INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

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PROTOCOL FOR TISSUE CULTURE PROPAGATION OF BANANA CV. NEY POOVAN (AB)

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ABSTRACT
Banana cv. Ney Poovan (syn. Elakki Bale AB) is the most preferred cultivar of Southern India. The cultivar is known for its special taste, aroma and the long keeping quality of its fruits. The demands for planting material of this cultivar is huge and tissue cultured plantlets are not available as the multiplication rate is very low. The present study was conducted with objective to investigate the effect of different concentrations of ascorbic acid, BAP and NAA on *in vitro* establishment, shoot multiplication and also tried different concentration of IBA and NAA for inducing adventitious root in banana *cv*. Ney Poovan (AB). During shoot multiplication data consisted of percent response, number shoots per explant, length of shoots and number of leaves. Out of various treatments, Ascorbic acid 175 mg/l, MS B + BAP 2.0 mg/l + 2 weeks dark incubation + 1 Week light incubation and half strength MS medium+ NAA 1.00 mg/l were found effective for tissue culture propagation of Banana cv. Ney Poovan.

KEY WORDS: Ney Poovan, Tissue culture, Shoot tip, Ascorbic acid, Shoot multiplication.

INTRODUCTION

The banana and plantains *(Musa* spp.*)* belonging to the family *Musaceae* are one of the world's most important fruit crops. It is originated in Malaysia through a complex hybridization process[13]. It is widely grown in the tropics and subtropics in all types of agricultural system, from small, mixed, subsistence gardens to large commercial monocultures. The crop serves in many developing countries as a staple food or the cornerstone of the country's economy. Ney Poovan (AB)(syn. Elakkibale) is the choicest diploid cultivar of banana, which is under commercial mono cultivation on a large scale especially in Karnataka and Tamil Nadu. In Kerala it is grown in backyards and now shifting to large-scale cultivation. Ney Poovan (AB) is a slender plant bearing bunches of 15-30 kg after 12-14 months. Dark green fruits turn golden yellow with a very good keeping quality. Fruit is highly fragrant, tasty, powdery and firm. Ney Poovan is tolerant to leaf spot but susceptible to Fusarium wilt and banana bract mosaic virus. *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. Mass multiplication through tissue culture plants could be done in a short time. They are cheaper to transport than conventional suckers and the coupling with virus indexing allows for safe movement, and exchange and conservation of germplasm. In addition, bananas produced using the tissue culture are reported to be more vigorous, higher yielding and produce better quality fruits than those produced by conventional means. For commercialization, it is necessary that consistent supplies of good quality bananas are produced to meet the increasing demand. Ney Poovanis not commercially propagated by the tissue culture industry because of the inherent problem in the initial establishment of the culture in the medium and the slow rate of multiplication[8]. Another problem of *in vitro* cultured explants, accompanied by darkening of culturemedium has been attributed to phenolic compoundsexuded from tissues and accumulating in the culturemedium. This process is initiated by browning of thesurface of plant tissues due to the oxidation of phenolic compounds resulting in the formation of quinines which are highly reactive and toxic to plant tissue. The browning phenomenon occurs in response to oxidation process of released phenolic compounds from injured tissue by phenol oxidase and formation of quinones[5]. Quinones negatively inhibit cell growth and can often result in death of cells (necrosis)[14]. Therefore, preconditioning of explants with media supplements such as, ascorbic acid[11] was necessary to limit the production of these harmful substances. Keeping these points in view, the present investigation "Protocol for tissue culture propagation of banana cv. Ney Poovan (*Musa* spp., AB group)" was carried out with the objective of minimization of browning, enhancement of shoot proliferation and induction of adventitious roots.

MATERIALS & METHODS

The present study was conducted at the Center for Horticulture Biotechnology, University of Horticultural Sciences, Bagalkot 587 104, Karnataka, India. Healthy and vigorously growing sword suckers of cv. Ney Poovan (3-4 month age), free from viruses and other diseases were selected as a source of explant (Fig. 1).

FIGURE 1. Mother plant and sword suckers of cv. Ney Poovan

Preparation of explant

The plant material obtained from the field was thoroughly washed in running tap water followed by washing with a detergent solution to remove adhering soil particles. Later, rhizomes were kept immersed in a fungicide solution of 1 % bavistin for half an hour, to further clean the planting material. The outer leaves, leaf base and corm tissue were trimmed using a sterilized stainless steel knife until the length of explant was 4-6 cm and the diameter, 3-4 cm. These trimmed suckers enclosing the shoot tip were washed with double distilled water. After trimming one more outer layer, they were soaked in a solution of 0.50 % bavistin + 0.05 % streptocycline for eight hours. After thoroughly washing with double distilled water, they were trimmed again, so that trimmed suckers were of 2-3 cm in length and 2-2.5 cm in diameter. These shoot tips were soaked in 0.05 % cetrimide for 30 minutes. After removing one more layer, the shoot tips were surface sterilized with 0.1% mercuric chloride in a closed container for 10 minutes. Further operations such as washing several times with sterile distilled water to remove all traces of chlorine, trimming of explants and inoculation were carried out under laminar air flow chamber (Fig. 2 A-C).

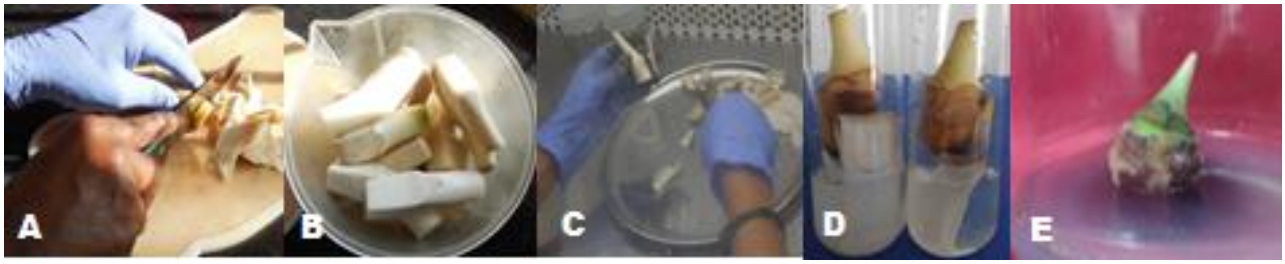

FIGURE 2. Establishment of aseptic culture: A) Trimming of suckers; B) Trimmed shoot tip; C) Trimming of shoot tip under laminar airflow; D) Inoculated shoot tips on liquid MS media; E) Established shoot tip.

Initiation of aseptic culture

Shoot tip explants were incubated in MS liquid medium containing 2 mg/lBAP, 35 mg/l adenine sulphate and different concentration of ascorbic acid concentration (25, 50, 75, 100, 125, 150, 175and 200 mg/l) (Fig. 2 D) for two weeks maintaining standard culture conditions of 25 ± 2^0 C temperature, 70% RH and photoperiodic cycle of 16 hours light and 8 hours dark period. After two weeks of incubation, all the explants were evaluated for their percent response, browning and percent contamination in liquid medium. Greening and swelling of the explants were utilized as important criteria for assessing the success in establishment (Fig. 2 E). Shoot tips that had turned dark brown/black and which did not swell were considered as non-established. Healthy and contaminant free explants were excised by removing discolored tissue and transferred to the semisolid medium supplemented with BAP (2 mg/l) and adenine sulphate (35 mg/l) and incubated for four weeks maintaining standard culture conditions. The browning degree in initiation stage was scored visually according to as follows:+++ = High browning;++ = Moderate browning; - = No browning

Effect of cytokinin andauxin on shoot proliferation in Ney Poovan (AB):

After 4 weeks, established banana explants were transferred onto proliferation media for shoot multiplication and development. The medium used for banana multiplication was Murashige & Skoog Medium (MS)[9]. Different concentration of 6-Benzylaminopurine

(BAP), Naphthalene acetic acid (NAA) hormones were used as culture medium for shoot induction, shoot multiplication and maintenance and regeneration of roots from multiplied shoots were inoculated onto half strength MS medium supplemented with different concentration of Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA) hormones and Activated charcoal 2 mg/l were used in the present experiment. All the media were autoclaved at 15 psi and 121˚C for 20 minutes. The culture jars containing explants were incubated in growth chamber at 26 ±2°C with 16 hour photoperiod (approximately 2000 lux) provided by cool white fluorescent tubes. The materials were 6 times sub cultured at a regular interval of four weeks into same medium to produce multiple shoots. Observations were recorded of percent response, number of shoots per explants, length of shoot (cm) and number of leaves per shoot. The regenerated plantlets after developing sufficient root system were deemed ready to transfer in soil. The plantlets were carefully removed from the culture vessels. The roots of the plantlets were gently washed under running tap water to remove agar attached to the roots. Observations were recorded on per cent rooting, number of roots, length of primary roots and length of secondary roots. Immediately after washing they were transferred to portrays containing a sterilized coco peat and kept under poly tunnel for weeks. Later plantlets transferred to poly bags containing sand, red soil and compost in 1:1:1 ratio (v/v). They were kept under shade house and sprayed with water regularly to maintain high humidity around the plantlets.

Statistical Analysis

The data were recorded at four week intervals after incubation. There were five explants for each treatment and three replications. Data recorded for different parameters were subjected to completely randomized design (CRD). Statistical analysis was done by (ANOVA) using software Wasp developed by ICAR Research Complex, Goa.

RESULTS & DISCUSSION

Effect of ascorbic acid on browning of medium

Browning phenomena is one of the most common problems associated with *in vitro* establishments of shoot tip explants. In the present investigation, the effect of antioxidant as ascorbic acid on browning and growth of banana Ney Poovan, were studied.

TABLE 1: Effect of ascorbic acid on browning intensity in banana cv. Ney Poovan (AB)

Treatments	Browning intensity
T_1: MS B + 2 mg/l BAP (Control)	+++
T_2: MS B + BAP 2 mg/l + Ascorbic acid 25 mg/l	+++
T_3: MS B + BAP 2 mg/l + Ascorbic acid 50 mg/l	+++
T_4: MS B + BAP 2 mg/l + Ascorbic acid 75 mg/l	+++
T_5: MS B + BAP 2 mg/l + Ascorbic acid 100 mg/l	++
T_6: MS B + BAP 2 mg/l + Ascorbic acid 125 mg/l	++
T_7: MS B + BAP 2mg/l + Ascorbic acid 150 mg/l	++
T_8: MS B + BAP 2 mg/l + Ascorbic acid 175 mg/l	-
T_9: MS B + BAP 2 mg/l + Ascorbic acid 200 mg/l	-

MS B: Murashige & Skoog Basal Medium

+++ = High browning; ++ = Moderate browning; - = No browning

The data demonstrated that when the initiation medium was free from the ascorbic acid, the highestbrowning was higher (Table 1 & Fig. 3 A). The browningphenomenon wasinhibited with concentrations of ascorbic acid 175 mg/l and 200 mg/l(Table 1 &Fig. 3 B-C).Highest browning intensity in control was attributed to oxidation phenols by oxygen radicals resulting in oxidative injury. Inhibition of browning at higher concentration of ascorbic acid was mainly due reducing activity of ascorbic acid therefore cells are protected from oxidative injury.

FIGURE 3. Intensity of browning in Ney Poovan on liquid MS medium: A) Control; B) Ascorbic acid 175 mg/l; C) Ascorbic acid 200 mg/l.

Phenolic secretions and other exudates in plants tissue culture systems lessen the efficiency of explant initiation, growth and development [6]. One of the major problems for several tissue culture systems is the lethal browning which result in death of the cultured explants that depend on the rate of oxidation of phenolic compounds, as well as the quality of the total phenols [7].

Effect of cytokinin and auxin on shoot proliferation

TABLE 2: Effect of cytokinin and auxin on shoot growth in banana cv. Ney Poovan (AB)

Treatments	Percent response	Number of shoots/ explants	Length of shoots (cm)	Number of leaves/shoots
T_1: MS B + BAP 2.0 mg/l (Control)	100 (89.53)	3.08	4.33	3.87
T_2:MS B + BAP 2.0 mg/l + 2 weeks dark incubation + 1 Week light incubation	100 (89.53)	6.41	9.15	7.53
T_3: MS B + BAP 2.0 mg/l + NAA 0.25 mg/l	100 (89.53)	6.27	8.67	7.98
T_4:MS B + BAP 2.0 mg/l + NAA 0.50 mg/l	100 (89.53)	5.07	5.59	4.45
T_5: MS B + BAP 2.0 mg/l + NAA 0.75 mg/l	100 (89.53)	3.21	5.66	4.77
T_6: MS B + BAP 2.0 mg/l + NAA 1 mg/l	100 (89.53)	3.40	3.77	4.26
T_7: MS B + BAP 3.0 mg/l + NAA 0.25 mg/l	100 (89.53)	3.22	3.49	3.10
T_8:MS B + BAP 3.0 mg/l + NAA 0.50 mg/l	100 (89.53)	3.78	3.55	3.78
T_9: MS B + BAP 3.0 mg/l + NAA 0.75 mg/l	100 (89.53)	2.87	4.16	4.56
T_{10}:MS B + BAP 3.0 mg/l + NAA 0.1 mg/l	100 (89.53)	3.37	3.05	3.71
T_{11}:MS B + BAP 4.0 mg/l + NAA 0.25 mg/l	100 (89.53)	3.60	3.85	4.17
T_{12}:MS B + BAP 4.0 mg/l + NAA 0.50 mg/l	100 (89.53)	5.45	8.20	7.10
T_{13}: MS B + BAP 4.0 mg/l + NAA 0.75 mg/l	100 (89.53)	3.16	3.41	4.30
T_{14}: MS B + BAP 4.0 mg/l + NAA 0.1 mg/l	100 (89.53)	3.60	3.11	4.57
T_{15}: MS B + BAP 5.0 mg/l + NAA 0.25 mg/l	100 (89.53)	3.530	3.590	3.950
T_{16}: MS B + BAP 5.0 mg/l + NAA 0.50 mg/l	100 (89.53)	3.470	3.505	3.405
T_{17}: MS B + BAP 5.0 mg/l + NAA 0.75 mg/l	100 (89.53)	3.440	3.435	3.725
T_{18}: MS B + BAP 5.0 mg/l + NAA 1 mg/l	100 (89.53)	3.585	3.160	3.435
SE m±	-	0.23	0.26	0.36
CD at 1%	NS	0.92	1.07	1.48

*Figures in parenthesis are arc sin transformation

Variable number of shoots were produced per explant in MS media supplemented with different concentrations of BAP and NAA (Table 2). Among the different concentrations, MS B + BAP 2.0 mg/l + 2 weeks dark incubation + 1 Week light incubation showed significantly maximum number of shoots per explants(6.41) and length of the shoots (9.15 cm) which was statistically on par with the treatment BAP 2.0 mg/l + NAA 0.25 mg/l(6.27 shoots per explant and 8.67 cm shoot length). A next best shoot growth was achieved with MS B+BAP 4.0 mg/l +NAA 0.50 mg/l (5.45 shoots/explants and 8.20 cm length of shoots) which was superior as compared to control treatments (Fig 4 A-D).Similar findings were also reported by[10] in banana cultivars Gros Michel, Bwara and Sukalindizi. Dark conditions enhanced higher number of shoots than light conditions suggesting that banana *in vitro* culture is a photomorphogenically process. Although light may be essential for plant development, darkness is also beneficial for plant morphogenesis. This could be true since *in vitro* photosynthesis was found to be unnecessary as opined by Hartman[4]. Increased shoot proliferation may also due to greater effectiveness of cytokinins and auxins under dark condition as they don't undergo photo oxidations.

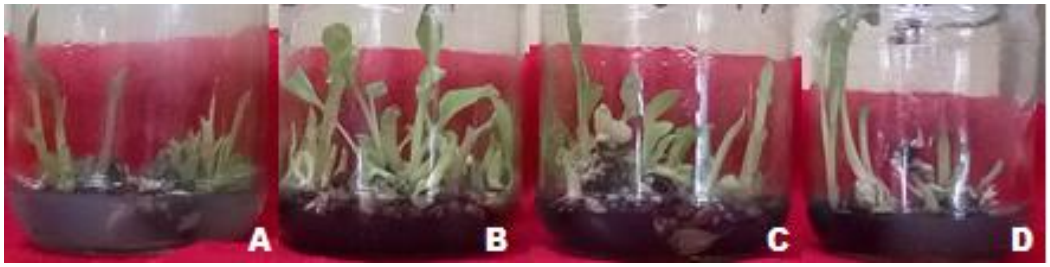

FIGURE 4. Shoot growth on media containing different concentration ofcytokinin andauxin: A) BAP 2.0 mg/l (Control); B) BAP 2.0 mg/l + 2 weeks dark incubation + 1 Week lightincubation; C) BAP 2.0 mg/l + NAA 0.25 mg/l; D) BAP 4.0 mg/l + NAA 0.50 mg/l.

The combination cytokinin and auxins also showed enhanced shoot growth (MS B + BAP 2.0 mg/l + NAA 0.25 mg/l), which suggest that there is strong synergistic effect of BAP-NAA interaction. Similar findings were also reported by Srangsam and Kanchanapoom[16,12] in banana. The concentration and combination of auxin and cytokinin in the nutrient medium is key factor which determines successful plant regeneration[15]. Synergistic mechanism if cytokinin-auxin interaction in which cytokinin is required to activate a protein expressed in response to auxin.

Effect of auxins on rooting of microshoots of banana cv. Ney Poovan (AB):

The data pertaining to the response of different auxins (IBA and NAA) on *in vitro* rooting of banana shoots are presented in Table 3.

TABLE 3. Effect of auxins on *in vitro* rooting of banana plantlets cv.Ney Poovan (AB)

Treatment (mg/l)	Percent response	Number of primary roots per shoot	Length of primary roots (cm)	Number secondary roots
T1: MS B + IBA 0.50	100 (89.41)	2.47	6.20	7.53
T2: MS B + IBA 1.00	100 (89.41)	2.33	7.55	7.80
T3: MS B + IBA 1.50	100 (89.41)	2.60	8.73	8.40
T4: MS B + IBA 2.00	100 (89.41)	2.20	9.20	9.47
T5: MS B + NAA 0.50	100 (89.41)	2.27	7.33	8.53
T6: MS B + NAA 1.00	100 (89.41)	3.26	7.10	8.73
T7: MS B + NAA 1.50	100 (89.41)	2.07	7.35	7.60
T8: MS B + NAA 2.00	100 (89.41)	1.60	6.43	7.02
T9: MS Basal media (Control)	100 (89.41)	1.34	3.28	5.44
S.Em ±	-	0.14	0.16	1.02
CD@ 1%	NS	0.43	0.53	4.33

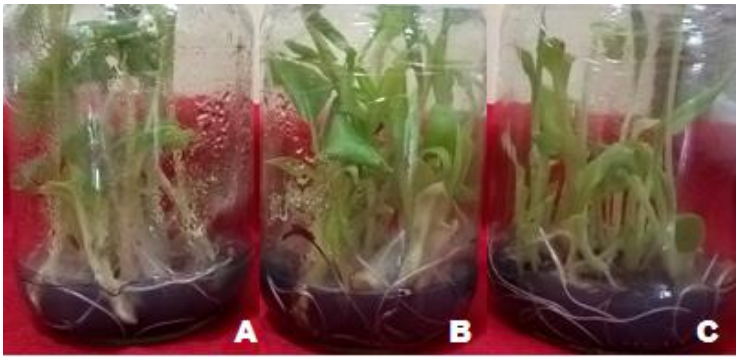

FIGURE 5: Effect of auxins on rooting: A) Control; B) MSB+ IBA 0.50 mg/l; C) MSB+ NAA 1.00 mg/l.

Media containing different concentration of auxins showed variable effects on rooting. MS medium supplemented with NAA 1 mg/l showed maximum number of primary roots per shoots (3.26) and good length of primary roots (7.10 cm) and number of secondary roots (8.74), while untreated control showed poor rooting (Table 3 and Fig. 5). NAAwas found to be effective at very low concentration for root initiation of banana according to Cronauer and Kirkorian [3]. NAA 1.0 mg/l is suitable for root initiation in Musa *sp*. similar finding were obtained 3.12 cm root length was highest in the medium supplemented with 3mg/l NAA. These results are conformity with the findings in banana [17&7].

CONCLUSION

The findings of present study indicates that phenolic browning was reduced by 175 mg/l ascorbic acid, shoot multiplication was increased with MS B + BAP 2.0 mg/L + 2 weeks dark incubation + 1 Week light incubation and half strength MS medium+ NAA 1.00 mg/L were found effective for *in vitro* rooting. So we concluded that the study tissue culture propagation protocol is successful for mass scale production of locally grown banana cv. Ney Poovan. This protocol can be employed for the commercial and production of disease free planting material.

ACKNOWLAGEMENT

Authors are thankful to Karnataka Biotechnology and Information Technology Services (KBITS), Department of Information Technology, Biotechnology and Science & Technology, Government of Karnataka for financial assistance (KBITS/126/BFC/5/2013-14).

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