In vitro multiplication of *Maytenus emarginata.* A tree of the Thar Desert

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***Abstract*- An in vitro method for cloning and mass multiplication of Maytenus emarginata, a highly drought resistant tree of the Indian Desert, has been developed. Shoot segments harvested from a "plus" tree (30-year-old) were cultured to produce multiple shoots (10-15 shoots/explant) on MS medium containing 0.1 mg/l IAA and 2.5 mg/l BAP. In vitro produced shoots were cut into segments and cultured on shoot proliferation medium supplimented with 1.0 mg/1 of BAP for further multiplication of the shoots. Isolated individual shoots were cultured on a filter paper bridge in half strength MS liquid medium containing 25 mg/l of IBA for 72 h in the dark at 28 + 2° C for induction of root. About 70-80 percent of shoots rooted. The treelets developed were hardened and transferred to pots. Thousands of plantlets can be obtained from a single explant. The protocol is highly reproducible and efficient.**

Abbreviations. IAA, indole-3-acetic acid; IBA, indole- 3-butyric acid; NAA, a- naphthalene acetic acid; NOA, B-naphthoxy acetic acid; BAP, 6– benzylaminopurine; Kn, 6-furfurylaminopurine; B5, Gamborg et al. (1968) medium; MS, Murashige and Skoog (1962) medium

Key Words: **Maytenus emarginata, Cloning, Tissue culture, Treelet, Regeneration.**

INTRODUCTION:

Maytenus emarginata (Celastraceae) is an important tree of the Indian Desert, as it is a drought and heat resistant biomass producer. The plant is valuable as it stabilizes the sandy soil and provides fodder and fuel. The plant yields timber and it has medicinal value (Bhandari 1990). During recent years ruthless cutting has resulted in disappearance of valuable germplasm from the arid and semi arid areas. Tissue culture biotechnology provides tools to mass multiply forest trees and to clone selected germplasm (Haissig et al. 1987; Cheliak and Rogers 1990). Though cloning of mature trees is difficult as with the increasing age the ability of shoots to root diminishes considerably. Revigoration and rejuvenation of a mature tree are difficult, though this has been achieved in several systems (Gupta et al 1989,Ahuja 1991). Our laboratory is working on multiplication and cloning of plants of the Indian Desert through tissue culture (Rathore et al. 1991,1992; Shekhawat et al. 1993). Establishment of cultures from shoot explants derived from mature "plus" tree of M. emarginata, proliferation of multiple shoots from axillary and nodal zones, and induction of roots from in vitro produced shoots and treelet formation are described in this communication.

MATERIAL AND METHODS;

In extensive field surveys of the Indian desert, a few plants of M. emarginata (Willd.) Ding-hou, were selected as "plus" trees on the basis of their straight and solid bole and large size. Explants viz apical shoots, shoot segments each with one node/axillary shoot bud were harvested from the mature tree (30-year-old) of M. emarginata. Explants were harvested periodically during all seasons. They were washed with tap water with a few drops of Tween-80, and then surface-sterilized with 0.1% HgCl2: (W/V) for 3-4 min. After thorough washing with autoclaved distilled water, the explants were kept in a sterilized antioxidant solution (aqueous solution of ascorbic acid 0.1% and citric acid 0.05% for 30 min). The explants were cultured on agar gelled media of full and half strength MS (Murashige and Skoog 1962) and BS (Gamborg et al. 1968), Various auxins (IAA, IBA, NAA) in the concentration range of 0.05-2.5 mg/l and cytokinins (Kn, 2-iP and BAP) ranging from 0.1-5.0 mg/l were either added to the medium alone or in various combinations (Table 1). The cultures were incubated at 28 ± 2° C, 60% relative humidity and 35-43 uEm's photon flux density for 12 h/day photoperiods. Shoots/shoot segments, (2.0-2.5 cm in length) were subcultured on MS medium supplemented with various combinations of auxins and cytokinins for further multiplication of shoots. Ascorbic acid (50 mg), citric acid (25 mg) and adenine sulphate (25 mg) were used as additives in the shoot induction and multiplication medium. Full and half strength MS basal,Hellers and White's basal (White 1943) agar gelled media were used with IBA, NAA and NOA (0.1-5.0 mg/l) for root induction from regenerated shoots (Table 2). In addition to agar media, shoots were also kept in half strength MS liquid medium (on a filter paper bridge) containing 2.5 mg/l of IBA for 12, 24, 48 and 72 h and then transferred to hormone-free 1/2 MS semi-solid medium (Table3). After of 35° C under 100 uEm's1 photon flux density for hardening. Rooted plantlets were washed thoroughly with water and transferred to pots containing sand: Vermiculite (3:1).

RESULTS AND DISCUSSION:

Shoot segments with one node (2.5 x 0.4 cm) harvested during the monsoon season (July-August) and in March were explanted for the establishment of cul- tures. Excessive browning at cut ends could be prevented by keeping the explant in antioxidant solution, On MS medium containing 0.1 mg/l of IAA, 2.5 mg/l of BAP and 25.0 mg/l of adenine sulphate,10-12 shoots developed from the nodal region of each of the explants, within 4 weeks. On B5 and half strenght MS media only 5-7 shoots regenerated from the nodal region. Kinetin was found to be less effective than BAP for shoot proliferation .Regenerated shoots could be further multiplied on the same medium but with 1.0 mg/l of BAP, each segment produced 15-20 new shoots within 4 weeks. At higher concentration of

BAP dwarf shoots were formed. These attained normal length by subculturing on MS medium + 0.1 mg/l of IAA + 0.1 mg/l NAA +0.25 mg/l BAP. Addition of auxins higher than 0.1 mg/l caused callusing at the cut ends of the explants. Incorporation of IBA and NAA in place of IAA in medium ,caused callusing from the explants. Shoots produced in vitro rooted best when it was treated with 25.0 mg/l of IBA in half strength MS medium for 72 h in the dark, followed by transfer to semi-solid, hormone-free half strength MS basal medium. Hundreds of plants were rooted ,hardened and transferred to field. The protocol described could be used for preservation and multiplication of depleting germplasm of M. emarginata in arid region and semi arid region of Rajasthan . The developed protocol is very potential for large scale field transfer and afforestation programme. Though initially this protocol was developed for an elite, but we found that this is equally applicable for the propagation of other mature plants of M. emarginata. propagation of other mature plants of M. emarginata.

Cytokinins	**Mg/l**	**Shoot number per node ± SD**	**Shoot length (cm) ± SD**
Control	0.0	2.2 0.4	1.8 0.4
BAP	0.25	5.3±0.7	2.4±0.3
	0.5	8.6±0.8	2.8±0.2
	1.0	10.2±0.8	1.6±0.2
	2.5	16.4±1.9	0.8±0.2
KN	0.25	4.2±0.6	2.6±0.4
	0.5	5.3±0.7	2.6±0.2
	1.0	6.1±0.3	1.9±0.3
	2.5	10.3±0.8	1.2±0.4
2-ip	0.25	4.3±0.5	2.1±0.3
	0.5	5.2±0.6	1.9±0.4
	1.0	5.9±0.4	1.9±0.6
	2.5	9.2±0.6	1.2±0.4

Table 1-Effect of cytokinins on shoot multiplication of M. emarginata on MS medium containing 0.1 mg/l 1AA.

Table 2-Effect of different media on root induction from cultured shoots of M. emarginata.

Media	**% of shoots rooted %**	**Root number ±SD**	**Root length (cm) ±SD**	**Shoot length (cm) ±SD**
MS	13	2.7±0.7	1.8±0.7	3.5±0.6
½ MS	27	4.0±1.1	2.3±0.9	3.9±0.6
Heller	22	4.9±0.8	1.9±0.4	3.2±0.8
White	18	4.9±1.3	1.5±0.4	2.8±0.4

Data scored on 28th day of treatment.

Each medium was supplemented with 2.5 mg/l IBA.

Table 3-Effect of pulse treatment of IBA (25 mg/l) for different time duration on root induction from cultured shoots of M. emarginata.

treatment	**% of shoots rooted %**	**Root number ±SD**	**Root length (cm) ±SD**	**Shoot length (cm) ±SD**
12 h	39	2.4±0.6	2.4±0.7	4.1±0.7
24 h	58	4.5±1.2	2.7±0.9	4.4±0.8
48 h	67	5.1±1.2	2.7±0.8	4.5±0.9
72 h	80	5.9±1.7	2.9±1.1	4.7±1.0

Shoots treatment with IBA 25 mg/l for 12, 24, 48 and 72 h in half strength MS liquid medium on filter paper bridge, thereafter cultured on hormone free-half strength MS agar gelled medium.

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