Indian Journal of Hill Farmg 15(1) : 18-21 (2002)

RAPID MICROPROPOGATION OF TWO ECONOMICALLY IMPORTANT BANANA CULTIVARS OF NORTH EAST INDIA

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ABSTRACT

Highly proliferate cultures of two banana cultivars of North East India were initially established from shoot tips on a modified MS medium supplemented with BAP (5mg/1) and IAA (0.2 mg/1). Maximum number of multiple buds per shoot-tip was obtained from large intact explant (5mm3) as compared to similar explant (2mm3) or the longitudinally splitted one. Subsequent but also bilayer media not only enhanced the rate of multiplication but also controlled browning of explants as well of the surrounding media. Regeneration of plantlets from these multiple buds with well -developed roots was achieved by transfeming them into the modified MS medium supplemented with BAP (0.2mg/1) and NAA (0.2mg/1)

INTRODUCTION

The two banana cultivars of North east India, Manohar (ABB) and Honda(AAA) yield fruits with commercially important quality attributes with respect to size, texture, flavour and keeping quality. However, they are grown locally only in small patches for short supply of planting materials. Congenitally, banana are propagated using various types of suckers that arise at the base of the main pseudostem, but the rate of multiplication by this method is limited to 5-20 per year (Vuylsteke, 1989). The planting material is always in short supply, particularly when a new clone is adopted for extended cultivation or a large number of planting materials of a specific pathogen tolerance clone is to be distributed to growers. Sterility and polyploidy of the edible banana constituted an important handicap for conventional breeding programmes (Grapin et.al., 2000). In this context, the technique of plant tissue culture could offer new possibility for banana in the propagation, conservation and genetic improvement (Cronaur and Krikorian,1986). Moreover, it is the technique par excellent to minimize somaclonal variation, which an important consideration when handling crop plants in vitro (Scowcroit, 1985). The main problem of which can be encountered during the course of in vitro culture procedures is the browning of explants as well as of the media. Browning is caused by oxidation of phenolic compounds in wounded tissues, which first appeared on the cut surface of the explant and subdivided shoot but clumps. We are standardized a serial treatment of explant with L-ascorbic acid to control browning. Further, a bilayer medium for subculturing is described which not only controlled browning but also increased the multiplication rate of the culture.

MATERIALS AND METHODS

Small suckers of Manohar (ABB) and Honda (AAA) were collected from the horticultural orchid of the Assam Agricultural University, Jorhat, Assam. They were washed thoroughly woth tap water. Shoot -tip cubes in the size range of 1.5-2.5 cm2, containing several ensheathing leaf bases enclosing the auxiliary bud subjacent corn tissue were surface sterilized with 0.1 % (w/v) Hgcl2 for 5 minute and rinsed with sterilized distilled water or a sterilize solution of antioxidant. Sterilized shoot-tips were systematically removing the ensheathing cones of leaf primodia to reduce their size to about 2-5 mm3. Shoot-tips were

pretreated with different combination and concentrations of antioxidants before and after surface sterilization and after escision. Murshige and skoog (1962) mineral salt containing sucrose 3% (w/v) glycine 2 mg/1, thymine - HCL 0.4 mg/1, pyridoxine - HCL 0.5mg/1, nicotinic acid 0.5 mg/1 and ascorbic acid 50 mg/1 were used to make basal medium which was further supplemented with various combinations and concentration of BAP, NAA an IAA (Table 1&2) and the pH was adjusted to 5.8. Semisolid media were made by using 0.8 % agar, 20/45 ml of which were poured into test tubes (25 mm x 150 mm) /flasks (100ml), plugged with non absorbent cotton and autoclaved for 20 minuts (15psi1210) explant were inoculated into the culture tube/flask under aseptic conditions and were maintained at 25 ± 20 with 12 hours of light (3000 lux) and 70 5 relatice humidity. Once the cultures were established, they were subcultured in to culture tubes (30 ml x 200 mm) / flask (250 ml) containing semisolid of the bilayer medium. For making bilayer media 30/60 ml of agar-solidified media in tubes. Flasks were autoclaved, allowed to cool and 10/20 ml of sterile liquid media poured onto the top. Subculturing was done routinely by subdividing the shoot-bud clusters with scalpel and transferring the divided clusters into fresh medium.

RESULTS AND DISCUSSION

Pretreatment of explant with different antioxidants (L-ascobic acid, citric acid and L-cystine) at different concentration prior to inoculation into the media was found to reduce browning and hence mortality of cultures. Ascorbic acid gave the best results among the antioxidant tested. Keeping the isolated shoot-tips in a solution of L-ascorbic acid (100 mg/1) for 4 hours in dark at 40C, followed by surface sterilization with Hgcl2 (0.1 % for 5 minutes), rinsing with sterilized distilled water containing 80 mg/1 ascorbic acid and finally treating them with 50 mg/1 ascorbic acid for d5 mites immediately after excision was found to be maximally effective in controlling browning and in increasing the subculturing duration as needed. Keeping isolated shoot tips in ascorbic acid solution for 4 hours caused loosening of leaf premordia, facilities their excision with minimum injury and consequently better survival of the cultures. Effectiveness of pretreatments of explants with various antioxidant solution in controlling browing of the explant has also reported by early workers (1986).

Isolated shoot-tip (5mm2) cultured on MS basal medium supplemented with BAP (5mg/1 and IAA (0.2 mg/1) was found to be optimal for establishment of culture and for followed by swelling of trhe basal com after 14-18 days of culture. Initial of the multiple buds formation started after 34 days of inoculation in this medium and tiny buds arising from the basalcorn was visible to the naked eye. Berg and Bustamente (1979) reported culture of banana meristem in kundo's medium supplemented with cosamino acid (1g/1) and 10 % (V/V)coconut mild using various combinations and concentrations of cytokinin and auxin in the culture media to produce multiple plantlets from apical buds. Doreswamy et.al (1983) could produce more than 35 plantlets from auxiliary buds on MS minimal salt minerals salt containing 2 % sucrose supplemented with 15 % (v/v) coconut mild and 10 mg/1 BAP

Size of the explants markedly affected multiple bud formation. Explants of 2 mm3 sizes, when cultured on the MS basal medium supplemented with BAP (5mg/1) and IAA (0.2mg/1), initial responses to establishment and multiple buds induction were comparatively slow (5-7 weeks) and the survival per cent of the explants was very low (40 %). The total number of multiple buds in case of 2 mm3 explants was quite low as compared to large explants i.e. 4.6 after 14 weeks of culture. On the other hand, early induction of multiple was observed (3-4 weeks) when 4-5 mm3 explants were cut longitudinally through apex into two halves and cultured separately. Total numbers of multiples buds per half explants (successfully established) x 2 was much higher as compared to that of intact number of multiple buds was quite low in case of cut explants as compared to that of intact ones (Table 2). Multiple shoot proliferation could be enhanced on modified MS media by splitting a small shoot-tip longitudinally through apex Cronaur and Krilirian (1986). They established rapidly proliferating shoot culture by adding in the medium 5 mg/1 BAP and obtained 18 new shoots per shoot-tip. They found that best size of the multiple shoot production was 1-2 mm3.

The number of multiple buds per explants significantly increased after every four weeks of sub culture in semisolid medium of the same composition and on an average of 25-35 multiple buds developed per shoot-tip after 20 weeks of culture (4th subculture). Formation of multiple buds was further enhanced when they were subcultured in bilayer medium of the same composition. A total of 40-45 shoot buds was recorded in this medium after 20 weeks of culture (3rd subculture). Lower semisolid media was used to give support to the explants and upper liquid media enhanced multiplication rate. Duration on each subculture in bilayer media was comparatively more (6-8 weeks, as media surrounding the explants did not turn brown due to diffusion of oxidized phenolics, thus reducing inhibitory effect of oxidized phenolics on growth of the culture. More number of multiple buds could also be produced in less time subculture.

Regeneration or shoots and rooting from the individual shoot could be induced by transferring the individually separated multiple buds to the basal MS media supplemented with various combination and concentration of BAP, NAA an IAA (Table 3). No detectable response wa seen when the shoot buds were transferred to MS basal medium without any growth regulator. A combination of 0.2mg/1 BAP and 0.2mg/1NAA was found to be the best for simultaneous regeneration of shoot as well as roots. Cronauer and Krikirian (1984) induced individual shoots to root by eliminating all hormones from the medium and found coconut milk to be effective for rooting. Doreswamy et.al (1983) reported induction of roots from cultured shootles by growing them on Ms mineral salt containing growth of leafy-root-rooted production during 6-8 weeks of culture on medium without any phutohormones and 1 % sucrose. They found darkness increased the number of rooted shoots.

In vitro regenerated plantlets were hardened sequentially. Individual plantlets were first transferred to half strength of MS basal medium for 4 weeks. In vitro hardening produced plantlets with well-proportioned shoot and roots that were capable of supporting each other and had high survival rate in soil. In vitro hardened plantlets were thoroughly washed with tap water to remove agar from the roots and then soaked in 0.2 % Bavistin (A fungicide) for 5 minutes. They were then transferred in to plastic pots (5 cm diameter) containing autoclaved mixture of soilrite - perlite (10:1 v/v), watered and kept in growth chamber for four weeks. About 80 % of the plantlets were established in the pots. These plantlets were then transferred to bigger pots (20 cm diameter) containing sand 1 part topsoil 1 part, FYM ½ part and composted leaf ½ part. They were acclimatized at 27 ± 5 0 C with 16 hours photoperiod for 4 weeks. All the plantlets survived in the soil. Acclimatized plantlets were then transferred to field and watered regularly.

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Table 1 Influence of various combinations and concentration of plant growth regulators in MS basal medium on explant establishment and multiple shoot bud proliferation after 20 weeks of culture

Plant growth Regulators (mg/1)		Percentage establishment	Percentage plant responded	Days to initiation of multiple buds	No. of shoot buds per responding explant
IAA	BAP				
0.0	0.0	16.5 ± 1.06^{d}	0.0	-	-
0.2	1.0	20.3 ± 0.28^{d}	0.0	-	-
0.2	5.0	90.5 ± 1.14^{a}	90.5 ± 1.26^{b}	34.0 ± 1.7^{c}	42.2 ± 1.02^{a}
0.2	10.0	80.6 ± 1.71^{b}	57.0 ± 0.27^{b}	28.0 ± 1.2^{c}	16.4 ±0.84^{b}
1.0	0.0	26.3 ± 0.11^{c}	16.5 ± 0.03^{c}	48.0 ± 2.6b	2.1 ± 0.03^{d}
1.0	0.5	28.7 ± 0.36^{c}	14.2 ± 0.23^{c}	53.0 ± 3.5^{a}	7.4 ± 1.21^{c}

Table 2. Influence of different explant size for establishment and multiple shoot proliferation cultured in MS medium supplemented with IAA (0.2mg/1) and BAP(5.0mg/1) after 14 weeks of culture

Size of explant	Survival %	No. of multiple buds
2 mm^3	40 ± 1.2^{c}	4.6 ± 0.5^{c}
4 mm^3	90 ± 1.9^{a}	11.4 ± 0.32^{a}
5 mm^3	95 ± 1.73^{a}	12.2 ± 0.08^{a}
5 mm^3/2	60 ± 2.04^{b}	8.2 ± 0.19^{b}

Table 3. Influence of various combinations and concentration of plant growth regulators in MS basal medium on rooting from the vitro regeneration shoots

Plant growth Regulators (mg/1)			Percentage shoot bud rooted	Days of initiation of rooting	No. of roots responding explant	Average length
IAA	BAP	NAA				
0.0	0.0	0.0	0.0	-	-	-
0.1	0.0	0.1	58.5 ± 0.83^{d}	16.0 ± 0.27^{b}	4.2 ± 1.06^{b}	5.9 ± 1.32^{c}
0.2	5.0	0.2	32.0 ± 1.15^{c}	25.4 ± 0.40^{a}	2.8 ± 0.06^{c}	3.2 ± 0.04^{d}
0.0	0.2	0.2	95.0 ± 1.28^{a}	7.6 ± 0.06^{d}	9.1 ± 0.07^{a}	6.8 ± 1.01^{a}
0.0	0.0	0.2	77.5 ± 2.21^{c}	10.2 ± 1.32^{c}	5.4 ± 0.16^{b}	6.1 ± 0.84^{c}
0.0	0.2	0.5	83.2 ± 0.13^{b}	8.5 ± 1.00^{d}	8.6 ± 0.73^{a}	5.6 ± 1.02^{c}

In each column, Mean ± SE flowed by different superscript letter diifer significantly (P=0.05) according to Ducan's multiple range test.