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Cloning of *Maytenus emarginata* (Willd.) Ding Hou – a tree of the Indian Desert, through tissue culture

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Summary. 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 mgl^{-1} IAA and 2.5 mgl^{-1} BAP. *In vitro* produced shoots were cut into segments and cultured on shoot proliferation medium but with only 1.0 mgl^{-1} of BAP to further multiply the shoots. Isolated individual shoots were cultured on a filter paper bridge in half strength MS liquid medium containing 25 mgl^{-1} of IBA for 72 h in the dark at 28 ± 2^0 C for induction of root(s). About 70-80 percent of shoots rooted. The treelets developed were hardened and transferred to pots. Around 20,000 plants can be obtained from a single explant within a period of 6 months. The protocol is highly reproducible and efficient.

Abbreviations. IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, α- naphthalene acetic acid; NOA, β-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 (Pierik 1990). Our laboratory is working on multiplication and cloning of plants of the Indian Desert through tissue culture (Arya and Shekhawat 1986; Rathore et al. 1991; Joshi et al. 1991). 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

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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% $HgCl_2$ (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 B5 (Gamborg et al. 1968). Various auxins (IAA, IBA, NAA) in the concentration range of 0.05 – 2.5 mgl^{-1} and cytokinins (Kn and BAP) ranging from 0.1 – 5.0 mgl^{-1} were either added to the medium alone or in various combinations. The cultures were incubated at 28 ± 2^0 C, 60% relative humidity and 35-43 $uEm^{-2}s^{-1}$ 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 mgl^{-1}), citric acid (25 mgl^{-1}) and adenine sulphate (25 mgl^{-1}) were used as additives in the shoot induction and multiplication medium.

Full and half strength MS basal and White's basal (White 1943) agar gelled media were used with IBA, NAA and NOA (0.1-5.0 mgl^{-1}) for root induction from regenerated shoots. In addition to agar media, shoots were also kept in half strength MS liquid medium (on a filter paper bridge) containing 25 mgl^{-1} of IBA for 12, 24, 48 and 72 h and then transferred to hormone-free 1/2 MS semi-solid medium. After initiation of roots these were kept at elevated temprature of 35^0 C under 100 $uEm^{-2}s^{-1}$ 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 cultures. Excessive browning at cut ends could be prevented by keeping the explant in antioxidant solution. On MS medium containing 0.1 mgl^{-1} of IAA, 2.5 mgl^{-1} of BAP and 25.0 mgl^{-1} of adenine sulphate, 10 – 12 shoots developed from the nodal region of each of the explants, within 4 weeks (Fig.1). On B_5 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 (Table 1). Regenerated shoots could be further multiplied on the same medium but with 1.0 mgl^{-1} of BAP, each segment produced 15 – 20 new shoots within 4 weeks (Fig.2). At higher concentrations of BAP dwarf shoots were formed. These attained normal lenght by subculturing on MS medium + 0.1 mgl^{-1} of IAA + 0.1 mgl^{-1} NAA + 0.25 mgl^{-1} BAP.

Table 1. Effect of cytokinins on multiple shoot induction from nodal shoot segments of M. emarginata cultured on MS medium supplemented with IAA (0.1 mgl^{-1}) over a period of 4 weeks.

Treatment (mgl^{-1})	Explants with shoot formation (%)	Number of shoots per explant ± SD	Shoot length (cm) ± SD
Control	60	1.6 ± 0.4	1.9 ± 0.5
Kn			
0.5	65	2.4 ± 0.6	2.4 ± 0.7
1.0	70	3.7 ± 1.1	2.5 ± 0.6
2.5	74	5.8 ± 1.3	1.9 ± 0.8
5.0	72	9.1 ± 2.2	1.4 ± 0.5
BAP			
0.5	68	3.1 ± 0.9	2.6 ± 0.9
1.0	78	5.4 ± 1.2	2.3 ± 0.8
2.5	84	11.8 ± 2.6	2.2 ± 0.6
5.0	79	15.0 ± 3.2	1.2 ± 0.4

Addition of auxins higher than 0.1 mgl^{-1} caused callusing at the cut ends of the explants. Incorporation of IBA and NAA in place of IAA in the shoot multiplication medium also caused callusing from the explant and subcultured shoots. Shoots produced *in vitro* rooted best when treated with 25.0 mgl^{-1} of IBA in half strenght MS medium for 72 h in the dark, followed by transfer to semi-solid, hormone-free half strenght MS basal medium at 28 ± 2^0 C (Table 2 & Fig.3 & 4). About 500 treelets have been obtained using this protocol and there is still more scope for scaling up of the producing of cloned plants. The protocol described could be used for preservation and multiplication of depleting germplasm of *M. emarginata* in aid in afforestation of arid and semi-arid lands. However, large scale field transfer and critical analysis of the performance of cloned trees have yet to be done. 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.*

Table 2. Effect of media and IBA on root induction from cultured shoots of *M. emarginata*.

	Additive (IBA; mgl^{-1})	Treatment Period	Shoots Rooted (%)	Root numbers ± SD	Root length (cm) ± SD	Shoot length (cm)± SD
MS *	IBA 2.5	28 d	13	2.7 ± 0.7	1.8 ± 0.7	3.5 ± 0.6
½MS *	IBA 2.5	28 d	27	4.0 ± 1.1	2.3 ± 0.9	3.9 ± 0.6
White *	IBA 2.5	28 d	18	4.9 ± 1.3	1.5 ± 0.4	2.8 ± 0.4
½ MS**	IBA 25	12 h	39	2.4 ± 0.6	2.4 ± 0.7	4.1 ± 0.7
½MS**	IBA 25	24 h	58	4.5 ± 1.2	2.7 ± 0.9	4.4 ± 0.8
½MS**	IBA 25	48 h	67	5.1 ± 1.2	2.7 ± 0.8	4.5 ± 0.9
½MS**	IBA 25	72 h	75	5.9 ± 1.7	2.9 ± 1.1	4.7 ± 1.0

(Data Scored on 28^{th} day of treatment)

* Semi-solid medium.

** Shoots treated with IBA 25 mgl^{-1} for 12, 24,48 and 72 h in half strength MS liquid medium on filter paper bridge, thereafter cultured on hormone-free 1/2 MS agar gelled medium.

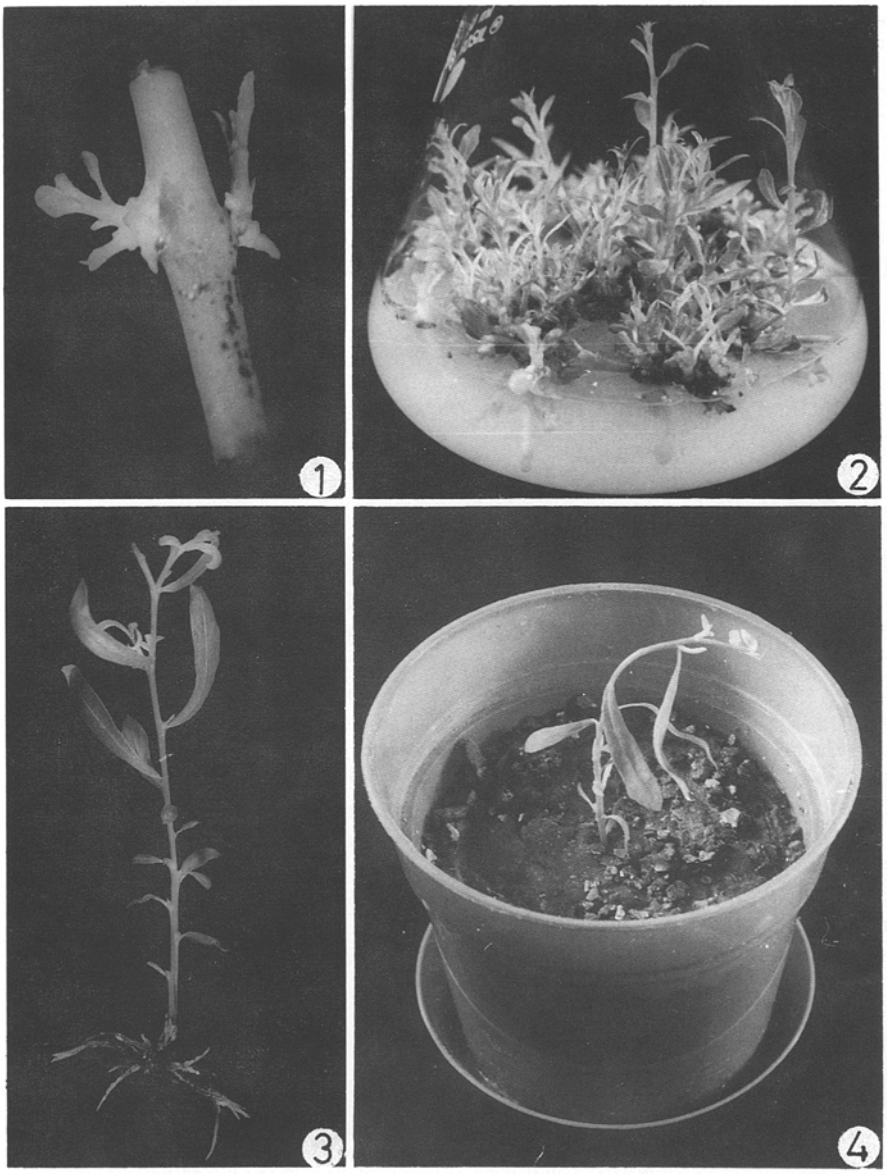

Fig. 1 to 4. Stages of *in vitro* clonal propagation of *M. emarginata.*

(1) Multiple shoots induced from nodal shoot segment on MS medium + IAA (0.1 mgl^{-1}) + BAP (2.5 mgl^{-1})

(2) 3 weeks old subcultured shoot segment multiplied on MS + IAA (0.1 mgl^{-1}) + BAP (1.0 mgl^{-1})

(3) Four weeks old plantlet rooted by two step method. First, the shoots were treated with IBA 25 mgl^{-1} in 1/2 MS liquid medium on a filter paper bridge for 72 h and in second step, treated shoot were cultured on hormone-free 1/2 MS agar gelled medium.

(4) 5-week-old potted treelet.

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