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Invitro germination of Bael (*Aegle marmelos* (L.) Corr.) seeds for clonal propagation

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ABSTRACT

Aegle marmelos, commonly known as Bael, is one of the most important medicinal plants of Indian tradition. All parts of the tree viz. leaf, fruit, bark and root have medicinal properties and have been used in many traditional medicine systems. The present study trial has been taken up at Horticultural College & Research Institute, Coimbatore to establish a protocol for in vitro germination of seeds to use it further for clonal propagation. The investigation revealed that the seeds without seed coat gave more number of multiple shoots (5.57) where as the seeds with seed coat gave single seedling. The days taken for germination was lesser in seeds without seed coat (14.76 days) than the the seeds with seed coat (20.59 days). The longest seedling was observed in the seeds with seed coat (2.09 cm) compared to that of seeds without seed coat (1.22 cm). the days taken for budbreak, number of shoots were higher in the MS medium supplemented with BAP 1.5 mgl^{-1}. Comparison between the explants from in vitro and field grown seedlings showed that the time taken for culture response was much earlier in the in vitro derived seedlings. All the explants showed better response in the basal MS medium supplemented with BAP 1.5 mgl^{-1}.

Keywords: *In vitro* germination, explants, field grown seedlings, MS medium, multiple shoots

INTRODUCTION

Aegle marmelos, commonly known as Bael, is one of the most important medicinal plants of Indian tradition. All parts of the tree *viz.* leaf, fruit, bark and root have medicinal properties and have been used in many traditional medicine systems. The leaves of *Aegle marmelos* are useful as laxative, astringent, expectorant and are useful in inflammation, diabetes and asthmatic complaints. The fruits are used to treat diarrhoea, dysentery and stomachalgia. The fruits extracts are also reported to show antiviral activities. *Aegle marmelos* is cultivated in marginal lands and conventionally it is propagated through seeds and root suckers (Ghosh *et al*, 2012). Seeds show short viability and vary with the source and size of seeds (Karthiyayini, 2017). Multiplication through root suckers is slow indicating the difficulty in propagation of *Aegle marmelos* (Neeraj *et al*, 2017). In terms of phytochemical extraction, improper collection of wild sources may cause extinction of the species. Hence, in spite of the available reports on micropropagation of *Aegle marmelos*, a complete study integrating both *in vitro* studies and phytochemical screening is essential. As the explants from trees show poor response *in vitro*, the seedlings germinated *in vitro* are good source of explants for clonal propagation. Hence, in the present study trial has been taken to establish a protocol for *in vitro* germination of seeds to use it further for clonal propagation.

MATERIALS AND METHODS

Fruits of *Aegle marmelos* were collected and the hard rind of ripen fruits was broken to extract the pulp. Repeated washing was done to get clean seeds, as the seeds are embedded in mucilage. The seeds were used with and without seed coat. MS medium developed by Murashige and Skoog (1962) was used with certain modifications supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg l^{-1}). The glasswares were soaked in 0.1 per cent potassium dichromate solution for 12 hours and washed thoroughly with tap water. After draining the water, they were sterilised in autoclave at 15 pounds per inch pressure at 121°C temperature for 20 minutes. The explants were washed with tap water followed by a washing with Tween 20 emulsifier. Then they were washed 2-3 times with distilled water. Surface sterilisation was done with ethyl alcohol

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(70 %) for 30 seconds. The explants so prepared were rinsed in 0.1% mercuric chloride for 3 minutes. The treated explants were taken to laminar airflow chamber and washed four times with sterile distilled water to make them free of sterilants. The culture tubes in the stand were kept inside the culture room maintained free of contamination. The culture room was maintained at a temperature range of 25 ± 2°C with 1500 lux light intensity using day light florescent tubes and relative humidity of 75 per cent. A photoperiod of 16 hrs light and 8 hrs darkness was maintained in the culture room. The number of days taken germination was counted and the mean was recorded. The length of shoots from collar region to the tip of the shoot was measured and the mean length was expressed in centimeter. The number of multiple shoots produced per explant was counted and the mean recorded. All experiments were conducted in Completely Randomized Block Design (CRD) and the data gathered from various experiments were subjected to statistical analysis as per the methods of Panse and Sukatme (1978) and the results were interpreted.

RESULTS AND DISCUSSION

In vitro germination of seeds

The percentage of germination of seeds at different levels of BAP showed significant difference from the control (Table 1). All treatments except lowest concentration of BAP showed the maximum germination percentage and the lowest response was found in the control. Among the two explants tried, there was no significant difference in the percentage of germination. The interaction effect between the explants and growth regulators was also not statistically significant. The effect of different concentrations of BAP on the time taken for germination was the shortest in BAP 1.5 mgl^{-1} followed by BAP 2.0 mgl^{-1}. It was the longest in the control (Table 1).The seeds with seed coat germinated earlier (14.76 days) when compared to the seeds without seed coat (20.59 days). The interaction effect between the explants and growth regulators was not statistically significant. The seeds with seed coat germinated normally into a single seedling in all the growth regulator treatments.

Table 1: Effect of growth regulators on in vitro germination of seeds

Treatment (BAP mgl^{-1})	Percentage of germination		Days taken for germination		Number of shoots formed		Seedling height (cm)	
	Seeds with seed coat	Seeds without seed coat	Seeds with seed coat	Seeds without seed coat	Seeds with seed coat	Seeds without seed coat	Seeds with seed coat	Seeds without seed coat
0.5	86.67 (68.86)	86.67 (68.86)	16.00	22.23	1.0	2.57	1.05	0.75
1.0	100.00 (89.09)	100.00 (89.09)	14.00	20.37	1.0	2.63	2.23	1.34
1.5	100.00 (89.09)	100.00 (89.09)	11.97	17.10	1.0	5.57	3.83	2.34
2.0	100.00 (89.09)	100.00 (89.09)	12.33	18.63	1.0	4.67	3.14	1.86
2.5	100.00 (89.09)	100.00 (89.09)	13.97	19.73	1.0	4.43	1.82	1.05
3.0	100.00 (89.09)	100.00 (89.09)	14.70	21.27	1.0	3.67	1.63	0.61
-	70.00 (56.99)	76.67 (61.22)	20.37	24.77	1.0	1.07	0.92	0.62
CD BAP	2.267		0.748		0.554		0.149	
Explant	4 241		1.399		-		0.278	
BAP x Explant	5.998		1.979		-		0.394	

**Values in parentheses are arcsine transformed values*

The results of the present study show that, there was highest germination response of both the seeds with and without seed coat even in the lowest concentration of BAP. Even in the control, without BAP, there was about 70 per cent germination of the seeds. The timetaken

for the germination of seeds was least in the BAP concentration of 1.5 mgl^{-1}. Thereafter the increase in the concentration of BAP has no additional advantage. Raghu *et al.* (2007) reported that concentration of BA beyond 1.0 mgl^{-1} has induced callusing in *Aegle marmelos* i*n vitro* cultures. In our experiments there was no callus initiation but the positive effect of BAP was only upto 1.5 mgl^{-1} which was in accordance with their report. Reports on difference among explants of different origin were reported in *Aegle marmelos* by Shahina Parveen *et al* (2015). But when the seed coat was removed and inoculated there was production of more than one shoot. The highest number of shoots (5.57) was observed in the treatment BAP 1.5 mgl^{-1} followed by BAP 2.0 mgl^{-1}. The lowest number of shoots was observed in the control (without BAP). The seedling height ranged between 0.61 cm and 3.83 cm among the different treatments (Table 1). The seedlings were longer (3.09 cm) in BAP 1.5 mgl^{-1} followed by BAP 2.0 mgl^{-1}. The shortest seedlings were observed in the control. The explants also showed significant difference for the seedling height. The longest seedling was observed in the seeds with seed coat (2.09 cm) compared to that of seeds without seed coat (1.22 cm). The interaction effect between growth regulators and explants was statistically significant. The longest seedling (3.83 cm) was observed when the seeds with seedcoat germinated in BAP 1.5 mgl^{-1} followed by BAP 2.0 mgl^{-1}. Seeds without seed coat showed the lowest shoot length

When the seeds with and without seed coat were compared, the later took comparatively longer time for germination. But when the number of shoots was considered, the seeds without seed coat produced more number of shoots whereas the seeds with intact seed coat produced single seedlings. Islam *et al*. (2006) has reported the regeneration of adventitious shoots from cotyledonary explants, but they have used cut bits of cotyledon. In the present study, only the seed coat was removed without damaging the inner tissues. It was reported by the same workers that the number of shoots produced was in the range of 10 and 15. But according to the present study, highest number of shoots was five. This was because only the shoots which are above 1.0 cm in length were considered and the count was taken 15 days after culture initiation whereas the reported literature shows the data on 30 days after culture initiation and includes all the shoots irrespective of their length. Gandhi *et al* (2018) reported comparatively lesser time of germination for the seeds than the present study. This may be attributed to the genetic characteristic of the seeds used than to the growing conditions and the culture medium composition which needs further exploration.

Multiple shoot induction

The growth regulator treatments showed bud break significantly earlier than the control. The minimum time taken for bud break was found in BAP 1.5 $mgl^{-1.}$ The explants in the medium without BAP took longer time for bud break. The shoot tips showed faster response in 5.76 days compared to basal nodal segments and axillary buds. The interaction effect of explants and growth regulator was significant. Bud break was earlier in shoot tips and basal nodal segments in BAP 1.5 mg l^{-1} followed by axillary buds in the same treatment (Table 2).

Table 2: Effect of growth regulators on initiation and multiplication of microshoots from *in vitro* seedling explants

Treatment (BAP mgl^{-1})	No. of days taken for bud break			Number of multiple shoots			Length of shoots (cm)		
	Shoot tip	Axillary bud	Basal nodal segment	Shoot tip	Axillary bud	Basal nodal segment	Shoot tip	Axillary bud	Basal nodal segment
0.5	5.20	6.77	7.30	1.13	5.43	12.07	1.21	1.05	0.84
1.0	5.80	6.50	5.63	1.07	9.67	17.57	1.85	1.92	1.36
1.5	3.17	3.67	3.17	1.07	11.87	26.40	3.13	2.60	2.08
2.0	3.90	5.37	3.77	1.10	13.20	21.97	2.64	1.73	1.72
2.5	5.20	6.47	5.93	1.00	8.27	19.37	2.04	1.70	1.53
3.0	6.90	7.27	7.83	0.97	8.07	18.20	1.61	1.11	1.56
Control	10.13	10.87	11.67	0.77	1.07	2.33	0.71	0.62	0.41
CD BAP	0.631			1.277			0.134		
Explant	0.413			0.85			0.087		
BAP x Explant	1.093			2.211			0.231		

The number of shoots ranged between 0.77 and 26.40 among the different treatments. The number of shoots was highest in G_3 BAP 1.5 mgl^{-1} which is significantly low in the control. Single shoots were produced by shoot tips in different growth regulator concentrations. The highest number of microshoots was produced by the basal nodal segments followed by the axillary buds. The length of shoots was the highest in BAP 1.5 mgl^{-1} and control showed the lowest shoot length. The explants differed significantly among themselves in the length of shoots produced. The longest shoots were produced by the shoot tips, whereas the shortest shoots were formed from basal nodal segments. The growth regulator treatments showed bud break significantly earlier than the control. The time taken for bud break was lowest in BAP 1.5 mgl^{-1}.There was no significant difference found among the explants. The interaction effect of explants and growth regulator was significant. Bud break was earlier in axillary in G_3 BAP 1.5 mgl^{-1} closely followed by shoot tips in the same treatment (Table 3). The axillary buds produced more number of shoots in BAP 1.5 mgl^{-1} and the lowest number of shoots was produced by the shoot titps in control. Shoot tips produced longer shoots followed by axillary buds in BAP 1.5 mgl^{-1}. Similar results were reported by many workers (Puspashree Puhan and Shiba Prasad Rath, 2012; Gandhi *et al.,*2018; Asha Gupta*et al.,* 2018).

Table 3: Effect of growth regulators on initiation and multiplication of microshoots from field grown seedling explants

Treatment (BAP mgl^{-1})	No. of days taken for bud break		No. of multiple shoots		Length of shoots (cm)	
	Shoot tip	Axillary bud	Shoot tip	Axillary bud	Shoot tip	Axillary bud
0.5	12.20	10.40	0.93	5.40	1.22	1.17
1.0	10.17	8.23	1.00	7.50	2.02	2.00
1.5	7.27	7.20	1.00	9.50	3.26	2.54
2.0	9.00	9.13	1.00	9.13	2.67	1.86
2.5	9.60	9.83	1.00	7.03	2.05	1.65
3.0	11.37	10.83	1.00	6.13	1.57	1.06
Control	15.13	16.43	0.67	1.13	0.63	0.53
BAP	0.978		0.687		0.208	
Explant	0.523		0.367		0.111	
BAP x Explant	1.384		0.971		0.294	

Comparison between the explants from *in vitro* and field grown seedlings showed that the time taken for culture response was much earlier in the *in vitro* derived seedlings. The number of shoots obtained was also comparatively higher. The *in vitro* seedling explants were comparatively younger and succulent and they might be activated readily by active absorption of growth substances. The field grown seedling explants took more time for releasing from the dormant phase and hence the time taken for budbreak and further development of multiple shoots was higher (Purohit *et al.,* 1995; Sen and Sharma, 1997; DAS *et al.,* 1999; Indhumathi, 2002).

All the explants showed better response in the basal MS medium supplemented with BAP 1.5 mgl^{-1}. The number of shoots increased upto 1.5 mgl^{-1} of BAP in the medium and beyond that concentration, there was no added effect due to increase in concentration. Similar results were reported by Islam *et al*. (2006) who reported that the number of shoots decreased with increase in concentration of BA beyond 1.5 mgl^{-1}. Tanaka and Sakanishi (1978) reported occurrence of shoot malformation if higher concentration of BAP was used in the medium. Though there was no malformation observed in the present study, the increase in BAP concentration beyond 1.5 mgl^{-1} has no added advantage. Shahina Parveen *et al* (2015) also reported that the numbers of shoots were obtained from axillary buds.

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