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PLANT REGENERATION FROM EXCISED COTYLEDON OF *AEGLE MARMELOS* CORR.

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Abstract

A protocol for excising and culturing cotyledon explants from seeds of different ages of *Aegle marmelos* was developed. Cotyledon explants formed callus and shoot buds on agarsolidified Murashige & Skoog (MS) medium containing several combinations of 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA) and gibberellic acid (GA_3). The highest frequency of adventitious bud forming explants and maximum number of shoots per explant were obtained from 110-150 days old cotyledons. BAP with IAA or GA_3 gave better result than BAP alone. Shoots were elongated by transferring explants with shoot buds to same basal medium containing 1.0 mg/l kinetin (kn) and 0.1 mg/l IAA. The *in vitro* regenerated shoots rooted when cultured on half strength MS medium containing 25 mg/l indole-3-butyric acid (IBA). Regeneration by this method may be useful for mass propagation of *A. marmelos*.

Introduction

In vitro methods have attained considerable success for improvement and yield of temperate fruit trees. However, little progress has been achieved for tropical fruits (Rao *et al.*, 1981). *Aegle marmelos* is a crassinucellate deciduous woody fruit tree of tropics and valued for its fruit (used medicinally), timber and especially valuable in afforestation in arid and semiarid areas for drought tolerance. No systematic efforts have been made to regenerate plants from mature and juvenile tissue of this plant. Only Arya *et al.*, (1981) demonstrated plant regeneration through organogenesis from hypocotyl derived callus. The purpose of this work was to evaluate suitable age of cotyledon in establishing high efficiency plant regeneration protocol for mass propagation of *Aegle marmelos*. Three growth regulator formulations were selected in the light of previous data which induced high frequency shoot formation from hypocotyl explants (Hossain, 1992).

Materials and Methods

The ripe fruits and fruits at different stages of development (70-210 days after pollination) were collected from 100 years old mature tree and brought to laboratory. The cotyledons were grouped into 8 categories (Table 1) according to the age of fruits which was determined from approximate date of pollination to date of harvesting of fruits. The fruits were cut into pieces and seeds were collected carefully and surface sterilized with 0.5% $HgCl_2$ (w/v) for 5 min, with shaking and subsequently rinsed 3 times with sterile distilled water. The sterilized seeds were then taken in sterilized Petri dish and seed coats were carefully removed. Cotyledons without embryo axes were aseptically isolated under dissecting microscope and cultured on MS medium (Murashige & Skoog, 1962) with 3 growth regulators formulations (Table 1) for induction of adventitious shoot buds. The explants with adventitious shoot buds were then

transferred to MS medium supplemented with 1 mg/l kinetin (kn) and 0.1 mg/l IAA for elongation and growth of shoot buds. The elongated shoots were rooted on 1/2 strength MS medium containing 1.0 mg/l IBA. Media were solidified with 7 g/l Difco Bacto-agar and adjusted to pH 5.6 prior to autoclaving for 15 min at 1.1 kg per cm^2 pressure. All cultures were maintained in a growth chamber at 28°C with a 16/8 h light/dark cycle.

Table 1. Effect of age of cotyledon and hormonal combinations on shoot regeneration in *Aegle marmelos*. Data recorded after 5 weeks of culture.

Days after pollination	Growth regulators mg/l BAP	IAA	GA3	% of bud forming explant	Number of shoots per explant
	0.5			-	-
70	0.1	0.1		-	-
	1.5		0.5	-	-
	0.5			8	2.5
90	0.1	0.1		15	4.2
	1.5		0.5	14	3.8
	0.5			56	14.7
110	0.1	0.1		72	18.5
	1.5		0.5	68	28.2
	0.5			38	9.2
130	0.1	0.1		65	21.9
	1.5		0.5	70	22.6
	0.5			32	9.8
150	0.1	0.1		68	18.5
	1.5		0.5	63	16.4
	0.5			23	7.5
170	0.1	0.1		61	14.3
	1.5		0.5	52	16.4
	0.5			14	4.5
190	0.1	0.1		48	12.5
	1.5		0.5	48	10.9
	0.5			15	5.2
210	0.1	0.1		45	10.6
	1.5		0.5	42	11.8

Results and Discussion

Morphogenetic changes on the cotyledonary explants were noticed within 2 weeks of culture. At the initial stage cotyledon attained a maximum size and became dark green in colour. Morphogenic potentialities of the explants were greatly influenced by the age of the explants and growth regulator formulations (Table 1). Cotyledons of 70 days did not show any organogenesis other than callusing. The explants other than this group produced adventitious shoot buds directly with or without some callusing. First shoot buds appeared as knob-like structures and oozed out on the upper surface of the explant within 3 weeks of culture. In the next 1-2 weeks the knob-like structures grew into adventitious buds (Fig.1A). At the later stage these adventitious buds were recognizable as monopolar structures developing procambial strands which established connections with the preexisting tissues of cultured explants.

Maximum frequency of shoot bud formation (56-72%) and the highest number of shoots per explant (14.7-28.2) were recorded in cotyledons of 110 days age group. Both 130 and 150 days age groups showed similar response in respect of shoot bud formation. No regeneration was observed in media containing IAA alone. Only BAP @ 0.5 mg/l could induce adventitious shoot formation at a lower frequency. The percentage of shoot bud formation and number of shoots per explant increased considerably when IAA or GA_3 was added with BAP. Both IAA and GA_3 equally influenced shoot regeneration. Cotyledons of 90 days age group showed the lowest organogenesis potential.

The explants, which produced shoot buds, were subcultured on the same basal medium with 1 mg/l kn and 0.1 mg/l IAA. Within 2-3 weeks of subculture the buds grew up and developed into shoots (Fig.1B). The regenerated shoots were excised and transferred individually to root inducing medium. Root induction was observed within 2 weeks of culture. The shoots which failed to produce roots within this period were unable to produce roots even after 6 weeks of culture. Approximately 70% of shoots rooted on this medium and primary roots ranged from 6-12 in number (Fig.1C).

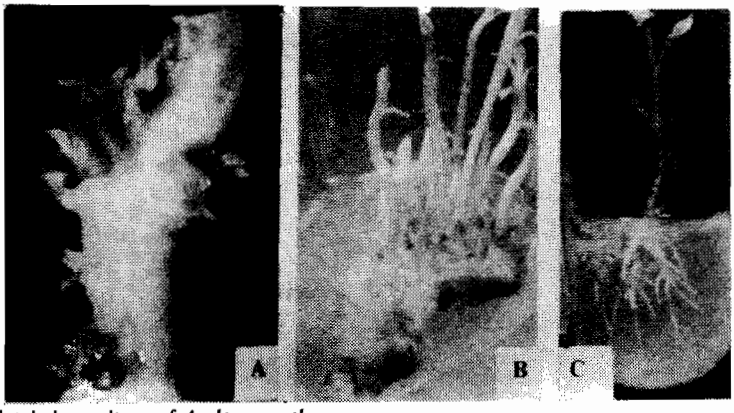

Fig.1 A-C. Cotyledon culture of *Aegle marmelos*.

A) Induction of adventitious shoot buds on cotyledon explant of 91-110 day age group on MS medium with BAP 1 mg/l and IAA 0.1 mg/l after 3 weeks of culture.

B) Development of shoots on MS medium with 1 mg/l kn and 0.1 mg/l IAA after 4 weeks of culture.

C) Induction of adventitious roots on *in vitro* raised shoot on 1/2 MS supplemented with 1.0 mg/l IBA.

The present experiments are reproducible for an efficient plant regeneration system from cotyledons with highest regeneration capacity at an age of 110-150 day. *Prunus persica* (Mante *et al.*, 1989), apple (Kouider *et al.*, 1985) and melon (Neidz *et al.*, 1989), cotyledons behaved similarly. This indicates that the developmental stage of the explant has a crucial effect on its response to regeneration treatments. Adventitious shoot regeneration from cotyledon explants has been reported in several other species (Rao *et al.*, 1981, Fazekas *et al.*, 1986; Dong & Jia, 1991; Ozcan *et al.*, 1992), indicating the potential of cotyledons as a suitable material for plant regeneration. The *in vitro* regeneration system described here is simple, rapid and reproducible and is potentially useful for mass propagation of *A. marmelos*.

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