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HIGH FREQUENCY IN VITRO REGENERATION VIA SOMATIC EMBRYOGENESIS IN MEDICINAL PLANT AEGLE MARMELOS (L.) CORR.

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Abstract

Aegle marmelos (L).Corr. commonly called Bael, belongs to family Rutaceae. It is middle sized, cylindrical, aromatic branched tree native to India and south East Asia. It has great mythological and medicinal significance protocol has been developed for regeneration of plantlets via somatic embryogenesis. Maximum callus induction observed in 2,4-D (2.0 mg/l) alone and combination of 2,4-D(0.5 mg/l) +KIN (2.0mg/l) + NAA (1.0 mg/l).Similarly, maximum induction of somatic embryogenesis and shoot induction from cotyledon explant was observed on Murashige and Skoog (MS) medium supplemented with BAP(2.0 mg/l)+NAA(0.5mg/l).

Keywords: Plant regeneration, Somatic embryogenesis, Callus induction.

Introduction

Medicinal plants comprised the exclusive source of life saving drugs in human being throughout the world. Traditional medicine system has become a topic of global importance. *Aegle marmelos* (L.) Corr. is belongs to family rutaceae. It is known with different names in different languages, commonly known as Bael. The Bael is a large tree, 8 to 10 meters in height and bears long thrones. It has a big stout trunk unusual branch with long leaves aromatic, usually three foliate. Flowers are sweet scented and greenish white. The fruit is large up to 5 to 15 cm in diameter, woody and smooth, globose, ovoid rind or grayish yellow. It has numerous seeds which are densely covered with fibrous hair and are embedded in a thick aromatic pulp. The flesh is eaten fresh or dried.

Bael tree is held sacred by the *Hindus*. The leaves of the tree are traditionally used as sacred offering to *Lord Shiva*, the God of health. *Aegle marmelos* (L.) Corr. is indigenous to Indian subcontinent and mostly found in Tropical and sub-tropical region .The medicinal properties of this plant have been described in the Ayurveda (T.Ramnathan et.al,2010).All parts of the tree used in preparing herbal medicine (Ranjoy Das.et al). Every parts of this plant *viz.*, stem, bark,root, leaves,fruit and flower at all stages of maturity have medicinal virtue(Malviya R.et.al).It has several chemical constituents viz. alkaloids like marmelosin, marmesin are major ones (Kala.et.al,), coumarin, Tannin, Steroids(Prabodh Chandra et al).

The bael plant has curative properties due to the presence of various complex chemical substances in them. It has a wide therapeutic importance in the treatment of diabetes, anemia, fractures, healing of wound, swollen joints, high bloodpressure, jaundice, diarrhea, typhoid.

The bael tree itself holistic and auspicious that its worship and it has great medicinal virtue. Conventionally, grafting and layering are carried out

to achieve this, but for large-scale propagation, they are not feasible methods. There is wide genetic variability in terms of quality, form and size of the fruit. Also, seeds have short viability and are prone to insect and fungal attack. Vegetative propagation through root suckers is possible, but it is so slow, difficult and cumbersome. For the large scale propagation it is not feasible method. A quick propagation method is need. *In vitro* micro propagation technique is alternative method used for multiplying disease free planting material in a large quantity within a short span of time.

Plant regeneration may be accomplished by employing callus, organ, cell and protoplast culture. Micropropagation may be accomplished by either organogenesis or somatic embryogenesis. Somatic embryogenesis has been achieved in number of angiosperms but success has been limited with woody species. The present communication comprises of high frequency plant regeneration via somatic embryogenesis using by zygotic embryo of *Aegle marmelos* (L.) Corr.

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Materials and Methods

Seed of *Aegle marmelos* (L.) corr. *(*Bael) were collected from the best quality and large size mature bael fruits. For seed germination, seeds were first washed under running tap water for 3 - 5 min. Floating seeds were considered to be empty and discarded. Later the seeds were dipped in 70% alcohol for 30 sec, followed by washing with distilled water. The seeds were surface sterilized with0.2% (w/v) mercuric chloride for 10 min. with continuous shaking. Finally it was washed four times with sterilized distilled water.

Culture media and Growth Conditions:

Full strength M.S medium (Murashige and Skoog, 1962) supplement with 3 %sucrose (Hi media Mumbai, India) ,0.2% Clerigel (Hi-media, Mumbai India) and different combination of Auxin (2,4-D,NAA,IBA) and Cytokinin (BAP,KIN) at the concentration 0.5,1.0…….3.0 mg/l was used as the callus induction medium. The pH of the medium was adjusted to 5.8 before the addition of Clerigel .Culture bottles were filled with 50 ml of the media. The media was autoclaved at 15 lbs. (121℃) for 15 min. Cotyledon explant were used to induce the embryonic callus from *Aegle marmelos* L. (Corr).The surface sterilized seeds were incubated to callus induction medium.

The cultures were incubated at 25 ± 2^0C with 16 h photoperiod with the light intensity of 3000 lux under cool white florescent tubes. All the experiment were conducted in 5 replicates and repeated for 3 times. The Number of days, frequency of callus formation and color of callus were determined after 4 weeks of culture. The morphology of embryonic callus was also observed every two weeks after the callus formation.

Development and plant regeneration from Somatic Embryogenesis:

In order to develop shoots from somatic embryos from the embryonic callus various hormones viz. NAA, 2-4-D, Kin and BAP were tried along with MS medium. After inoculation slow growing, compact, yellowish green with nodular callus formation was observed. The callus was transferred to the medium similar to the initial medium. After 1-2 weeks, yellowish green colored, smooth zygotic embryos were noticed, which have different developmental stages. Meanwhile dense shoot formation was noticed from these embryos.

Results and Discussion

Effects of different Auxins and Cytokinins on the cotyledon explants for callus induction:

Effects of different Auxins and Cytokinins were studied by using cotyledon as an explants. The basic culture medium utilized in present piece of work was Murashige and Skoog medium (MS) supplemented with different concentration of Auxins and cytokinins. Results obtained during experiment revealed that when 2,4-D and NAA tried alone and in combination of BAP and KIN, shows callus induction. Maximum proliferation of callus was achieved on (2 mg/ L) 2, 4 D when it was tried alone and in combination (2 mg/L) BAP and from (0.2 mg/L to 0.4 mg/L). Increase in concentration of NAA subsequently increased the proliferation of cotyledons tissue. Highest rate of callus induction was recorded on MS medium supplemented with NAA, with (1mg/L + 0.5mg/L) KIN (2.0 mg/L) of and 2, 4 D (0.4 mg/L)and NAA with (2.0 mg/L) of BAP(Table 2). Callus induced in present study were showing variability in terms of color and texture (Plate.1). Yellowish, Green, Light Green, White, creamish color callus were frequently observed with different type of texture viz. smooth, rough, crystalline. Callus developed in present piece of work were showed somatic embryogenesis with different types of shapes .In this study, cotyledon the medium containing combination of BAP (2.0 mg/L)explants capable to induce somatic embryogenesis in + NAA (0.4 mg/L), similar results

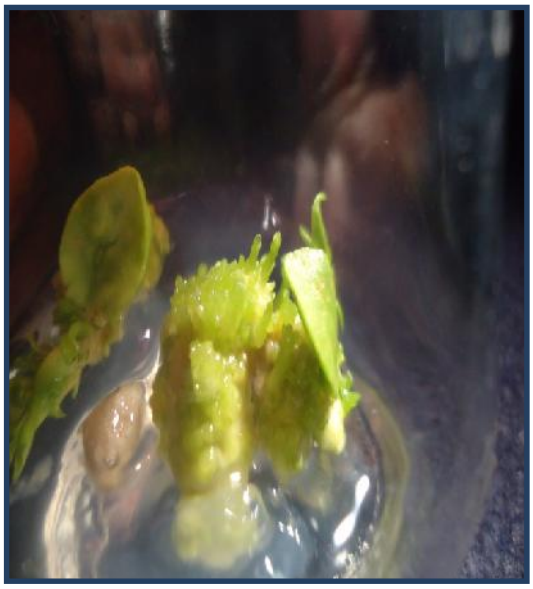
reported by (Hegde et. al 1994) and (Onay et, al 1995) in which the somatic embryo were grown from cotyledon explant. The yellowish, yellowish green, yellowish light green, Cream colored, compact, glossy callus was induced from cotyledon.

Table 1: Effect of different concentration of PGR's on direct somatic embryonic callus from cotyledon explant of *Aegle marmelos* (L.) Corr.

Concentrations of Plant Growth Regulator (mg/l)				Frequency of Callus formation	Frequency of Somatic embryonic callus	Color of callus/ somatic embryo
NAA	2,4-D	BAP	KIN			
0.1	-	2.0	-	+	+	Yellowish green
0.2	-	2.0	-	+++	+++	Yellowish light green
0.3	-	2.0	-	++++	++++	Yellowish green
0.4	-	2.0	-	+++++	+++++	Whitish
-	1.0	-	-	+	-	Creamish
-	1.5	-	-	+++	-	Creamish
-	2.0	-	-	++++	-	Creamish
-	2.5	-	-	+	-	Creamish
1.0	0.5	-	0.5	+	+	Yellowish
1.0	0.5	-	1.0	+++	+++	Yellowish
1.0	0.5	-	1.5	+++	++++	Yellowish
1.0	0.5	-	2.0	+++++	+++	Creamish

+ : very weak ; +++: Moderate; ++++: Profuse; +++++: Very profuse

Plate:1. Effects of different Auxins and Cytokinins on the cotyledon explants for callus induction:



Development and Plant Regeneration from Somatic embryos:

Somatic embryo development was highly depended on morphology of the embryonic callus. The development of somatic embryos was represented by the green colored with smooth surfaced structures on the compact, yellowish, yellowish light green callus. When viewed under the microscope, the cotyledonary stage and the multiplication of somatic embryos were observed. The organized structures were induced either directly from explant or from intervening compact callus. The organized structure obtained from cotyledonary explant on MS media fortified with various concentration of NAA,BAP,KIN, 2,4-D (0.5 to 2.5 mg/L) without an intervening of callus, most of the organized structures were developed from intervening callus on media fortified with BAP +NAA (0.5 to 2.0 mg/L) and 2,4-D+ KIN +NAA(0.5 to 2.0 mg/L). No organized structures were observed on explant on media with 2,4-D alone (0.5 to2.5 mg/L) (Table 2). According to Arnold et. al. (2002), somatic embryo were noticed with bipolar and bear typical embryonic organ. The radial, hypocotyls, cotyledons and somatic embryos were developed and differentiated from the somatic cell without vascular connection from the original tissue. The organized structures were identified as somatic embryos on the basis of their globular shaped, heart shaped, torpedo shaped morphology (Plate.2). A histological examination of one of the heart shaped structures exhibited a bipolar organization with future shoot and root apices. After six week, somatic embryos were actively developed radical and apices active within 15days, cotyledons were turned dark green, shoot were elongated. Rhizogenesis was achieved by sub- culture fully elongated shoots on MS medium containing 1.0 mg/L of NAA.

Plate.2. Development and Plant Regeneration from Somatic embryos:

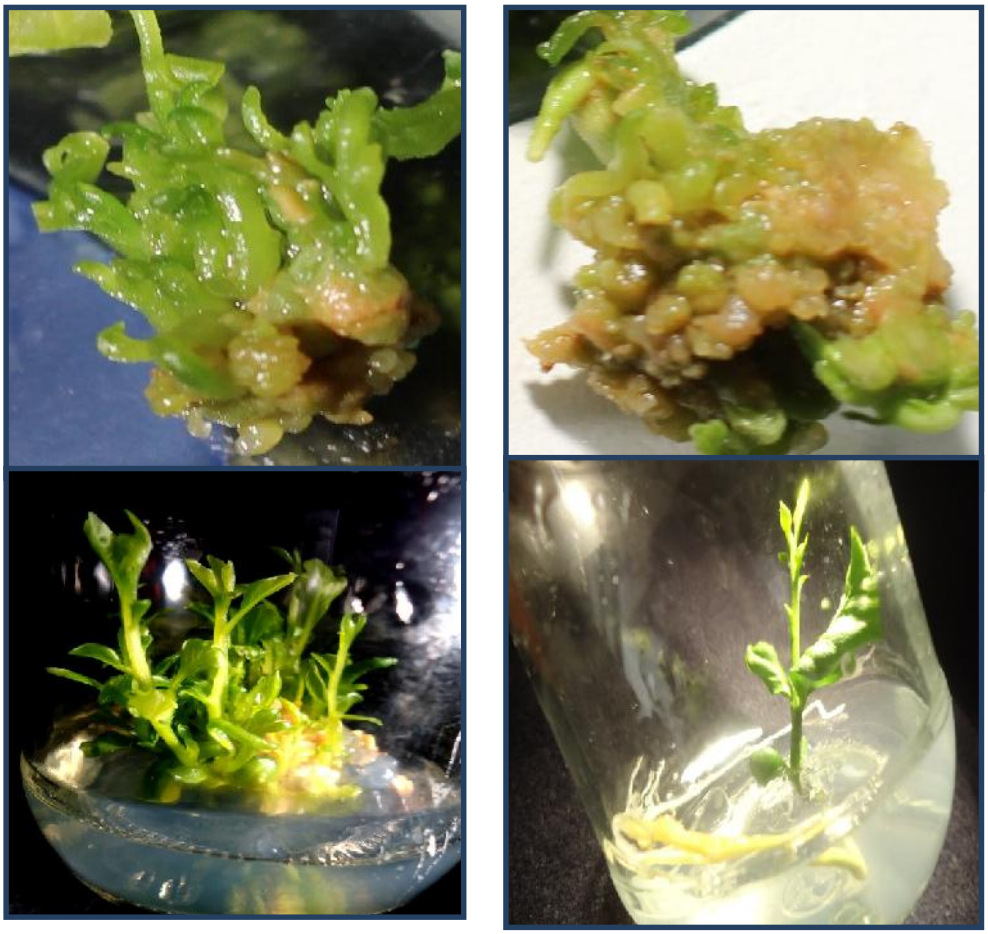


Table 2 :Effect of PGR's on Shoot induction From Embryonic Callus Of *Aegle marmelos* (L.) Corr.

Concentrations of PGR's (mg/l)				No of shoot induced	Mean ± SE
NAA	2,4-D	BAP	KIN		
0.1	-	2.0	-	10	8± 0.577
0.2	-	2.0	-	22	18± 1.632
0.3	-	2.0	-	36	28± 1.527
0.4	-	2.0	-	52	45 ± 2.848
-	1.0	-	-	-	-
-	1.5	-	-	-	-
-	2.0	-	-	-	-
-	2.5	-	-	-	-
1.0	0.5	-	0.5	07	5 ± 0.577
1.0	0.5	-	1.0	12	9 ± 0.881
1.0	0.5	-	1.5	32	28 ± 1.201
1.0	0.5	-	2.0	25	19 ±1.452

Conclusion

The result presented here indicates that *in vitro* regeneration of complete plantlets is possible using callus culture through somatic embryogenesis of *Aegle marmelos.* Physiological state of the explant plays an important role in the regeneration of plants. Embryonic callus originated from somatic embryos were capable of producing plants. The mature embryos are known to be a good source for initiating callus which possesses high regenerative capacity as has been shown in peach (Hammerschlag et al. 1985), oak and linden (Chalupa et al.1990).

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