, sand and farmyard manure (1:1:1), hardened under greenhouse conditions, and transferred to the field, where 75% of the plants survived.

Keywords: AAB group, Acclimatization, *In vitro* regeneration, *Musa paradisiaca* Shoot multiplication.

Introduction

Banana (*Musa* spp.) is one of the most important fruit crops in the world and ranks second among fruit crops in India after mango. It is valued for its high nutritional content, medicinal properties, year-round availability, and affordability, making it an important food source for people of all socioeconomic groups. India is the largest producer of bananas; however,

its export potential is limited because of the short shelf life of ripe fruits (Ganapathi *et al.* 1999). The edible parts of banana are rich in carbohydrates, minerals, potassium, phosphorous and other nutrient elements (Kumar *et al.* 2018). The North-Eastern region of India, including North Bengal, is considered one of the important centers of diversity for banana. As a result, several locally adapted cultivars with desirable agronomic traits are found in this region (Uma *et al.* 2005). Some banana ecotypes have also received a status of cultivars, among them, the cultivar 'Malbhog' (*Musa paradisiaca* L., AAB group) is highly valued because of its pleasant aroma, sweet taste, good fruit quality, and relatively longer post-harvest shelf life (Roy *et al.* 2010). Owing to these desirable characteristics, the cultivar has high commercial demand and is widely cultivated in eastern and north-eastern India.

Banana is propagated mainly through suckers. However, the natural rate of multiplication is slow, producing only 5–20 suckers per plant during its growth period. In addition, sucker-derived planting material often carries pathogens and insect pests, which reduce crop productivity and affect fruit quality. Therefore, the availability of healthy and disease-free planting material remains a major challenge for banana cultivation. Plant tissue culture offers an efficient alternative for the rapid multiplication of elite banana

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cultivars. Micropropagation enables the production of large numbers of genetically uniform, disease-free plantlets within a short period of time using a limited number of mother plants. It also ensures a continuous supply of quality planting material throughout the year, thereby supporting commercial cultivation and sustainable agriculture (Razdan 1993).

Although several protocols have been reported for the micropropagation of different banana cultivars, efficient regeneration methods are often cultivar-specific. There are a number of published studies describing successful *in vitro* regeneration protocols for *Musa* spp. by using shoot tip and other explants (Martin *et al.* 2006, Khaldun *et al.* 2007, Farhani *et al.* 2008, Srivastava *et al.* 2012, Rahman *et al.* 2013, Suman *et al.* 2013a, Ngomuo *et al.* 2014, Ferdous *et al.* 2015, Lakshmi *et al.* 2019, Selvakumkar and Parasurama 2020). However, only a few studies have been reported about micropropagation technique of banana cv. 'Malbhog' (AAB group). Hence, the present study aimed to formulate and standardize the micropropagation technique in banana cv. 'Malbhog' (AAB group) for the benefit of farmers. Information on *in vitro* propagation of the commercially important cultivar 'Malbhog' is still limited. Therefore, the present study was undertaken to develop a simple, efficient, and reproducible protocol for multiple shoot induction, rooting, and successful acclimatization of *M. paradisiaca* L. cv. 'Malbhog' (AAB group) using shoot tip explants.

Materials And Methods

Plant Material and Explant Preparation

Healthy suckers of *M. paradisiaca* L. cv. 'Malbhog' (AAB group) were collected from the experimental farm of the Department of Plant Breeding and Genetics, Nalanda College of Horticulture, Noor Sarai, Nalanda, Bihar, India. The outer leaf sheaths and roots were removed, and an apical shoot tip (2–3 cm long) containing the apical meristem and a small portion of the corm was excised and used as the explant.

Surface Sterilization

The excised explants were washed thoroughly under running tap water to remove adhering soil and debris. They were pretreated for 30 min in a solution containing 0.2% ascorbic acid, 0.4% citric acid, 0.1% Bavistin, and 0.1% streptomycin sulphate to minimize microbial contamination and tissue browning. The explants were then surface sterilized with 10% sodium hypochlorite solution for 10 min, followed by 0.1% mercuric chloride (HgCl₂) for 5 min. Finally, they were rinsed in sterile 1% potassium chloride (KCl) solution for 5 min and washed three to four times with sterile double-distilled water to remove traces of the sterilants. Under aseptic conditions, the outer two to three leaf sheaths were carefully removed, and the shoot tips

(1.0–2.0 cm) containing the apical meristem were used as explants for inoculation.

Culture Medium and Incubation

The explants were inoculated on solid Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with different concentrations and combinations of indole-3-acetic acid (IAA) and 6-benzylaminopurine (BAP). The cultures were maintained in culture tubes and bottles under aseptic conditions. The cultures were incubated in a temperature-controlled culture room at 25 ± 2°C with a relative humidity of 65–75%. A 16 h light/8 h dark photoperiod was provided using cool white fluorescent lamps at an intensity of approximately 2000 lux (Fig.1).

Shoot Multiplication and Root Induction

Shoots regenerated from the explants were subcultured on fresh MS medium containing the selected concentrations of IAA and BAP to enhance shoot multiplication. Individual shoots were separated from the cluster of regenerated shoots and transferred to rooting medium consisting of half-strength MS medium supplemented with NAA (0.5, 1.0, 1.5, 2.0 and 2.5) mg L⁻¹ NAA for one to three weeks.

Acclimatization of Plantlets

Well-rooted plantlets were gently removed from the culture vessels, and the adhering culture medium was carefully washed off with sterile distilled water without damaging the roots. The plantlets were transplanted into earthen pots containing a sterilized mixture of soil, sand and well-decomposed farmyard manure in a ratio of 1:1:1 for primary hardening for one month. The potted plants were maintained under greenhouse conditions for gradual acclimatization and hardening before transfer to the field.

Data Recording and Analysis

Observations were recorded at 10–15 day intervals. The parameters recorded included shoot elongation, percentage of cultures showing multiple shoot formation, number of shoots per explant, rooting response, and survival of hardened plantlets after field transfer. All experiments were conducted using at least three independent replicates. Data are presented as mean values. The treatment showing the highest response was identified based on the percentage of responding cultures and the mean number of shoots per explant.

Results And Discussion

The shoot tip explants (2–3cm) of *M. paradisiaca* L. cv. 'Malbhog' were inoculated in different concentrations of auxin (IAA) and cytokinin (BAP) alone and different combinations and concentrations used in the culture medium, showed positive response. The same response was observed by Kumar *et al.* (2024) and Kumari and Kumar (2025) in banana tissue cultures after using auxin (IAA) and

cytokinin (BAP). The cultured explants remained healthy after inoculation, and shoot elongation was observed within 12–14 days of culture on most of the media tested (Fig. 1, A–C). The step was repeated 2–4 times to get required number of shoots. The response varied depending on the concentration and combination of plant growth regulators (Table.1).

Shoot Elongation

Elongation of existing shoot was recorded in cultures grown on media containing either IAA or BAP alone, as well as their combinations (Table 1). Media containing lower concentrations of plant growth regulators produced better shoot elongation than those containing higher concentrations. Similar observations have been reported in other banana cultivars by Akbar and Roy (2006), Ali *et al.* (2011), Suman *et al.* (2013a) and Ahmed *et al.* (2014a), also reported that lower concentrations of auxin and cytokinin favoured shoot growth.

Multiple Shoot Induction

Multiple shoots originated from the basal region of the cultured shoot tips (Fig. 1D), which has also been reported by

Jafari *et al.* 2011. No multiple shoot formation was observed on MS medium supplemented with IAA alone (0.5–1.5 mg L⁻¹) as earlier reported by Suman *et al.* 2013a, although the culture showed elongation of the existing shoot. It indicates that auxin alone was insufficient to induce shoot proliferation. In contrast, BAP alone promoted multiple shoot formation, although the response was lower than that obtained with a combination of IAA and BAP. Among the treatments tested, MS medium supplemented with IAA (0.5 mg L⁻¹) and BAP (2.5 mg L⁻¹) showed the highest multiple shoot formation (78.66%) and mean number of shoots per explant (7.56) with a mean shoot length of 5.36 cm after the second subculture (Table 2). In a report published by Selvakumkar and Parasurama (2020) found that MS media containing lower concentration (2mg L⁻¹ BAP) with IAA (1mg L⁻¹) was the most favourable concentration for shoot regeneration of banana cultivars, Grand Naine and Elakki, but in the present investigation it was 0.5 mg L⁻¹ IAA and 2.5 mg L⁻¹ BAP which showed the optimum result. A comparable response (75.45%) multiple shoot induction was obtained on MS medium containing 0.5 mg L⁻¹ IAA and 3.0 mg L⁻¹ BAP producing an average of 6.62 shoots per explant, followed

Table 1: Effect of IAA and BAP on shoot elongation, shoot multiplication and number of shoots per explants in *M. paradisiaca* L. (AAB group) cv. 'Malbhog'.

Growth regulator (mg L ⁻¹)	Shoot elongation (%)	Multiple shoot formation (%)	Mean shoots per explant
MS+IAA (0.5 mg/L)	42.22	Nil	Nil
MS+IAA (1.0 mg/L)	24.89	Nil	Nil
MS+IAA (1.5 mg/L)	21.56	Nil	Nil
MS+IAA (2.0 mg/L)	18.32	Nil	Nil
MS+BAP (1.5 mg/L)	48.22	24.24	1.42
MS+BAP (2.0 mg/L)	50.48	30.80	1.74
MS+BAP (2.5 mg/L)	62.28	38.64	3.33
MS+BAP (3.0 mg/L)	55.54	36.34	3.20
MS+BAP (3.5 mg/L)	48.34	34.24	2.22
MS+BAP (4.0 mg/L)	37.56	22.16	1.22
MS+BAP (5.0 mg/L)	19.68	Nil	Nil
MS+BAP (6.0 mg/L)	10.27	Nil	Nil
MS+IAA (0.5 mg/L)+BAP (2.0 mg/L)	64.22	52.15	4.52
MS+IAA (0.5 mg/L)+BAP (2.5 mg/L)	68.75	78.18	6.83
MS+IAA (0.5mg/L)+BAP (3.0 mg/L)	62.47	66.68	5.45
MS+IAA (0.5mg/L)+BAP (3.5 mg/L)	56.45	54.66	4.48
MS+IAA (0.5mg/L)+BAP (4.0 mg/L)	48.75	40.88	2.24
MS+IAA (1.0 mg/L)+BAP (2.0 mg/L)	49.68	20.48	3.53
MS+IAA (1.0 mg/L)+BAP (2.5 mg/L)	52.68	50.00	4.54
MS+IAA (1.0 mg/L)+BAP (3.0 mg/L)	50.62	37.46	3.52
MS+IAA (1.0 mg/L)+BAP (3.5 mg/L)	48.68	28.67	2.78
MS+IAA (1.0 mg/L)+BAP (4.0 mg/L)	40.56	20.45	1.47

Table 2: Effect of IAA and BAP on shoot elongation, shoot multiplication and number of shoots per explants after second subculture in *M. paradisiaca* L. (AAB group) cv. 'Malbhog'

Growth regulator (mg L ⁻¹)	Shoot elongation (%)	Multiple shoot formation (%)	Mean shoots per explant
MS+IAA (0.5 mg/L+BAP (2.0 mg/L)	65.24	50.15	4.52
MS+IAA (0.5mg/L+BAP (2.5 mg/l)	65.87	78.66	7.56
MS+IAA (0.5mg/L+BAP (3.0 mg/l)	63.89	75.45	6.62
MS+IAA (0.5mg/L+BAP (3.5 mg/l)	60.22	67.54	5.88
MS+IAA (0.5mg/L+BAP (4.0 mg/l)	52.23	46.45	3.68

by (67.54%) multiple shoot induction on MS medium containing 0.5 mg L⁻¹ IAA and 3.5 mg L⁻¹ BAP producing an average of 5.88 shoots per explant (Table 2). Muhammad *et al.* (2007) and Ahmed *et al.* (2014a) found highest response of multiple shoots in 2.0 mg L⁻¹ IAA and 4.0 mg L⁻¹ BAP. These results demonstrate that a lower concentration of auxin combined with a relatively higher concentration of cytokinin is optimum for shoot multiplication in the 'Malbhog' cultivar. Similar findings have been reported by Kulkarni *et al.* (2004), Muhammad *et al.* (2007), Jafari *et al.* (2011), Suman *et al.* (2013a) and Ahmed *et al.* (2014a). Media supplemented with lower concentration of auxin combined with a relatively higher concentration of cytokinin supports multiple shoot formation, which has also been observed in tissue culture of *Trichosanthes dioica* Roxb. (Komal R. 2021). Cytokinins stimulate cell division and axillary bud development, whereas a low level of auxin helps maintain organized shoot formation. This hormonal balance favours efficient multiple shoot regeneration in banana tissue culture. However, Shivkumar and Visalakshi (2021), found

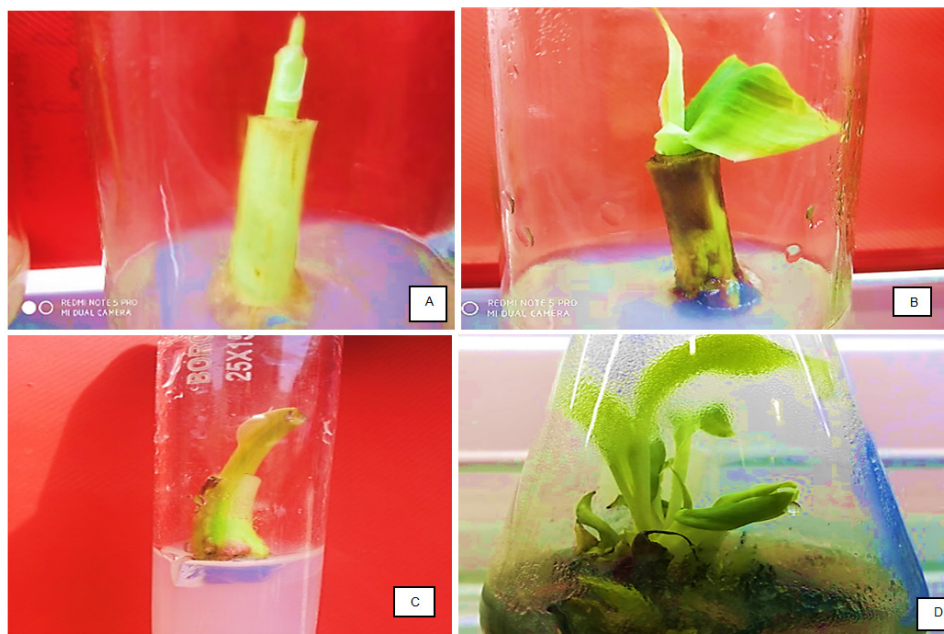
Table 3: Effect of NAA on differentiated shoot on rooting in *M. paradisiaca* L. (AAB group) cv. 'Malbhog'

Growth regulator (mg L ⁻¹)	Root formation(%)	Number of roots/shoot
1/2MS+NAA (0.5 mg/L)	52.30	3.44
1/2MS+NAA (1.0 mg/L)	86.65	6.65
1/2MS+NAA (1.5 mg/L)	70.65	4.44
1/2MS+NAA (2.0 mg/L)	40.44	3.56
1/2MS+NAA (2.5 mg/L)	22.42	2.28
1/2MS+NAA (3.0 mg/L)	Nil	Nil
1/2MS+NAA (3.5 mg/L)	Nil	Nil

that for multiple shoot induction TDZ containing media was found to be better for shoot multiplication rate than BAP.

Root Induction

Optimum rooting response was achieved on half-strength MS medium supplemented with 1.0 mg L⁻¹ NAA, where 86.65% of the regenerated shoots produced healthy roots within 10-12 days (Table 3), and an average root length of

**Figure 1:** (A-D) Explant showing existing shoot elongation and shoot proliferation. (A-C) Growth and elongation of the existing shoot within 12-14 days after culture. (D) Multiple shoot formation after 25 days of culture on media supplemented with MS + 0.5mg/l IAA + 2.5 mg/l BAP

6.65cm was found after 15 days. When the concentration of NAA was increased to 1.5 mg L⁻¹ or decreased to 0.5 mg L⁻¹, root formation dropped to 70.65% and 52.30% respectively. An average root length of 4.44cm and 3.44cm was observed when the concentration of NAA was 1.5 mg L⁻¹ and 0.5 mg L⁻¹ respectively. This indicates that 1.0 mg L⁻¹ NAA alone is the optimal concentration for root induction from the regenerated shoots. The regenerated roots were well developed and suitable for successful transplantation. Similar rooting responses on NAA supplemented media have been reported by Acharjee *et al.* (2004), Strosse *et al.* (2004), and Suman *et al.* (2013a). Vani *et al.* (1999) suggested that media supplemented with BAP, IAA and 0.1% charcoal showed profused rooting from shoot tip culture. Although Ahmed *et al.* (2014b) reported better rooting with IBA supplemented media, the present study demonstrated that NAA was effective for root induction in the 'Malbhog' cultivar. In contrast Selvakumkar and Parasurama (2020) reported that half strength MS media containing activated charcoal 200 mg L⁻¹ and 20 mg L⁻¹ adenine sulphate (AS) was favourable concentration for root regeneration in Grand Naine and Elakki cultivars.

Acclimatization

Rooted plantlets were successfully transplanted into earthen pots containing a sterilized mixture of soil, sand and well-decomposed farmyard manure in a ratio of 1:1:1 for primary hardening for one month. The potted plants were maintained under greenhouse conditions for gradual acclimatization and hardening before transfer to the field. Approximately 75% of the transplanted plantlets survived under field conditions. The regenerated plants exhibited normal growth and morphology and were comparable to the mother plants, indicating successful clonal propagation. The present investigation established an efficient and reproducible protocol for the rapid micropropagation of the commercially important banana cultivar 'Malbhog'. Among the different media tested, MS medium supplemented with 0.5 mg L⁻¹ IAA and 2.5 mg L⁻¹ BAP was most effective for multiple shoot induction, whereas half-strength MS medium containing 1.0 mg L⁻¹ NAA proved suitable for root induction. The protocol developed in this study can be used for the large-scale production of healthy, true-to-type planting material and may contribute to sustainable banana cultivation. The protocol described in this study provides a simple and effective method for the large-scale production of healthy, true-to-type planting material of the 'Malbhog' banana cultivar and has potential applications in sustainable banana cultivation.

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