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Shoot Proliferation of *Aegle marmelos* from nodal stem segment as explant

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ABSTRACT : Rapid shoot multiplication of Aegle marmelos (L.) Corr. (Rutaceae), a herbal tree, was achieved by using nodal stem segments from 20 year old tree cultured in Murashige and Skoog (MS) nutrient medium. Nodal explant growth depended on cytokinin supply. 2mg^{-1} kinetin induced bud proliferation. Repeated sub culturing resulted in callus free shoot multiplication.

INTRODUCTION

Aegle marmelos Correa. Beal fruit (J.C.Yorston, 1892) member of family Rutaceae is an important ayurvedic medicinal tree of India. It is a medium sized armed deciduous tree up to 8.0m high (P.K. Warrier, 1993) with sharp thorns and yellowish brown bark. The leaflets are 3 to 5 and ovate lanceolate. The flowers are 3 cm and greenish white. Its fruit is nearly spherical. The tree is frequent in Northern part in dry deciduous forests and often planted near temples (N.P. Singh, 1988). Various parts including fruits possess medicinal properties and has been extensively used in ayurvedic and folk medicine (Current science, 1995). Cooling drinks are prepared by mixing the pulp of ripe fruits of Aegle marmelos (Harbhajan Singh, 1978). Aegle marmelos is an astringent and has antiviral, antihelminthic, anti-inflamatory, and antimicrobial (against vibrio cholera and salmonella) properties (Martin Ingrouille, 2006). Leaves of Aegle marmelos are good for stomach ailments, diarrhea, worms and dysentery (Ashwani Kumar, 2008). The use of plant for allergy, cough, bronchitis and snake bite has also been described in ayurvedic system of medicine (Tripathi, 2003). Leaves of Stone apple stimulate pancreas to secrete insulin (Nadkarni, 1976). The fruit of this tree contain marmelosin, young bark contain coumarin and leaves contain 0.6% essential oils .The growth is slow and trees don't start to fruit till they are 5 to 8 years old (Narian Singh Chouhan, 1999). Micropropagation is one of the innovative methods of asexual reproduction that is effective for invitro propagation of medicinal plants. The objective of the study was to develop invitro regeneration system of Aegle marmelos from nodal stem segment as an explant followed by shoot proliferation.

Culture Medium and culture conditions

Nodal explants were inoculated onto semi solid MS medium (Murashige and Skoog, 1962) containing 3% sucrose enriched with different concentration of Kinetin (0.5, 1.0, 2.0 mg^{-1}) and with different concentration of BAP (0.5, 1.0, 2.0 mg^{-1}). pH of the medium was adjusted to 6.8 using 0.1N NaoH or 0.1% HCL before gelling with 0.8% agar(w/v). Media was steam sterilized at 121°C and 15 psi pressure for 15 min. After surface sterilization and inoculation of nodal stem segments cultures were incubated in culture room maintained at 25+3°C under 16/8 h light dark cycle.

MATERIAL AND METHOD

Study was undertaken at Institute of Biotechnology and Allied Sciences (IBAS), Sikar. Nodal stem segments used as explant for present study were collected from 20 year old tree of Aegle marmelos situated in temple near the Institute. Sharon and Marie (2000) reported that nodal explants were preferred over meristem to produce large number of identical clones of Bixa oveliana. Shoot twigs with 2 to 3 axillary buds collected from mother tree were kept under tap water for 5 min. and were rinsed with non-phytotoxic detergent 2% Tween 20 for about 5 min. They were washed in distilled water to make explant free from detergent. Explants were then disinfected with 1% sodium hypochloride for 10 min. Explants were than washed with double distilled water twice for 10 min. then were transferred to laminar air flow hood and were sterilized with 0.1% (w/v) mercuric chloride for 1 min. Lastly explants were washed with double distilled water and sterile water for 5 min. After sterilization, the explants were transferred on MS medium supplemented with different concentration of growth regulators.

Statistical Analysis. 15 replicates were used in each treatment when separate growth regulators were taken. 10 replicates were taken when combination of cytokinin was taken. All experiments were repeated thrice. Average number of multiple shoots and shoot length were determined after 71 days.

RESULTS AND DISCUSSION

Shoot Proliferation : When explants were grown on MS media devoid of any growth regulators no morphogenetic response was observed. Sprouting of axillary buds was observed in 21 days of culture on MS medium enriched with different concentration of cytokinin. It is mandatory to augment the medium with combination of different cytokinin in order to induce shoot proliferation. Of the two cytokinin (kinetin and BAP), kinetin supplemented

media was most effective in shoot induction, proliferation, and multiplication. Cytokinin concentration has been several times reported to be decisive for shoot proliferation and elongation of many medicinal plant species (Saxena *et al*, 1998; Rout *et al*, 2000; Rout, 2004). Within 7 days of incubation explant swelling was observed. Shoots commenced to emerge from axillary bud in 14 days in kinetin supplemented media where as in BAP supplemented media less responsive explants were observed.

Fig. 1. Nodal explant growth after 21 days supplemented with $2mg^{-1}$ kinetin.

Fig. 2. Growth after 25 days.

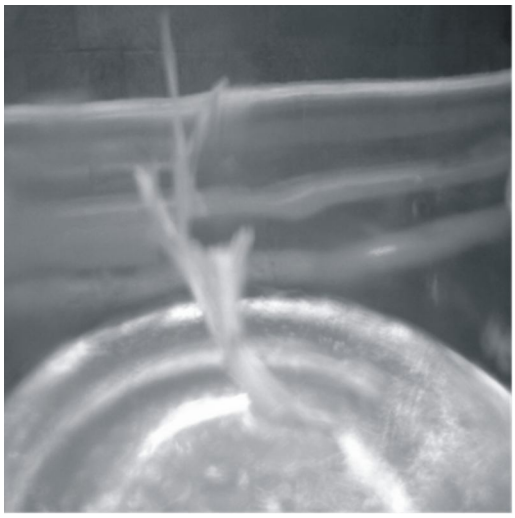

Fig. 3. Growth after 2 months (subculturing 1).

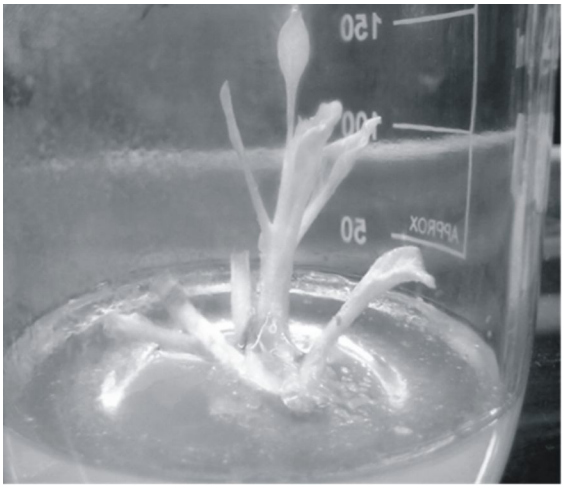

Fig. 4. Growth after 67 days.

Fig. 5. Nodal explant shoot multiplication after 71 days.

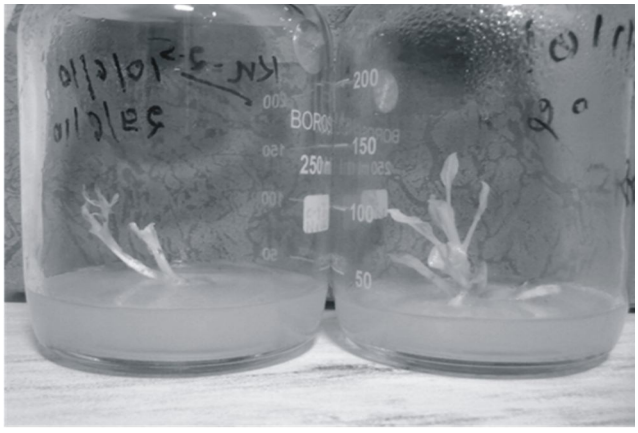

Fig. 6. Growth after 81 days of subculturing 2.

Maximum response was recorded in kinetin 2 mg^{-1} where number of shoots per explant were highest (08) with an average length of 2 + 2.5 cm. Reduction in number of shoots in all the concentrations higher than the optimal level has been reported for several plants (Sudha and Seeni, 1994).

Table 1 : Effect of different concentration of growth regulators in MS medium for shoot proliferation.

Supplements	No. of explant	No. of responsive explants	Average no. of shoots	Average length of shoots	Average no. of leaves	Average leaf leaf area
MS + 0.0	15	-	-	-	-	-
MS + BA						
0.5	15	-	-	-	-	-
1.0	15	-	-	-	-	-
2.0	15	4	5	3.5	5 ± 6	2.5 ± 3
MS+kinetin						
0.5	15	-	-	-	-	-
1.0	15	9	3	2 ± 2.5	4 ± 5	1
2.0	15	11	8	4 ± 4.5	9 ± 10	3
MS + BA + Kn						
1.0 ±2.0	10	2	4(callus)	2 ± 2.5	3 ± 4	2
2.0 + 2.0	10	3	8 ± 9	3 ± 4	7 ± 8	2

In BAP and Kinetin enriched media callus was formed from nodal stem segment. Callus developed into shoots(8 to 9)with average length of 3 to 4 cm but on subculturing the shoots were degenerated and no response was observed. MS medium augmented with BAP showed comparatively less response from kinetin.

Subculturing was carried out after four weeks on to fresh medium with same composition. For kinetin the number of shoots per explant increased with increase in number of subcultures.

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