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In Vitro Seed Germination and Clonal Propagation through Epicotyls Explants of *Aegle Marmelos*

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Abstract: *An efficient and rapid in vitro clonal propagation of medicinally important Aegle marmelos (L.) family Rutaceae was designed from epicotyls explants. The explants were obtained by in vitro seed germination in presence of phytoregulators. Response of kinetin was found more significant in in vitro seed germination. Multiple shoots were formed on Murashige and Skoog (MS) medium supplemented with different concentration of Benzyl amino purine (BAP)/Kinetin alone or in combination. Maximum number of 19.35±0.324 shoots were formed on MS medium supplemented with BAP and kinetin (1.5mg/l+1.5mg/l). In vitro rooting was done on different concentration of auxins (Indole 3-butyric acid-IBA, Indole 3-acetic acid-IAA and Naphthalene acetic acid- NAA). IBA at 1.0 mg/l was found best for rooting. The method standerised could be used for large scale production of this medicinally important tree.*

Keywords: *epicotyls, in vitro, seed germination, phytoregulators etc.*

1. INTRODUCTION

Tissue culture protocols have been extensively used for *in vitro* propagation, germplasm conservation and production of pharmaceutically important bioactive compounds, Nalawade, et. al.; (2003); Phatak and Heble (2002). Plant tissue culture provides the possibility of applying different growing conditions including phytoregulators to induce a response of germination in seeds. Tissues obtained from *in vitro* seed germination are frequently used in micropropagation because they are usually easy to establish in culture and form the basis of development of suitable nutrient media, Aitken-Christie and Thorpe (1984). Despite great advancements of plant tissue culture techniques the success with woody plants has continued to be a challenging task, Mc Cown (2000).

Aegle marmelos family Rutaceae is an out breeder and is routinely propagated by seeds for cultivation, Singh et. al.; (1976). Seeds have short viability and are prone to insect attack, Purohit and Vyas (2004). Indiscriminate collection resulted in disappearance of this plant and species is reported to be vulnerable in Western Ghats of Kerala, Tamil Nadu and Karnataka states of India, Ravikumar and Ved (2000). Therefore there is need of its conservation and one of the means is micropropagation.

The present study was aimed at developing a highly efficient and reproductive protocol for rapid shoot multiplication of *Aegle marmelos*. For this purpose, epicotyl explants were selected obtained from in vitro seed in presence of phytoregulators.

2. MATERIAL AND METHODS

Seeds were obtained from fruits collected from Keoladeo National Park, Bharatpur and plant was certified as *Aegle marmelos* by Botanical survey of India, (BSI), Jodhpur. Seeds were germinated in vitro on MS medium with 3% sucrose and 0.8% agar and in presence of growth regulators including Benzyl amino purine, Kinetin and Gibberelic acid. Seed cultures were placed in dark for 4 days and then shifted to 16 hours photoperiod. Epicotyl explants were excised from 2-3 week old cultures and were used for further set of experiments.

Epicotyl explants obtained from in vitro seed germination were cultured on MS medium supplemented with different concentrations and combinations of phytohormones to obtain multiple shoots. Cultures were incubated at 26 ± 2^0C under 16 hours photoperiod for 5 weeks.

Replicates were taken for each treatment. Periodic observations were recorded and results were subjected to statistical analysis.

3. RESULTS AND DISCUSSIONS

Results indicated that presence of growth regulators significantly affected seed germination. Kinetin was found most effective that showed germination in 12±0.25 days (Fig. A). Gibberelic acid was found better after kinetin by showing germination in 13±0.29 (Table 1) days. Here kinetin was found better, though Gibberelic acid has shown good germination response in other plants of Rutaceae family including *Dictamus albus* and *Ruta chalepensis*, Martyn et. al.; (2009). Earlier research workers have worked on in vitro seed germination of *Aegle marmelos* by maintaining moisture level and chemical treatment, Sharma et. al.; (2011); Nayak and Sen (1999).

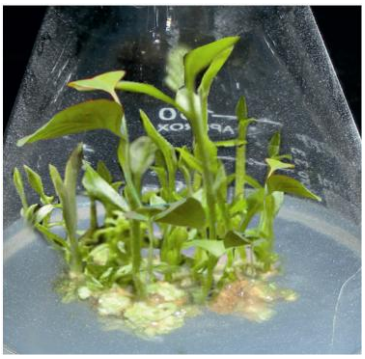
Table1. *Effect of Phytohormones on in vitro germination of Aegle marmelos seeds*

S.No	Phytohormone concentration (mg/l)	Days taken for germination	Germination Index
	Control : MS basal medium	NIL	NIL
	BAP		
1.	0.5	20.75±0.270	1.34
2.	1.0	19.00±0.163	2.18
	Kinetin		
3.	0.5	21.03±0.313	1.28
4.	1.0	12.10±0.259	1.68
	Gibberelic acid		
5.	0.5	19.03±0.269	1.42
6.	1.0	13.73±0.295	1.69

Table2. *Effect of BAP and Kinetin on shoot bud proliferation through in vitro germinated seedling from in vitro germinated seeds*

S.No.	Cytokinin Concentration (mg/l)	No. of Shoots buds per explants Mean±S.E.
	BAP+Kinetin	
1.	0.5 -	08.41±0.434
2.	1.0 -	11.41±0.434
3.	1.5 -	10.50±0.452
4.	2.0 -	9.18±0.463
5.	2.5 -	7.70±0.528
6.	3.0 -	5.80±0.466
7.	- 0.5	8.36±0.491
8.	- 1.0	9.67±0.449
9.	- 1.5	13.15±0.405
10.	- 2.0	10.10±0.314
11.	- 2.5	8.45±0.578
12.	- 3.0	4.66±0.372
13.	0.5 + 0.5	12.07±0.548
14.	1.0 + 1.0	16.35±0.439
15.	1.5 + 1.5	19.35±0.324
16.	2.0 + 2.0	15.50±0.452
17.	2.5 + 2.5	12.08±0.378

FigA. *BAP (1.0 mg/l)*



FigB. *BAP+Kn (1.5+1.5 mg/l)*

Effect of Phytohormone on in vitro seed germination and multiple shoot proliferation from epicotyl explant

Epicotyl explants obtained from seed germination showed good response in shoot induction, elongation and proliferation in presence of BAP and kinetin. BAP and kinetin in combination (1.5mg/l+1.5mg/l) regenerated maximum 19.35±0.324 (Fig. B) number of shoots. On treatment with BAP alone (1.0mg/l) also showed good response (11±0.43). Increase in concentration of BAP and kinetin level lesser number of shoots were formed. The overall response of shoot formation on Kinetin was found better then BAP when explants were treated with individual phytohormones. Plant regeneration from epicotyl explants have been reported earlier by researchers, Siddique et. al.; (2013); Barik et. al.; (2005); Costa et. al.; (2004).

In present study, rooting was achieved on half strength MS medium fortified with IBA (1.0 mg/l). Increase in concentration of auxin didn't affected root induction. IBA has been found to be a potent auxin for root induction in other members of Rutaceae family also, *Phellodendron amurense*, Wang et.al.; (2011); *Citrus limon*, Tornero et.al.; (2010); *Citris mitis*. In present work, various physical factors like light intensity at 2000-2500 lux, temperature at 28±2^{0}C and high relative humidity played a crucial role in establishment of plantlets.

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