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In vitro Propagation of *Aegle marmelos* through Nodal Explants

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Abstract: ***A protocol for micro propagation of bael [Aegle marmelos (L.) Corr.] was developed. The nodal explants of trees were used to initiate cultures. Two cytokinins, viz., 6-benzylaminopurine (BAP) and kinetin (Kn) were used in varied concentration (0.1–2 mg/l) for shoot multiplication. BAP (2 mg/l) was found better than Kn, where a 3-fold increase in the number of shoots was recorded in 4 weeks. A synergistic influence of cytokinin and auxin was also observed in the present study. A combination of 0.5 mg/l BAP and 0.1 mg/l IAA induced the formation of maximum number (4.5) of shoots (2.5 cm). For rooting of in vitro shoots, different auxins, namely, NAA, IAA and IBA (0.1–2 mg/l) were tested. IAA (0.01 mg/l) was found better than NAA and IBA. It was concluded that elite cultivars of bael can be micropropagated, without undergoing callus phase, using the BAP (0.5 mg/l) plus IAA (0.1 mg/l) for shoot multiplication and IAA (0.1 mg/l) for rooting, to produce true-to-type in vitro plants. The in vitro raised plantlets were acclimatized with 60% success.***

Keywords: invitro, explants, sterilization, shooting, bael

1. Introduction

Aegle marmelos is a medicinal plant widely used in Indian system of medicines for many diseases. It belongs to the family rutaceae. It is medium sized tree, commonly known as bael tree. In pharmacology all parts including root is used for many purposes. The fruit pulp marmalosin is a patented drug in India as it is used as laxative and diuretic. In Siddha and Unani, its suggested as a plant of unique healing powers. It also contains number of phytochemicals. In Charaka Samhita, it prescribed that the tender fruits of *Aegle mermelos* with buttermilk is good for diarrhoea. In case of diarrhoea with blood, it is prescribed as tender fruits mixed with jaggery, honey and oil. We find many such references in ancient and in most recent works proving the importance of this red listed tree. This tree has the wonderful capacity to acts as a potent anti-helminthic, hypoglycaemic, cardiac stimulant, anti-diarrhoeal and antiviral agent (Khare, 2004) [18]. All parts of this tree all used as ingredients in many ayurvedic preperations like Bilva Taila, asamoolaarishta, Gangaadharab Choorna, Amritaarishta, Mahaanaaraayana taila, Chyawana praasa, Pushyaarvuga choorna. The root of this tree being the major medicinally useful part, afforestration causes a serious threat for its survival in nature. The timber is good for furniture. Seed and root propagation is very slow and the progeny formed is not uniform also. Conventional methods of propagation are very slow. It is season bound also. So *in vitro* propagation is suitable for rapid multiplication.

2. Review of Literature

This tree is cited as one of the red-listed medicinal species of South India (Ravi kumar & Ved, 2000)[30]. Many workers namely Arya et al, (1981)[5], Arya & Shekhawat (1986)[4] Hossain *et al*, (1993, 1994, 1994a)[9,10] Islam *et al.* (1993, 1994, 1995, 1996a, 1996b)[11], Arumugam & Rao (1996, 2000)[2,3], Ling & Iwamasa (1997)[20], Ajith kumar & Seeni (1998)[1], Islam (2006)[12], Prematilake *et al* (2006)[24],Pranati & Behera (2007)[23], Raghu *et al*, (2007)[27], Das *et al* (2008)[7], Rajesh Pati *et al*, (2008)[28], Neha *et al.* (2010)[22], Rekha Warrier *et al* (2010)[31], Ramanathan *et al* (2011)[29], Puspasree & Thirunavoukkasu (2011)[26], Kuldeep & Narendra (2011)[19]etc attempted to raise this hardy tree through *in vitro* techniques In the current report, efforts were made to find out, which is the best suitable explant material and best media formulation for rapid clonal propagation of *Aegle marmelos.* Bhajaj (1997)[6], conducted an experiment in this plant and proved that the nodal explants are very in growth and proliferation . Kuldeep & Narendra (2011)[19], explained the importance of the position of nods is important in shoot initiation. Raghu *et al.* (2007)[27], Rajesh Pati *et al.* (2008)[28], also conducted such works. Arya & Shekhawat (1986), Ajithkumar & Seeni (1998), conducted *in vitro* propagation works and stressed the importance of auxin and cytokinine for proliferation. Islam *et al.* (2007), generated callus using different concentrations and combinations of BAP with 2, 4- D, NAA and IBA while culturing node. As per Raghu *et al.* (2007)[7], low concentrations (0.1- 1 mg/L) of BAP and KN phytohormones can induce multiple shoots from nodal segments. Multiple shoots formation was induced by Das et al. (2008) by using combination treatments of BAP + NAA . Neha *et al.* (2010), successfully cultured nodal segment on MS media with many plant growth regulators to attain the multiple shoot-lets. MS medium with different concentrations of BA, KN and GA3 either individually or in combination is better for shoot proliferation in the view of Puspashree *et al.* (2012)[26].

3. Materials and Methods

Plants were selected from different parts of Thrissur District. Explants were selected from healthy twigs collected and cut it as pieces of 10 cm length. Leaf, internode, nodal segments were taken as taken as explants. Explants were washed thoroughly with tap water then by teepol for 10 minutes and then with bavistine and again with distilled water. Then it was taken in to the laminar air flow chamber there it was treated with 0.1% mercuric chloride for 2 minutes and then with distilled water. Then the explants were inoculated in MS medium fortified with Kinetin and IAA(2.0 -1 mg/l) each. Different media concentrations were used. Then they were incubated in

culture room. Rooting hormones were provided for root initiation. It was ½ strength MS+IBA 10.0 mg/l and IAA 1.0 mg/l.

All these growth regulators were taken in different concentrations of BAP alone in 05t0 3mg/l BAP in 0.5, 1 and 2mg/l concentration along with IAA in 0.1, 0.2, 03, 0.5 mg/L each. We were also evaluated explants for responses. In total, 20 combination treatments were tested for explants establishment purpose. Again Kinetin and IAA combination was there. Kinetin in three concentration (0.5, 1 and 2mg/l) each concentration combined with 0.1, 0.2, 03, 0.5 mg/L concentration of IAA.

Maintenance of cultures: All the cultures were maintained under 12 hr light and 12 hr dark cycle in a culture room at 23 ± 2^0 C with 55 ± 5 % relative humidity. White cool fluorescent lights controlled by a timer were used to provide about 1000 Lux intensity light for obtaining morphogenesis. Cultures were regularly monitored for callusing, direct or indirect differentiation of shoots/roots at regular intervals.

4. Results

Of the different explants used, the nodal explants produced, it was direct morphogenesis in most of the cases, though indirect morphogenesis interfered sometimes in the presence of some strong callus inducing growth regulator. In case of explants like, leaf bits, internode it was indirect morphogenesis, and also poor growth. After 25 days multiple shoot proliferation was noticed in medium. Maximum proliferation was noticed for the nodal explants in medium containing Kinetin 2.0 mg/l +IAA 1.0mg/l. This treatment produced 8.98 micro shoots and maximum leaves/ explants. After shoot formation rooting medium was applied and While more number of roots (2.33 and 2.0), root length (4.8.0and 3.33 cm) were recorded with MS +IBA 10 +IAA 1.0 mg/l. Then they were transferred to the small pots in the green house and after 4 weeks to large field pots.

5. Discussion

Due to low shoot proliferation, basal callusing, vitrification and difficulty in rooting the *in vitro* propagation of *Aegle marmelos* is a challenging task. Different explants materials were used to test the regeneration potential of each of them following tissue culture methods. Of the various explants used, nodal explants proved to be the best suited material for the fast adventitious plantlets development. Bhajaj (1997)[6], pointed out that the nodal explants are very in growth and proliferation. Kuldeep & Narendra (2011)[19], mentioned that the explants from 4-8th nodal segments are the best ones for the shoot initiation and not the terminal tender ones. Raghu *et al*. (2007)[27], Rajesh Pati *et al*. (2008)[28], also conducted such works. All these result are also in agreement with this work. Arya & Shekhawat (1986)[4], Ajithkumar & Seeni (1998)[1], conducted *in vitro* propagation works and stressed the importance of auxin and cytokinine for proliferation. Islam *et al*. (2007)[13], derived callus to regenerate plantlets using different concentrations and combinations of BAP with 2, 4- D, NAA and IBA while culturing node. Raghu *et al*. (2007)[27], used low concentrations (0.1- 1 mg/L) of BAP and KN phytohormones to induce multiple shoots development from nodal segments. Multiple shoots formation was induced by Das et al. (2008) by using combination treatments of BAP + NAA and attained about 22.7 multiple shoots. Neha *et al*. (2010)[22], successfully cultured nodal segment on MS media provided a range of plant growth regulators to attain the multiple shoot-lets. According to Puspashree *et al*. (2012)[26] MS medium with different concentrations of BA, KN and GA3 either individually or in combination is better for shoot proliferation.

Rooting hormones were provided for root initiation .It was ½ strength MS+IBA 10.0 mg/l and IAA 1.0 mg/l. After initiation of root, rooted plantlets were successful acclimatized to the *in vivo* conditions following suitable measures. Arya & Shekhawat (1986), Ajithkumar & Seeni (1998), Pranati & Behera (2007), Rajesh Pati et al. (2008), Hossain *etal*. (1994a), Kuldeep & Narendra (2011), Puspasree & Thirunavoukkarasu (2011) etc were also used½ strength MS media supplemented with IBA in different concentrations. From these the results, it appears ½ strength liquid MS medium fortified with different concentrations of IBA and IAA is good for root initiation.

Table1: Growth rate of various explants on different concentrations of growth hormones

Growth regulators	% of explant showing proliferation	No of shoot per culture	Average length of shoot per culture
BAP			
0.5	30	3.1±0.35	1.33±0.32
1.0	32	3.25±0.38	2.11±0.36
1.5	36	3.32±0.62	2.58±0.16
2.0	38	3.75±0.28	3.28±0.24
2.5	38	3.99±0.45	3.64±0.58
3	31	3.02±0.39	3.22±0.14
BAP+IAA			
0.5+0.1	31	3.13±0.54	1.21±0.12
0.5+0.2	35	3.34±0.22	2.22±0.32
0.5+0.3	38	3.36±0.24	2.61±0.34
0.5+0.5	38	3.86±0.06	2.25±0.38
1.0+0.1	41	5.36±1.47	4.41.±0.36
1.0+0.2	42	5.32±0.66	4.46±0.43
1.0+0.3	42	5.26±0.78	4.28±0.82
1.0+0.5	34	4.5±0.48	3.44±0.68
2.0+0.1	32	4.23±0.89	3.22±0.24
2.0+0.2	30	3.16±0.36	2.24±0.38
2.0+0.2	30	3.36±0.62	2.13±0.28
2.0+0.5	28	2.82±0.55	1.89±0.54
KINETIN+IAA			
0.5+0.1	32	3.28±0.38	2.26±0.12
0.5+0.2	32	2.98±0.33	2.49±0.44
0.5+0.3	32	2.69±0.28	2.26±0.42
0.5+0.5	32	2.38±0.44	2.34±0.88
1.0+0.1	34	3.23±0.22	2.50±0.18

1.0+0.2	34	3.56±0.26	2.78±0.14
1.0+0.3	34	3.58±0.23	2.88±0.16
1.0+0.5	36	3.62±0.18	2.98±0.34
2.0+0.1	**40**	**3.62±0.26**	**3.26±0.24**
2.0+0.2	34	3.16±0.18	3.14±0.24
2.0+0.2	32	2.54±0.22	1.69±0.22
2.0+0.5	28	2.24±0.16	1.98±0.24

6. Conclusion

In conclusion we can say that even though the *in vitro* propagation is a tedious task due to many reasons the successful propagation of *Aegle marmelos* can be achieved through the nodal explants by using MS medium fortified with Kinetin and IAA(2 -1 mg/l) each.

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