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Direct Shoot Regeneration And *Agrobacterium Rhizogenes* Mediated Rooting In *Aegle Marmelos* (L.) Corr. Serr. Nodal Explants

R ANITHA*, MK LAVANYA

Department of Plant Biology and Plant Biotechnology, Ethiraj college for women, Ethiraj salai, Egmore, Chennai-600008, India.

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ABSTRACT

Aegle marmelos is one of sacred medicinal tree in India, which has numerous medicinal properties. Micropropagation of *A. marmelos* is challenging and direct regeneration of shoot is simpler and can be carried out with ease. Direct shoot regeneration was observed with MS medium supplemented with 0.5 mg/l IAA + 2 and 3 mg/l kinetin after 6 weeks of incubation. However, direct shoot regeneration was observed in nodal explants co-cultured with *Agrobacterium rhizogenes* in 5, 10 and 15 minutes exposure after 3 days. Instead of hairy root formation, after 3weeks of incubation, tumors developed in explants exposed to 10 and 15min of exposure. It is evident from this study that response to *A. Rhizogenes* differs from plant to plant and is highly specific.

Keywords: *Aegle marmelos, Direct Shoot induction, Agrobacterium rhizogenes, Root induction*

Introduction

Aegle marmelos (L.) Corr. (Rutaceae) (Bael) is an armed spiny medicinal tree sparsely distributed throughout India on the plains and in hilly tracts up to 1300 m elevation [1]. *Aegle marmelos* is native to the Indo- Burma continent [2] and now being cultivated as a backyard tree in India, Bangladesh, Pakistan, Srilanka, Burma and Thailand. The plant is listed in earliest Ayurvedic medicinal texts viz. All parts of the tree like root, bark, leaves and fruits are highly medicinal. It is astringent, cooling, carminative, laxative, restorative, stomachic, used in dysentery, diarrhoea, flatulence, fever, vomiting, colic, febrifuge, tonic and good for heart and brain [3-4]. Antidiabetic property [5-6], Antidiarrheal activity [7-8], Antiulcer activity of seeds [9], Antifungal activity of leaves [10], Antitumour and Antimutagenic activity [11-12] of this plant are clinically evaluated. The plant has been widely used for antibacterial, antioxidant [13], pesticidal, antidote and antiinflammatory properties [14].

**Corresponding Author*
Dr. R Anitha
Department of Plant Biotechnology, Ethiraj College for Women, Ethiraj Salai, Egmore, Chennai 600 008, India.
Email: anitha.rajasekaran023@gmail.com

Indiscriminate collection has resulted in the disappearance of this plant from the wild sources and the species is reported to be vulnerable in the Western Ghats of Kerala, Tamil Nadu and Karnataka states of India [15].

Aegle marmelos is conventionally propagated through seeds. Seeds have short viability and are prone to insect attack. Vegetative propagation through root suckers is slow, difficult and cumbersome. Based on the studies conducted on seed propagation, the germination percentage of seed was very low under natural and controlled conditions [16]. Root being the major medicinally useful part, destructive harvesting poses a serious threat to the sustenance of the tree. Propagation through tissue culture is a viable alternative in this species. Many woody plants, economically important for its medicinal values are often difficult to root, both in conventional and *in vitro* propagation. In some cases, it was possible to improve *in vitro* rooting with hormone application, etiolation, or the use of polyamines [17-18]. However, the difficulty of rooting is still one of the major obstacles to successful micropropagation. Recently, many attempts to overcome this problem have been carried out on fruit trees and woody species using *Agrobacterium rhizogenes*. Hence this study attempts to evaluate the direct shoot regeneration and root induction using *Agrobacterium rhizogenes*.

Materials and Methods

Collection of explants for Direct Shoot Regeneration

Approximately 4 weeks old stem cuttings with four to five nodes were collected from the tip of the lower branch of *Aegle marmelos* tree (After leaf excision, the stem was cut into single node pieces, thoroughly washed under running tap water for 10 mins. The explants were surface sterilized with 70 % ethanol for 2 min followed by three washing in distilled water. The explants were then taken to the laminar airflow chamber and surface sterilized with 0.1 % (w/v) mercuric chloride for 3 min followed by three washing in sterile distilled water.

Direct regeneration of shoots [19]:

Under aseptic conditions the nodal explants were inoculated vertically into the culture medium consisting of full strength Murashige and Skoog (MS) basal medium with 3% (w/v) sucrose, 0.7% (w/v) agar, 0.5 mg/l IAA and 1-3 mg/l kinetin. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 min. The explants were incubated under alternative light condition at 25 °C.

Agrobacterium rhizogenes **mediated rooting [20]:**

Agrobacterium rhizogenes was obtained from Microbial Type Culture Collection, Chandigarh (MTCC-532). *A. rhizogenes* culture was revived in Nutrient broth for 48 hrs. The nodal explants collected from 4 weeks old plants were surface sterilized with 70% ethanol and 0.1% mercuric chloride and incubated with *A. rhizogenes* in half strength MS broth for different time intervals such as 5, 10, 15, 20 and 25 min. The explants were blotted dry and co-cultured in MS broth for 48 hrs. After incubation the explants were again blotted dry and transferred to MS agar with 3% sucrose without any exogenous auxin and incubated at 25 °C in dark. The explants were observed for the formation of adventitious rooting for a period of 4 weeks.

Results and Discussion

1. Direct regeneration of shoot:

Direct regeneration of shoot was observed in 0.5 mg/l IAA + 2 mg /l and 0.5 mg/l IAA +3 mg /l kinetin. (Better regeneration of shoot was observed at 3 mg/1 kinetin induction. (Figure-1). .Regeneration of shoot was initiated after 6 weeks of incubation A similar observation was reported in the earlier literature [21], where 2mg/l kinetin was sufficient to induce shoots without calli formation. On the contrary the proliferation of shoot from axillary buds at 1 mg/l kinetin was also reported [22]. Shoot induction was observed in

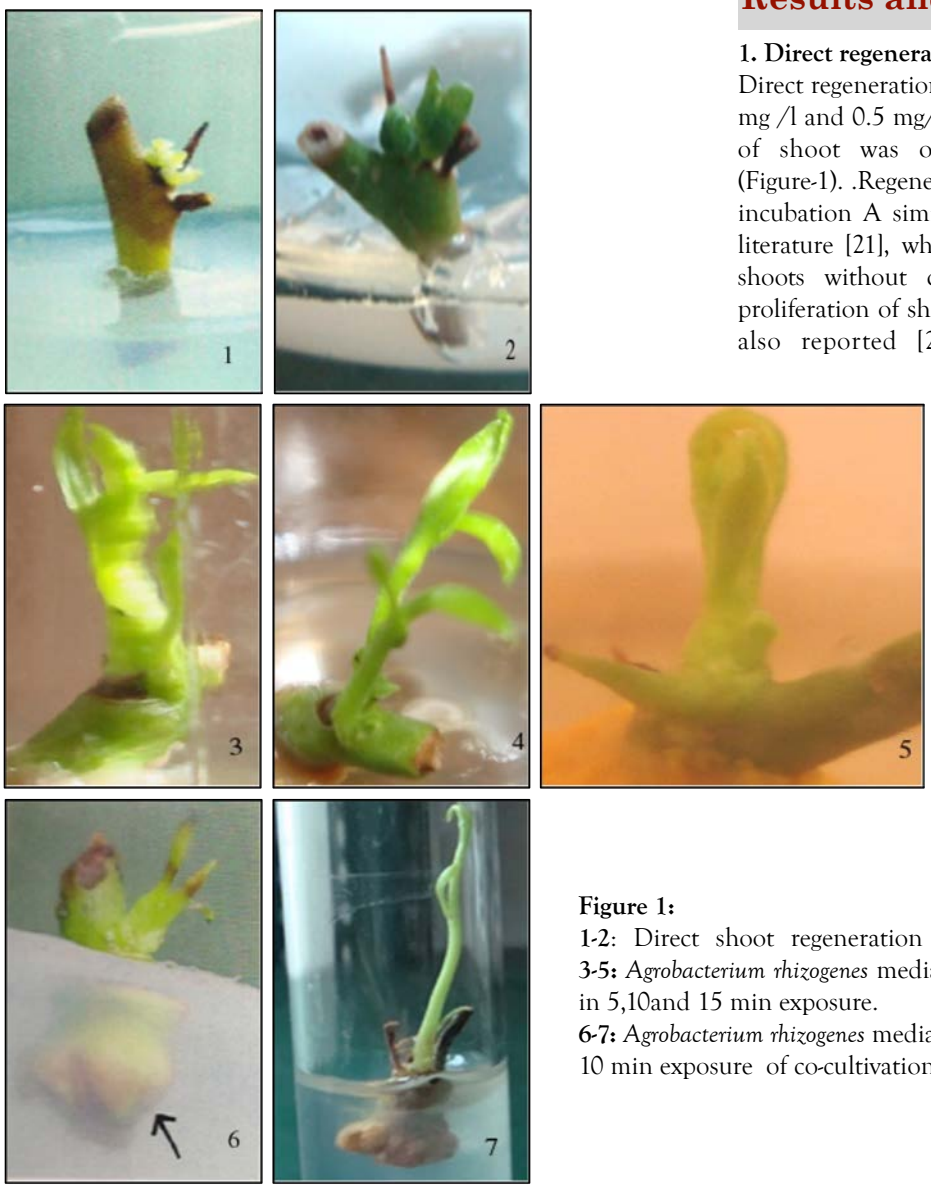

Figure 1:

1-2: Direct shoot regeneration in 2 and 3 mg/l Kinetin after 6weeks. **3-5:** *Agrobacterium rhizogenes* mediated direct shoot regeneration after 3 weeks in 5,10and 15 min exposure.

6-7: *Agrobacterium rhizogenes* mediated tumour induction after 3weeks in 5 and 10 min exposure of co-cultivation.

cotyledonary explants at 0.2 mg/l IAA and 1.5 mg/L BA [23].

2. *Agrobacterium* **mediated induction:**

The nodal explants of 4 week old plant was co-cultured with *Agrobacterium rhizogenes* after an incubation of 5, 10, 15, 20 and and 25 minutes and transferred to MS agar. After 3 days of incubation direct shoot regeneration was observed in 5, 10 and 15 minutes incubated explants. After 3 weeks of incubation shoot growth increased and tumor induction was observed in 5 and 10 min incubated explants instead of rooting (Figure-1).

Agrobacterium rhizogenes is a soil bacterium responsible for the development of hairy root disease on a range of dicotyledonous plants [24]. In the present study when *A. marmelos* nodal explants were inoculated with *A. rhizogenes* prominent tumor induction was observed. A similar observation was reported, where after 42 days of *A. rhizogenes* inoculation induced tumors in the epicotyl of *Cicer arietinum* [25]. The absence of rooting in *A. marmelos* explants and tumor induction showed different response because most of the species do not appear to be affected by infection and hormonal supply may be required for good rooting. The formation of a globular tumor in 5-8 days after infection is reported [26]. The efficiency to induce hairy root in different *A. rhizogenes* strains may vary from one species to another. In terms of hairy root induction, different populations [26] exhibit varying degree of response. It is reported that a few strains of *A.rhizogenes* did not induce hairy root in any of the population. These results show that genotype is an important factor for hairy root induction via *A. rhizogenes*.

Successful direct regeneration of shoot in the explants suggests that cytokinin synthesis dominated the production of auxins. This may be a reason for direct regeneration of shoot in *A. rhizogene* infected nodal explants. The synthesis of cytokinins in the explants also indicated successful incorporation of Ri plasmid from *A. rhizogenes* into the host. The Ri plasmid has region for both auxin and cytokinin production. The expression of these region depended on the strain of *A. rhizogenes*.

Conclusion

In the present study it was observed that the absence of rooting, along with tumorous growth indicated that certain strain of *A.rhizogenes* need an exogenous auxin supply for successful rooting. Successful shoot regeneration indicates the varied response of *A.marmelos* to *A.rhizogenes.*

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References

1. Anonymous.**Wealth of India–Raw Material**.National Institute of Science Communication, Council of Scientific and Industrial Research(CSIR), New Delhi,1:A (revised) 2003: 5-91.
2. Hooker JD: **The Flora of British India**. Reeve, U.K, 1975:516-517.
3. Sivarajan VV and I Balachandran: **Ayurvedic drugs and their plant sources**. Oxford and IBH, New Delhi, India, 1994:515-516.
4. Warrier PK, VPK Nambiar and C Ramankutty: **Indian Medicinal Plants. A compendium of 500 species**. Vol. 1. Orient Longman Pvt.Ltd, Madras, India. 1994:62-66.
5. Kamalakkanan N and PS Prince: **Hypoglycaemic effect of water extracts of** *Aegle marmelos* **fruit in streptozotocin diabetic rats**. *J Ethnopharmacol* 2003, **87**:207-210.
6. Kar A, BK Choudhary and NG Bandyopadhyay: **Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats.** *J Ethnopharmacol* 2003, **84**:105-108.
7. Shobam FG and M.Thomas: **Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhea.** *J Ethnopharmacol* 2001, **76**:73-76.
8. Mazumder R, Bhattacharya S, Mazumder A, Pattnaik AK, Tiwary PM and Chaudhary S: **Antidiarrhoeal evaluation of** *Aegle marmelos* **(Correa) Linn. root extract**. *Phytother Res*. 2006, **20(1)**: 82-84.
9. Goel RK, RN Maiti, M Manickam and AB Ray: **Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa**. *Ind.J.Exp.Biol.* 1997, **35:**1080-1083.
10. Rana BK, Singh UP and Taneja V: **Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of** *Aegle marmelos. J Ethnopharmacol*. 1997, **57**:29-34.
11. Annapurani S and R Priya: **Antimutagenic, antitumourogenic and antigenotoxic effects of polyphenols extracts of selected medicinal plants**. *Ind J Nutr Diet* **1999,** 36:431-435.
12. Lampronti L, D Martello, N Bianchi, M Borgatti, E Lambertini, R Priya, S Jabbar, MS Choudhuri, MT Khan and R Gambari: **Invitro antiproliferative effects on human tumor cell lines of extract from the Bangladeshi medicinal plant** *Aegle marmelos* **Correa**. *Phytomedicine* 2003, **10**:300-308.
13. Dhalwal K., Shinde VM, Namdeo AG and Mahadik KR: **Antioxidant Profile and HPTLC Densitometric Analysis of Umbelliferone and Psoralen in** *Aegle marmelos*. *J Pharmaceutical Biology* 2008, **46(4)**: 266 –272.
14. Arul V, Miyazaki S and Dhananjayan R: Studies on the anti-inflammatory, antipyretic and analgesic properties of the leaves of Aegle marmelos Corr.", *Journal of Ethnopharmacology* 2005, 96(4):159-163.
15. Ravikumar K and DK Ved: **Hundred red listed Medicinal plants of conservation concern in Southern India.** 1st Edn.Foundation for Revitalization of local Health Traditions (FRLHT), Anugraha, Bangalore, India 2000.
16. Raghu AV: **Studies on Variability , Conservation and Propagation of Dasamula group of medicinal plants**. Ph.D Thesis, University of Calicut, Kerala, India. 2006.

17. Damiano C, Archilletti T, Caboni E, Lauri P, Falasca G, Mariotti D and Ferraiolo G: *Agrobacterium* **mediated transformation of almond:** *in vitro* **rooting through localised infection of** *A. rhizogenes***.** *Acta Horticulturae* 1995, **392**:161-169
18. Rugini E, Pellegrineschi A, Mencuccini and Mariotti D: **Increase of rooting ability in the woody species kiwi (***Actinidia deliciosa* **A. Chev.) by transformation with** *Agrobacterium rhizogenes rol* **genes.** *Plant Cell Reports* 1991, **10**:291-295.
19. Neha Parihar, Anant Sharma and Sanjay Kumar. **Shoot Proliferation of** *Aegle marmelos* **from nodal stem segment as explants.** *Biological Forum* 2010, **2(2)**: 109-111.
20. Karthikeyan A, Palanivel S. Parvathy S and R Bhakya raj: **Hairy root induction from hypocotyl segments of groundnut (***Arachis hypogaea* **L.).** *African Journal of Biotechnology* 2007, **6 (15)**:1817-1820.
21. Rekha Warrier, Jasmine Viji and P Priyadharshini: **In Vitro Propagation of Aegle Marmelos L. (Corr.) From Mature Trees through Enhanced Axilliary Branching**. *Asian J. Exp. Biol. Sci*. 2010, **1(3)** :669-676
22. Puhan and Thirunavoukkarasu, M: **Direct organogenesis of** *Aegle marmelos* **(L.) Corr. From cotyledon.** *African Journal of Biotechnology* 2011, **10(82)**:18986-18990.
23. Islam,R, Karim,MR, Rahman.SM., Hossain,M and OI. Joarder: **Plant regeneration from excised cotyledon of** *Aegle marmelos* **Corr**. *Pak.J.Bot* 1994,**26**:393-396.
24. Tepfer, D: **Genetic transformation using** *Agrobacterium rhizogenes*. *Physiol. Plant* 1990, **79**:140-146.
25. Khawar KM, Ozcan S: **Hairy root transformation in Turkish chickpea (Cicer arietinum L) cultivars.** *Biotechnol iotechnol Equip* 2004, **18:** 51–54.
26. Georgina EN, Xochitl AA, Juan EO, Claudia DC, Olivia S, Enrique M, Gabriel G, Nayeli SG, Jorge A, Carmen Q, Dongxue Li, Peter M Gresshoff and Federico Sánchez: *Agrobacterium rhizogenes* **Transformation of the** *Phaseolus* **spp.: A Tool for Functional Genomics.** *Molecule plant and microbial interaction* 2006, **19(12)**: 1385.

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