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HIGH FREQUENCY ADVENTITIOUS PLANT REGENERATION FROM RADICLE EXPLANTS OF *AEGLE MARMELOS* CORR.

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Abstract

A procedure was developed which allows high frequency adventitious shoot regeneration from radicle tissues of *Aegle marmelos*. Adventitious buds were initiated on Murashige & Skoog's (MS) medium containing various combinations of benzyladenine (BA) and naphthaleneacetic acid (NAA). The medium containing 1.0 mg/l BA and 0.2 mg/l NAA produced highest number of shoots per explant with maximum frequency of regeneration. Shoots were elongated by transferring explants with shoot buds to a medium with a low concentration (0.1 mg/l) of BA. The shoots grown in medium containing 25 mg/l indolebutyric acid (IBA) for one week, when transferred to basal medium produced adventitious roots. Maximum rooting (80%) with 3-6 roots per shoot was achieved.

Introduction

Aegle marmelos Corr. (Rutaceae), commonly known as bael, thrives under arid, semiarid and sub-humid conditions (Arya, 1986). The plant is valued for its strong and durable timber, edible fruits and immense medicinal properties (Heywood & Chant, 1982). It offers great potential for horticultural, industrial and commercial exploitation,. Bael is seed propagated because it is very difficult to propagate vegetatively. Hence it is desirable to raise bael populations adopting tissue culture technology, as this technique permits multiplication of superior genotypes. Plantlets have been recovered from different organs of bael such as cotyledon, hypocotyl and leaf from seedlings (Arya *et al.*, 1981; Islam *et al.*, 1993, 1994; Hossain *et al.*, 1995) and embryonic tissues (Hossain *et al.*, 1994; Islam *et al.*, 1995). There does not appear to be any report using radicle tissues as an explant. The present report describes the totipotency of radicle tissues of *Aegle marmelos*.

Materials and Methods

Seeds were collected from ripe fruits of *A. marmelos* and washed thoroughly under running tap water to remove outer mucilaginous sheath. The seeds were surface sterilized by immersing in 0.1% HgCl2 for 10 min and then rinsed three times in sterile distilled water. The seeds were decorated and germinated on MS medium (Murashige & Skoog, 1962) with or without 1.0 mg/l BA. Radicle tissues from 10 day old seedlings were excised and cultured on the same basal medium supplemented with different concentrations and combinations of BA and NAA. After 5 weeks, the explants with adventitious shoot buds were transferred to a medium containing 0.1 mg/l BA for

elongation of shoots. The elongated shoots were grown on 25.0 mg/l IBA for one week and then transferred to hormone free medium for adventitious rooting. pH of the medium was adjusted at 5.8 gelled with 0.7% Difco Bacto-agar and autoclaved for 15 min (121°C and 108 KPa). The cultures were grown at 26 ± 1°C at 55-60% R.H under a 16 h photoperiod (50-70 μMol $m^{-2}s^{-1}$).

Results and Discussion

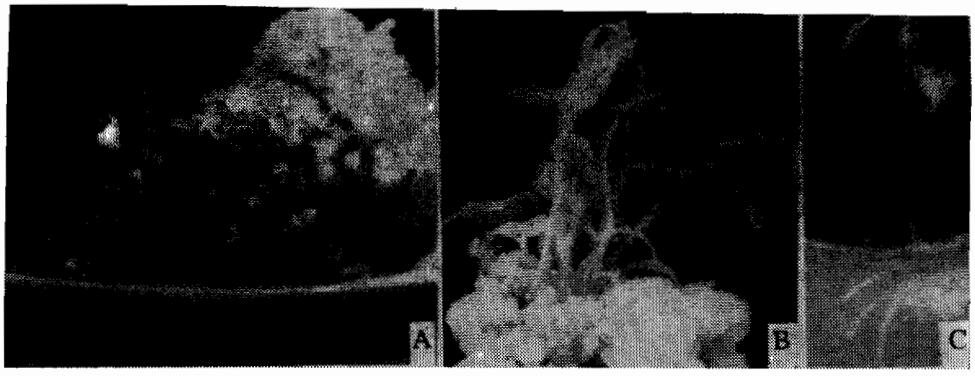
In a preliminary experiment radicle tissues from 10 day old seedlings raised on medium with or without BA were cultured on one shoot induction medium fortified with 1.0 mg/l BA and 0.2 mg/l NAA. Seedlings grown on BA-containing medium were characterized with stunted and thick radicle, and reduced hypocotyl and bushy thick shoots. The radicle explants taken from such seedlings produced atleast 2-3 times greater number of shoots than those from seedlings revised on medium without B A. Redicle tissues from seedlings grown on BA-containing media were used in subsequent proliferation experiments.

Morphogenetic changes of radicle explants were noticed within 2 weeks of culture. At the initial stage the explants increased in size and became green in colour. With increasing time in culture the explants either developed adventitious bud primordial directly or formed callus alongwith adventitious buds depending on growth regulator formulations (Table 1). In media with BA (1.0-2.0 mg/l) alone or in combination with

Table 1. Effect of growth regulators on adventitious shoot formation from radicle tissue of *Aegle marmelos*

Growth regulators (mg/L)		Shoot profliferating cultures (%)	No. of shoots per culture
BA	NAA		
0.5	-	51.2 de	10.3 f
1.0	-	54.3 cd	12.3 cde
2.0	-	44.2 e	10.6 ef
	0.1	61.6 bc	13.2 c
0.5			
	0.2	58.2 cd	12.6 cd
	0.1	68.3 b	18.6 b
1.0			
	0.2	78.8 a	25.8 a
	0.1	54.5 cd	10.8 a
2.0			
	0.2	57.7 cd	11.2 def

Means within a column having the same letter are not significantly different (P=0.05) according to Duncan's Multiple Range Test. Data on percentage of explants forming shoots were subjected to arcsin transformation before analysis and were transformed back to percentages for presentation.



Figs.1. Plant regeneration from radicle explants of *Aegle marmelos*.

A) Adventitious bud formation on the surface of radicle on MS + 1.0 mg/l BA and 0.2 mg/l NAA, after 4 weeks of culture.

B) Shoot elongation on MS + 0.1 mg/l BA after 4 weeks of culture.

C) Adventitious root induction from *in vitro* regenerated shoot grown on MS + 25.0 mg/l IBA for one week and then transferred to hormone free medium, 3 weeks after transfer.

0.1 mg/l of NAA the explants developed shoot buds directly. In case of direct organogenesis bud primordial appeared as knob-like structures on the surface of the explants (Fig.1A). On the other hand, in media containing BA @ 1.0-2.0 mg/l and 0.2 mg/l of NAA, shoot bud formation was always preceded by an early stage of callus growth. However, no correlation between amount of callus and number of shoots per culture was observed. By the end of 5th week of culture, the shoot buds had grown into shoots. Maximum frequency (78.8%) of adventitious shoot proliferating cultures was found in medium containing 1.0 mg/l BA and 0.2 mg/l NAA, where highest number of shoots (25.8) per explant were recorded followed by 1.0 mg/l BA and 0.1 mg/l NAA.

The explants that developed shoot buds were transferred to a medium containing a lower concentration (0.1 mg/l) of BA for elongation of shoots. Shoot elongation was best achieved when the regenerating part of the explant was cut into smaller pieces containing 4-5 shoots and subcultured onto the elongation medium. The developing shoots had sturdy growth with deep green leaves (Fig.1B). During elongation of shoot formation of callus at the base of the shoots also occurred. The regenerated shoots were excised individually and transferred to rooting medium (MS + 25.0 mg/l IBA) for one week and then transferred to basal medium. The percentage of rooted shoots reached above 80% with 3-6 roots per shoot within 4 weeks (Fig.1C).

Results of the present experiment present a reproducible and efficient plant regeneration system through organogenesis from radicle explants of *A.marmelos*. The system comprised 3 cultural steps: induction of adventitious shoot buds, elongation of shoots and root induction. Juvenile plants such as cotyledon, hypocotyl and various parts of seedlings have been reported as suitable explants for plant regeneration in many tree species (Rao *et al.*, 1981; Narayanaswamy, 1994). There is need to determine a method for regeneration of explants derived from mature trees to exploit the potential of cellular manipulation of *A. marmelos* clones.

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