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In vitro Regeneration and Acclimatisation of Banana cv. Malbhog

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Banana (*Musa paradisiaca*) cv. Malbhog is a very popular banana cultivar in the North Eastern part of India. A method has been developed to multiply it *in vitro* to standardise the best multiplication and rooting media for banana and hardening of rooted plantlets for field transfer using different potting mixtures. Media formulation using BAP alone or in combination with NAA were used for shoot initiation and multiplication. Minimum number of days for shoot initiation (18.4), the longest length of shoot (5.1 cm) and a maximum number of shoots/ explant (4.2) was obtained on PGR combination NAA 0.5 + BAP 5.0 mg/l.Half strength MS media supplemented with IBA 1.5 mg/l was found significantly higher over all other auxin treatments for various rooting parameters. This combination gave the best response in terms of root formation frequency (66.7%), least number of days for root formation (18), maximum no. of roots/explant (3.8) and longest length of the root (4.0 cm). Maximum survival frequency was found in case of coco peat (89%) followed by sterile soilrite (79%). Plant morphology in terms of plant height, leaf length, leaf width, leaf number, etc. coco peat gave better result over other potting media.

Keywords: Banana; Malbhog; micropropagation; NAA; BAP.

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1. INTRODUCTION

Banana is the second major fruit crop after mango in Bihar. Banana is grown on an area of 34.31 thousand ha with an annual production of 1526.50 thousands MT and a productivity of 44.06 MT/ha in Bihar [1]. In Bihar "Malbhog (*Musa paradisiaca* AAB group)" is a very popular variety with high commercial value. It has high demand in the market 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 banana growers. *In vitro* banana production technology is a superior technology over traditional method (Sucker-propagated) of banana production with respect to optimal yield, uniformity, disease-free planting material and true to type plants. They are cheaper to transport than conventional suckers coupling with virus indexing, allows for safe movement and exchange of germplasm [2]. So, use of tissue culture technique and development of micropropagation protocol in the elite cultivar of Bihar such as Malbhog will save them from extinction and will help in their further expansion in new areas.

2. MATERIALS AND METHODS

Banana cultivar Malbhog was procured from Germplasm Block, Department of Horticulture, Rajendra Agricultural University, Pusa, Bihar. Sword suckers were used for micropropagation experiment. Superfluous tissues were removed by trimming away the outer leaf sheaths, leaf bases and corm tissues until a 6.5 to 8.5 cm cube enclosing the shoot apex is obtained. The trimmed suckers were then washed with Dettol and Tween-20 for 20 minutes, 0.2% Bavistin and Tween-20 for ½ hours, dipping in streptocycline (0.05%) for 3 hours. Surface sterilisation was done firstly by rinsing in ethanol (70%) for 30 seconds and finally rinsing with $HgCl_2$ (0.1%) for 25 minutes. Then, the sterilised explants were dispensed into culture medium containing 50 ml media in each jar. P^H of the medium was adjusted to 5.8, autoclaved at 15 Psi pressure and 121°C temperature for 15-20 minutes. Cultured plants were incubated in an air-conditioned culture room with a controlled temperature of 25±2°C and light intensity of 2000-3000 lux for a photoperiod of 16 h of light. The regenerated explants were cut aseptically and cultured on multiplication media containing BAP (1, 2.5 and 5.0 mg/l) and NAA (0.5 and 1.0 mg/l) either individually or in combined form. The cultures were incubated in multiplication media for 6 weeks and then observations were recorded for a number of days taken for shoot initiation, number of shoots/explant and length of shoot. The cultures were grown for 4 cycles and further inoculated in rooting media for root induction. Half MS media containing three different levels of IAA (0.5, 1.5 and 2.5 mg/l) and IBA (0.5, 1.5 and 2.5 mg/l) were used for root induction. Observations were recorded for percent root formation in regenerated shoots, no. of days taken for root formation, no. of roots per shoot and root length (cm) after six weeks of subculture. Rooted shoots that were 4-5 cm high and had several well-ramified roots were ready to transplant. Plantlets were removed from culture container and agar was gently washed from roots under running tap water. They were then subjected to primary hardening in different potting media such as Coco peat, Coco peat: Soil (1:1 v/v), Sterile soilrite and Sterile soilrite: Soil (1:1 v/v). Observations were recorded for maximum survival of plantlets and morphological characteristics of hardened plants (Height, Girth, leaf length, leaf width and number of leaves of plants) after 1 ½ months.

Each treatment consisted of 15 plants and the data were analyzed by running one way analysis of variance (ANOVA). The means were compared using the critical difference to find the difference at 5% ($P<0.05$) level. The results are expressed as a mean ± SE of five replications.

3. RESULTS AND DISCUSSION

The response of cytokinin (BAP) either alone or in combination with auxin (NAA) on shoot regeneration and proliferation of Malbhog banana is presented in table 1.In micropropagation technique, establishment includes all steps right from culturing till the explants are able to produce multiple shoots. Data were recorded after six weeks. Very weak or much less response was observed in hormone-free media for shoot regeneration. Only one shoot and shortest length of shoot (0.98±0.06 cm) were obtained on hormone free media. It also took the longest duration for shoot initiation (41.40±0.98 days). Comparatively much better response was obtained on MS media fortified with either cytokinin or combination of auxin and cytokinin. Of the various combinations tested, MS + NAA (0.5 mg/l) + BAP (5.0 mg/l) gave consistently best result for various multiplication parameters like a number of shoots/ explant, shoot length and number of days

required for shoot initiation being 4.00±0.32, 5.10±0.07 cm and 18.40±1.03 days respectively. The second best result was obtained on NAA 0.5 + BAP 2.5 mg/l combination. In this combination, number of shoots produced/ explant was 3.20±0.37, length of shoots 4.66±0.17 cm and number of days for shoot initiation being 21.80±0.80 days. Overall, a higher and good quality of shoots were obtained on hormonal combination NAA 0.5 + BAP 5.0 mg/l. Cytokinin (BAP) alone did not give a much better response as compared to cytokinin (BAP) and auxin (NAA) combination indicating that addition of auxin good for best response of shoot regeneration. Analogical results were obtained by Sipen and Davey (2012) in *Musa* spp *Pisang Nangka* on medium supplemented with BAP 5 mg/l and IAA 0.2 mg/l. Previous researchers [3,4] also indicated that 5 mg/l (22.2 µM) BAP was the optimum concentration for most banana cultivars.BAP stimulates cell divison thereby increasing bud break and thus increases the multiplication rate. Ahirwar et al. [5] found highest frequency of shoot regeneration (52.25), number of shoots regenerated per explant (3.25) and shoot length (4.69 cm) at BAP concentration 5 mg/l or combination of 7.5 mg/l BAP + 0.3 mg/l NAA while Banerjee and Langhe [6] carried out rapid clonal propagation and multiplication onMS media supplemented with BAP 2.30 mg/l and IAA 1.80 mg/l. Arinaitwe et al. [7] reported in *vitro* bud initiation from banana being cultivar dependent. Al-Amin et al. [8] obtained only single shoot and shorter shoot length (1.05 cm) at 20 and 30 DAI when explants cultured on MS medium without growth regulator which is in accordance with our finding.

Well- developed, healthy and elongated shoots obtained after multiplication phase was now transferred on ½ MS media containing either IAA or IBA. The data for various rooting parameters were recorded over a period of six weeks.A remarkable increase in shoot and root length was obtained at the end of six weeks of culture. In ½ MS media containing no auxin very less rooting response percentage (21.60±2.04) was observed. Rooting can be stimulated when individual shoots are transferred to ½ MS basal medium alone [9,10]. However, auxins may

Table 1. Shoot formation in suckers of Malbhog variety of banana cultured on solid MS medium supplemented with various concentration of BAP and NAA (15 explants per treatment, data recorded at the end of 6 weeks)

Treatment	Composition(mg/l)	No. of days required for shoot initiation	No. of shoots/ explant	Length of shoots (cm)
T_1	Control	41.40±0.98	1.00±0.00	0.98±0.06
T_2	BAP 1.0	38.00±1.38	1.40±0.24	1.80±0.37
T_3	BAP 2.5	29.00±1.18	2.20±0.20	2.80±0.37
T_4	BAP 5.0	22.60±1.03	3.40±0.24	4.06±0.15
T_5	NAA 0.5+ BAP 1.0	28.60±1.2.06	1.80±0.37	2.40±0.24
T_6	NAA 0.5+ BAP 2.5	21.80±0.80	3.20±0.37	4.66±0.17
T_7	NAA 0.5+BAP 5.0	18.40±1.03	4.00±0.32	5.10±0.07
T_8	NAA 1.0+BAP 1.0	33.00±1.79	1.40±0.24	2.00±0.31
T_9	NAA 1.0+BAP 2.5	27.20±0.86	3.00±0.45	3.68±0.22
T_{10}	NAA 1.0+BAP 5.0	25.20±0.66	3.60±0.24	4.00±0.23
$CD_{0.05}$		3.57	0.83	0.71

Table 2. Effect of different levels of IBA and IAA on rooting response of microshoots of Malbhog (15 replicates/ treatment, data recorded after 6 weeks)

Treatment	Composition (mg/l)	Percent (%) root formation	Duration (days) for root formation	No. of roots/ explant	Length of root (cm.)
T_1	Control	21.60±2.04	32.20±0.86	1.80±0.37	1.00±0.00
T_2	IBA 0.5 mg/l	53.33±2.04	21.60±0.51	3.40±0.24	3.44±0.13
T_3	IBA 1.5 mg/l	66.67±2.64	18.00±0.95	4.20±0.37	4.00±0.07
T_4	IBA 2.5 mg/l	45.00±2.04	23.80±0.37	3.40±0.24	2.56±0.04
T_5	IAA 0.5 mg/l	43.33±3.12	26.60±0.40	2.00±0.32	1.12±0.07
T_6	IAA 1.5 mg/l	50.00±2.64	24.20±0.58	3.80±0.20	3.04±0.05
T_7	IAA 2.5 mg/l	58.33±2.64	26.80±0.51	3.40±0.24	2.74±0.05
$CD_{0.05}$		7.18	1.83	0.84	0.20

induce further root initiation [11]. On this treatment, a minimum number of roots/ explant (1.80±0.37) and minimum length of root (1.00±0.00 cm) was obtained. Similar results were obtained by Al-Amin et al. [8] who obtained 2.00 cm root length with control treatment. In this treatment maximum days (32.20±0.86) for root formation was observed. As the concentration of auxin (either IAA or IBA) was increased there was a marked increase in various rooting parameters but, enhanced rooting response was observed only upto certain threshold level and beyond that, it was decreased. So, it indicates that neither very less nor much more concentration of hormones is required for an optimum response. For various rooting parameters like root formation percentage (66.67±2.46), number of days for root formation (18±0.95), number of roots per shoot (4.20±0.37) and root length (4.00±0.07 cm)IBA 1.5 mg/l gave significantly superior result over other concentrations of IBA.(Table 2). IBA was proved superior rooting hormone than IAA.The action of IBA may be lies in the fact that IBA may be converted into IAA through a similar process to β-oxidation of fatty acids and the conversion of IBA to IAA then suggests that IBA works as a storage sink for IAA in the plants. Rahman *et al*. [12] also reported IBA better than NAA in rooting of shoots in banana cv. Agnishwar. The next best rooting response percentage (58.33±2.64) was obtained on ½ MS media containing IAA 2.5 mg/l while number of roots/ explant (3.80±0.20) was found at IAA 1.5 mg/l. Length of root; 3.44±0.13 cm and days for root formation; 21.60±0.51 days was found at IBA 0.5 mg/l. Govindaraju et al. [13] obtained the maximum percent of adventitious root formation in half strength MS medium supplemented with IBA 1.5 mg/l and NAA 1.0 mg/l.Many workers have also found indole butyric acid (IBA) 1 mg/l for inducing rooting within one to four week in banana [14,15,12]. Babylatha [16] observed maximum rooting in half strength MS media supplemented with 5.0 mg/l IBA.

Well rooted plants were taken out from culture bottles and were washed thoroughly to remove traces of agar. The rooted shoots were then transferred to multiwell portrays containing different potting media such as coco peat, coco peat: soil (1:1 v/v), sterile soilrite and sterile soilrite: soil (1:1 v/v) (Table 3) The temperature was maintained at 28°c and 70-80% relative humidity in the greenhouse. Observations were recorded for the best potting media in which maximum survival, length, girth, number of leaves, length of leaves and width of leaves may be obtained. Out of various potting medias tested, coco peat was found significantly best for above all the characters. Maximum survival percentage of rooted shoots were obtained on coco peat (89.00±2.45) followed by sterile soilrite (79.00±1.87) (Table 3). The results of present experiment agreed with the finding of Shankar et al. [17] who found the survival rate of the plantlets in coconut coir pith to be 84.44% during primary hardening. Similar results were found by Rai et al. (2012) who recorded survival rate of 96 % on medium containing coco peat and sand in the ratio of 2:1 among different growth media *viz.,* soil, sand and cocopeat (1:1:1), soil sand and farmyard manure (1:1:1) and mixture of cocopeat and sand (2:1). Maximum height (7.98±0.19 cm) of plants was achieved on coco peat (7.98±0.19 cm) (Table 3) Maximum value for leaf character was also found highest in coco peat media being 6.10±0.23 cm, 4.12±0.27 cm and 4.20±0.37 for leaf length, leaf width and a number of leaves, respectively. (Table 3). Girth of plants was found statically non-significant to each other.The current finding is in accordance with Uzibara et al. [18] who also found cocopeat to be far superior to other potting media in terms

Table 3. Effect of different potting mixtures on hardening and different morphological features of micropropagated banana cv. Malbhog plantlets (15 replicates/ treatment, data recorded after 1 ½ months)

Treatments	Potting mixtures	Response (%)	Height (cm)	Girth (cm)	Leaf length (cm)	Leaf width (cm)	No. of leaves
T_1	Coco peat	89.00±2.45	7.98±0.19	2.20±0.11	6.10±0.23	4.12±0.27	4.20±0.37
T_2	Coco peat: soil (1:1 v/v)	69.00±3.67	6.12±0.19	1.93±0.19	4.98±0.13	3.72±0.14	4.00±0.31
T_3	Sterile soilrite	79.00±1.87	7.20±0.19	2.04±0.17	5.16±0.08	3.88±0.10	4.20±0.37
T_4	Sterile soilrite: soil (1: 1 v/v)	66.00±2.45	5.36±0.18	2.00±0.11	4.92±0.07	4.92±0.07	3.80±0.2
$CD_{0.05}$		5.66	0.55	NS	0.44	0.16	NS

of percentage survival of plantlets (95.00%), plantlet height (5.58 cm), number of leaves (3.20), plantlet diameter (4.59 mm), number of primary roots per plantlet (5.20), length of primary roots (5.18 cm) and number of secondary roots per plantlet (25.50). Coco peat is an ingredient that can keep the soil loose, which in turn, enable roots to spread out easily and thus, giving more breathing space and aeration and consequently better plant growth is achieved.

4. CONCLUSION

Media formulation using BAP alone or in combination with NAA were used for shoot initiation and multiplication. Minimum number of days for shoot initiation (18.4), the longest length of shoot (5.1 cm) and a maximum number of shoots/ explant (4.2) was obtained on PGR combination NAA 0.5 + BAP 5.0 mg/l.Half strength MS media supplemented with IBA 1.5 mg/l was found significantly higher over all other auxin treatments for various rooting parameters. Plant morphology in terms of plant height, leaf length, leaf width, leaf number, etc. coco peat gave better result over other potting media.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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