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IN VITRO PROPAGATION OF *ORMOCARPUM SENNOIDES* (WILLD) DC. PRODR. FROM SHOOT TIP EXPLANT

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SUMMARY

***Ormocarpum sennoides*, a medicinal tree has been successfully micropropagated using shoot tip explant on Murashige and Skoog (MS) basal medium and MS medium with different concentrations (0.5, 1.0, 2.0, 3.0 and 4.0 mg l^{-1}) of benzyladenine (BA), adenine sulphate (Ads) and kinetin (Kn). A maximum of 10 shoots were observed at 3.0 mg l^{-1} BA. The regenerated shoots were transferred to MS medium fortified with different concentrations (0.5 – 2.0 mg l^{-1}) of gibberellic acid (GA_3) and 3.0 mg l^{-1} BA for shoot elongation. The maximum of 2.0 – 2.5 cm long shoots were observed at 1.0 mg l^{-1} GA_3. The regenerated shoots were excised and transferred to rooting medium containing indole acetic acid (IAA), α-naphthalene acetic acid (NAA) and 3-indole butyric acid (IBA) at different concentrations. The highest of 60 % of rooting was observed at 0.3 mg l^{-1} IAA, IBA and NAA. The *in vitro* derived plantlets were hardened and acclimatized in soil.**

Key words: In vitro* propagation, medicinal tree, *Ormocarpum sennoides.

INTRODUCTION

Ormocarpum sennoides is a rare valuable medicinal tree belonging to the family Leguminosae. The roots of this plant are considered to be toxic and stimulant and are used in the treatment of lumbago. An application prepared by rubbing the root bark in oil is used in paralysis. This plant is considered to be a fish poison and probably contains a rectone and related compounds (Anonymous 1948). In the present study, *in vitro* propagation of *Ormocarpum sennoides* (Willd) Dc. Prodr. from shoot tip explant is described.

MATERIALS AND METHODS

The shoots of about 2 cm long from apex were collected from the garden grown plants. They were first washed with running tap water. Then they were surface sterilized with 0.1% bavistin for 15 minutes and were rinsed in distilled water thrice. They were taken to the laminar air flow chamber where they were treated with 0.1% $HgCl_2$ for 3 minutes and washed in sterile distilled water. The shoot tips were excised and transferred to MS (Murashige and Skoog 1962) basal medium and MS medium with different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) of BA for multiple shoot formation. Sucrose (3%) was added with MS medium as a carbon source. Then the pH of all media was adjusted to 5.8 prior to autoclaving at 15 lb for 20 minutes. The cultures were incubated at 24±2°C under 2000 lux light intensity provided by white fluorescent lamp for 16 hours photoperiod. All the results were statistically analyzed. Subculturing was carried out regularly at 2 weeks interval.

For shoot elongation, the regenerated shoots were transferred to MS medium with 3.0 mg l^{-1} BA and different concentrations (0.5, 1.0 and 2.0 mg l^{-1}) of GA_3.

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The elongated shoots were excised and transferred to MS medium with various concentrations (0.1, 0.2, 0.3 and 0.4 mg l^{-1}) of auxins like NAA, IAA and IBA individually and in combined form for rooting. The rooted plantlets were hardened and were successfully acclimatized in soil condition.

RESULTS AND DISCUSSION

The present study of *in vitro* propagation of *Ormocarpum sennoides* (Willd) Dc. Prodr. includes, multiple shoot formation from shoot tip explant, elongation of regenerated shoots and rooting and hardening of elongated shoots.

Multiple shoot formation from shoot tip explant: In this method axillary or terminal buds were forced to produce multiple shoots with higher concentrations of cytokinin. Shoot tip explants of *Ormocarpum sennoides* were inoculated on MS basal medium and MS medium with different concentrations (0.5,1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) of BA for multiple shoot formation. The shoot buds were initiated after 7 days of inoculation from the shoot apex in all the concentrations tried. The maximum of 10 shoots were observed at 3.0 mg l^{-1} BA (Plate 1a) followed by hormone free medium where 5-6 shoots were obtained (Table 1). For multiple shoot induction BA has played a key role. Similar results were observed in *Adhatoda vasica* (Sangeetha *et al*. 2004) and *Baliospermum montanum* (Shanthi and Xavier 2005).

Table 1. Multiple shoot formation of *Ormocarpum sennoides* (Willd.) DC. Prodr. from shoot tip explant on MS basal medium and MS with different concentrations (0.5-5.0 mg l^{-1}) of BA.

BA (mg l^{-1})	Response (%)	No. of shoots (Mean ± SD)	Average length shoots in cm (Mean ± SD)
None	60	6.1 ± 1.0	3.0 ± 0.5
1.0	68	5.1 ± 0.1	2.3 ± 0.78
2.0	79	5.3 ± 0.2	2.3 ± 0.78
3.0	82	10.1 ± 1.6	2.5 ± 0.8
4.0	64	5.4 ± 1.4	2.17 ± 0.72
5.0	57	5.0 ± 0.2	2.14 ± 0.71

In the present study, a maximum of 10 shoots were obtained per explant. Atta-Alla and Van Staden (1997) produced an average of 6.6 shoots of *Yucca alifolia* on MS medium with 4.4, 8.9 or 17.8 µm BA +1.1 or 2.7 µm NAA or 1.1, 2.3 or 4.5 µm thidiazuron, 0.5 or 1.1 µm NAA. They used different combination of hormone for multiple shooting. In our study, single growth hormone (BA) was found to be sufficient for producing multiple shoot. However, the combination of 2 cytokinins was needed for multiple shoot formation in *Mussaenda erythrophylla* (Maity *et al*. 2001).

In the present study 5 – 6 shoots were obtained in MS basal medium. In contrast to that combination of hormones were used in S*apindus mukorossi* (Philamina and Rao 1999) and *Carica papaya* (Agnihotri *et al.* 2004). In *Pisonia alba* the maximum of 8 – 10 multiple shoots were reported on MS medium supplemented with BA and Kn (1.0 mg l^{-1}) (Jagadish Chandra *et al*. 1999).

Elongation of regenerated shoots: The regenerated shoots from shoot tip explants were inoculated on MS medium with 3.0 mg l^{-1}BA and different concentrations (0.5, 1.0 and 2.0 mg l^{-1}) of GA_3. Of these different concentrations of GA_3 tried, the best results on shoot elongation were observed at 1.0 mg l^{-1} GA_3 (Plate 1b). The shoots attained a length of 2.0 – 2.5 cm within 20 days of inoculation (Table 2). From this study, it is inferred that GA_3 at 1.0 mg l^{-1} was the best for the elongation of regenerated shoots from shoot tip explant. Similar findings were observed is *Canavalia virosa* (Kathiravan and Ignacimuthu 1999) and *Ruta graveolens* (Shanthi and Xavier 2001). In contrast to

Table 2. Effect of different concentrations (0.5, 1.0 and 2.0 mg l^{-1}) of GA_3 on elongation of regenerated shoots of *Ormocarpum sennoides*

Hormone in mg l^{-1}		Response (%)	Shoot length (Mean ± SD)
BA	GA_3		
3.0	0.5	43	1.9 ± 0.8
3.0	1.0	87	2.5 ± 0.3
3.0	2.0	52	2.1 ± 0.3

the present study, BAP and IBA were used to induce elongation of multiple shoots in *Vigna mungo* (Agnihotri *et al*, 2001) and *Momordica charantia* (Agarwal and Kamal 2004).

Rooting and hardening of regenerated shoots: The elongated shoots were excised from the shoot clumps and inoculated on MS medium containing different concentrations (0.1, 0.2, 0.3 and 0.4 mg l^{-1}) of auxins like NAA, IAA and IBA individually and in combined form. The root initiation was observed after 15 days of inoculation. Of these different concentrations of auxins tried, the maximum percentage (60%) of rooting was observed at 0.3 mg l^{-1} of IBA, IAA and NAA each (Plate 1c). The roots were long, pale white, linear and robust with root hairs, with an average of 2 – 4 cm long. It was followed by 0.2 mg l^{-1} of NAA + IAA + IBA combination, which produced 41% rooting (Table 3). The auxins at low concentrations produced only 2 roots without any root hairs. Auxins used individually and in combinations of two did not show any positive results for rooting. After 30 days of inoculation on rooting medium, the rooted plantlets were removed from the culture tube and washed with distilled water. These *in vitro* derived plantlets were transferred to plastic cups containing vermiculite for hardening and maintained for about 15 days (Plate 1d). A maximum of 65% of plantlets were hardened successfully.

Table 3. Effect of different concentrations (0.1, 0.2, 0.3 and 0.4 mg l^{-1}) of auxins (IAA, IBA and NAA) on rooting of regenerated shoots of *Ormocarpum sennoides.*

Hormones in mg l^{-1}			Response (%)	No. of roots /shoot (Mean ± SD)	Root length (cm) (Mean ± SD)
IAA	NAA	IBA			
0.1	0.1	0.1	15	3.32 ± 0.4	3.78 ± 0.89
0.1	0.2	0.1	10	2.4 ± 1.1	4.14 ± 1.03
0.1	0.3	0.1	-	-	-
0.1	0.4	0.1	-	-	-
0.2	0.1	0.2	23	4.1 ± 0.98	3.18 ± 0.72
0.2	0.2	0.2	41	5.24 ± 0.55	4.0 ± 1.20
0.2	0.3	0.2	12	3.9 ± 0.90	2.74 ± 0.42
0.2	0.4	0.2	-	-	-
0.3	0.1	0.3	-	-	-
0.3	0.2	0.3	22	3.46 ± 0.48	3.22 ± 1.4
0.3	0.3	0.3	60	4.78 ± 0.86	3.9 ± 0.56
0.3	0.4	0.3	20	3.72 ± 1.7	3.62 ± 0.94
0.4	0.1	0.4	-	-	-
0.4	0.2	0.4	-	-	-
0.4	0.3	0.4	16	3.9 ± 0.26	3.58 ± 1.52
0.4	0.4	0.4	20	3.46 ± 1.02	3.66 ± 0.87

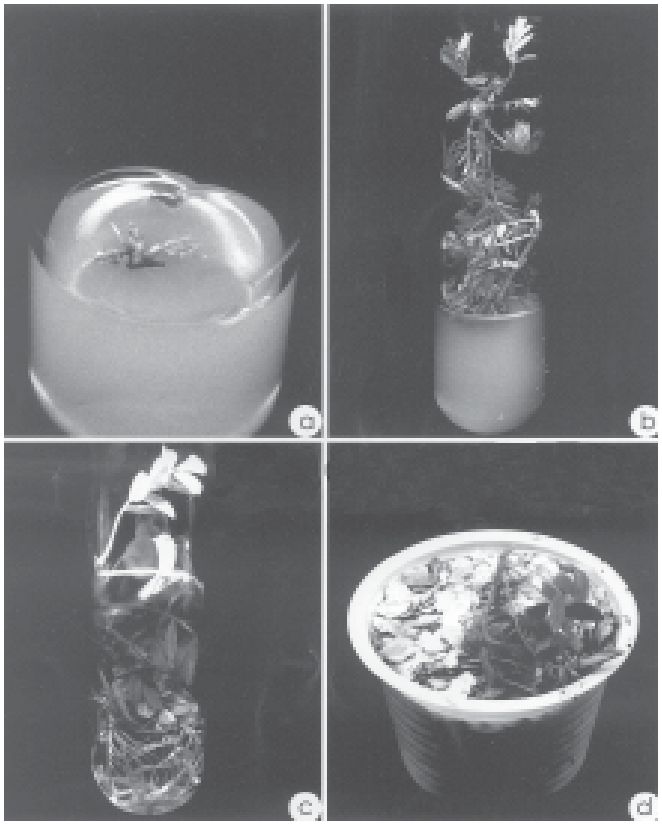

Plate 1. *In vitro* propagation of *Ormocarpum sennoides* (Willd) Dc. Prodr. from shoot tip explant. (a). Initiation of shoot buds from shoot tip explants of *O. sennoides*, (b). Elongation of shoots from shoot tip explants of *O. sennoides*, (c). Rooting of regenerated shoots from shoot tip explants of *O. sennoides*, (d). Hardening of *in vitro* derived shoots from shoot tip explants of *O. sennoides*

For rooting, MS medium was used in the present study. Similar results were reported in *Mussaenda erythrophylla* (Maity *et al*. 2001), *Baliospermum montanum* (Shanthi and Xavier 2005) and *Garcinia indica* (Meera *et al*. 2005). In our study, the combination of three auxins (NAA + IAA + IBA) were essential for root formation. A maximum of 60% of rooting was observed at 0.3 mg $l^{-1}$of IAA + IBA + NAA

combination. Similar results were observed in *Enicostemma littorale* (Shanthi and Xavier 2003). The present study shows that through tissue culture we can have mass production of plantlets of *Ormocarpum sennoides* tree in a short span of time.

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