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Plant regeneration of *Aegle Marmelos (l.)* corr. *from cotyledon* explants through *In vitro* studies

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ABSTRACT

Regeneration of whole plant from cotyledon explants was developed for Aegle marmelos (L.) corr. This method involved organogenic calli formation in a MS-basal medium supplement with BAP (Benzyl Amino Purine) and Zeatin-6-furfurylamine (0.5-2.5mg/l) in combination with NAA (0.5mg/l) under dark conditions. The well developed calli later developed shoots when transferred to hormone free medium and under illumination. Developed shoots continued to grow in MS medium free of hormones and produced roots at the presence of IBA (0.5-3.0mg/l and BAP (1.0mg/l)).Rooted plants survived well under acclimatization.

Key words: *Aegle marmelos*, organogenic calli, shoots regeneration

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INTRODUCTION

Aegle marmelos (L.) corr;commonly known as 'beli' or bael, it belongs to the family of Rutaceae. It is generally considered as an under-utilized fruit crop in India but the whole plant has valuable medicinal properties. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants [5]. The fruits are a good source of minerals and vitamins **[**8**]** and all parts of the plant (stem, bark, roots, leaves and fruits) are used in Ayurvedic medicines **[**7**]**. Root bark is used in intermittent fever and as fish poison, as a remedy for palpitation of heart and melancholia. Juice of the bark with a little cumin in milk is valued as remedy for poverty of seminal fluid. Alcoholic extract of roots having hypoglycaemic activity Bael is reported to contain a number of cumarins, proteins, carbohydrates, phenols, alkaloids, sterols and essential oils. Roots, barks and fruits are hypoglycaemic, astringent and febrifuge and they are antidiarrhoel and antivenin [10].

Bael fruit is a heterozygous out -breeding plant that can be easily propagated by seeds. However the seedlings show great variations in form, size, texture and quality of rind and numbers of seeds. Hence, vegetative multiplication is desirable in maintaining superior characters conventionally, grafting and layering are carried out achieve this, but for large -scale propagation method is they in need.

Nuclleus culture of *Aegle marmelos (L.)* corr.has been attempted to develop plants with seedless fruits [4]and *in vitro* auxiliary shoot proliferation is reported for rapid clonal propagation [1-2].

Micropropagation using tissue culture is an alternate method used to multiply mainly vegetatively propagated crops. A tissue-t-o plant regeneration system is thus an important pre-requisite for application of this technology for *Aegle marmelos (L.)* corr. In addition, this technology can be applied in crop improvement through involved variations and

mutations. Within this context, the objective of this study was to develop an efficient method of plant regeneration system using cotyledon tissue of *Aegle marmelos (L.)* corr.

MATERIALS AND METHODS

The explants source selected for the study of *Aegle marmelos (L.)* corr. seeds were selected and germinated *in vitro* using MSmedium[9]. When the seeds germinated, cotyledon tissues were obtained from part of the seeds while the rest was allowed to grow and produce plantlets to obtain axenic leaf tissue.

Cotyledon pieces were obtained from axenically germinated seeds and cultured on agar solidified MS medium containing sucrose (3%w/v) and different concentrations of cytokinin and auxin (BAP, Zeatin and NAA) and each treatment consisted of 10 replicates. Cultures were incubated at 25°c with illumination. Once calli were initiated, globular shaped organogenic calli were selected and sub cultured in a fresh medium and incubated under dark conditions to promote further calli proliferation.

Shoot Culture

Proliferated calli were sub cultured in fresh medium and it transferred to light condition were the calli turned into green in colour. The well developed calli transferred and allowed into shoot induction medium (MS medium) containing various concentrations and combination of growth hormones (BAP with NAA IAA). The shoots were promoted in the corner of callus tissues. The shoots (3cm) were separated and cultured in an MS medium for shoot elongation.

Root Formation

Shoots that were 3-4 cm in length and with 4-5 leaves were separated and cultured in MS basal medium (MS medium, sucrose (3%w/v) and solidified with agar (0.7%w/v) and added several growth regulators in various combination to promote root initiations and growth (IBA 0.1-3.0 with BAP 1.0mg/l) and (IBA 0.1-3.0 with Kin (1.0mg/l) with activated charcoal (0.5mg/l). Each treatment consisted of 10 replicates.

Acclimatization of plants

The rooted plantlets were transferred to a potting mixture of compost: sand (1:1) in 5cm diameter pots. They were kept inside a poly chambers for 28 days and the poly cover was gradually removed. The plants were allowed to grow in a plant house with application of a liquid fertilizer one a week.

RESULTS AND DISCUSSION

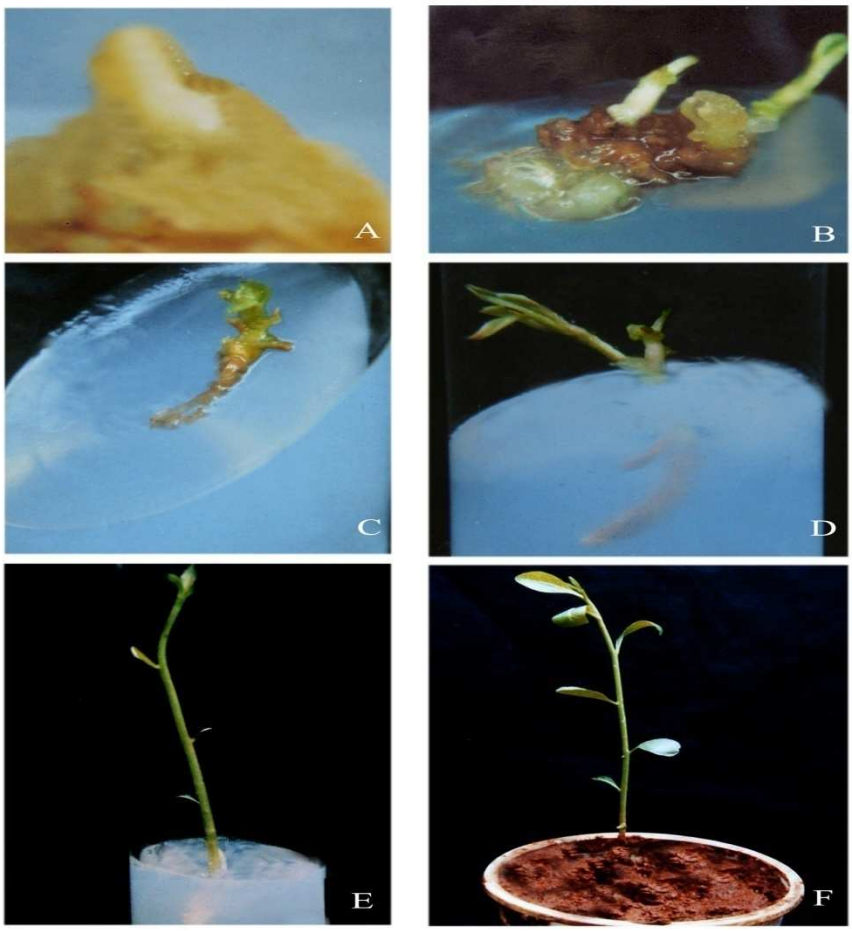
Calli formation was observed in cotyledon explants at 45-55 days. This response was highly observed in MS medium containing BAP (2.0mg/l and NAA 0.5mg/l) and Zeatin (2.0mg/l and NAA 0.5mg/l) under dark conditions 92% and 95% respectively (Fig 1) and Table-1 (Plate1). They were cream in colour and globular shaped, with an embryonic nature. The calli proliferated rapidly when separated and sub cultured in a fresh medium in 30 days for further studies.

Table 1 Effect of growth hormone on callus induction of frequency of *Aegle marmelos (L.)*Corr. *in* cotyledon explants.

S.NO	MS+Hormone concentration		Callus induction frequency (%)	Average number of shoots production
	BAP	**NAA**		
	0.5	0.5	-	-
	1.0	0.5	55	3-5
	1.5	0.5	75	7-10
	2.0	0.5	92	10-15
	2.5	0.5	85	8-12
	Zeatin	**NAA**		
	0.5	0.5	-	-
	1.0	0.5	68	2-4
	1.5	0.5	80	8-13
	2.0	0.5	95	12-18
	2.5	0.5	82	8-12

This result showed that, out of the several concentrations of BAP and Zeatin combination with NAA was tested; BAP with NAA (2.0mg/l+0.5mg/l) and Zeatin with NAA (2.0mg/l+0.5mg/l) is suitable concentration for callus induction of cotyledon explants in *Aegle marmelos (L.)*Corr. Since the growth regulators 2, 4-D is promoting abnormal cell divisions that can induce mutations; by [2-6] reported BAP at 1.5mg/l induced calli from young leaves of *Aegle marmelos (L.)*Corr.

PLATE-1



(A)Intitation of the Callus from Cotyledon Explants (B) Intitation of the Shoot bud(C) Intitation of the Root formation (D)& (E) Elongation of Shoots with buds and Shoots maturation (F) Plantlets in Plastic cups

When the calli were sub cultured into the above said medium and it allowed for further analysis. The shoot buds calli turned into green. Then the shoot buds calli sub cultured in the MS + hormone free medium from that each calli produced different number of shoots and its allow to growing healthy shoots for further studies.

Table 2 Effect of growth hormones on root induction frequency of *Aegle marmelos*

S.NO	MS+Hormone concentration		Average no of roots production
	IBA	**BAP**	
	0.5	1.0	1.3
	1.0	1.0	1.4
	1.5	1.0	3.5
	2.0	1.0	6.8
	2.5	1.0	3.6
	3.0		-
	IBA	**KIN**	-
	0.5	1.0	-
	1.0	1.0	-
	1.5	1.0	-
	2.0	1.0	1.5
	2.5	1.0	2.5
	3.0	1.0	3.0

As reported by [6] similar response was observed even with *in vitro* grown shoots. They found, that the 2-3 leaves from the distal parts of the *in vitro* shoots were the most responsive in terms of calli using and organogenesis[3] proved with *Flacourtia Jangowas(Lour),* a woody medicinal plants ,that all explants do not have the equal potential to regenerate shoots buds and only nodal segments derived callus produced shoot buds.

Enlargement of shoot base occurred to prior root initiation frequency was varied according to the hormone concentration. The IBA (2.0mg/l) and BAP (1.0mg/l) concentration was showed high number of roots compare to other concentration. The lowest response of root induction was Observed in IBA (0.5mg/l) and BAP (0.1mg/l) Table -2 The similar results was also reported by[1-2]**).***in vitro* auxiliary shoot proliferation is observed for rapid clonal propagations.

Plants were successfully aclimalitiazed under the given conditions and are now growing well in the plant house.
It is a well known fact that the plants regenerated via a callus phase may carry features that are different to the mother plant (somoclonal variations) and the performance of *Aegle marmelos (L.)*Corr. plants resulting from the present systems has to be evaluated in the field to detect (I) the suitability of this system as a method of micro propagation and (ii) type of changes that may have generated by this method.

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