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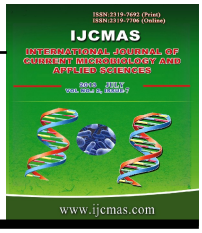

Original Research Article

Micropropagation of *Heliotropium keralense* - a globally endangered and endemic medicinal plant through indirect organogenesis from roots

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A B S T R A C T

Keywords

Micro-propagation; indirect organogenesis; *Heliotropium keralense*.

An efficient protocol was established for rapid micropropagaion and conservation of *Heliotropium keralense* from root explants. Murashige and Skoog's (MS) medium supplemented with 2,4-D (0.5 *mg*/l) and 2 i P (4.0 mg/l) was the most effective combination for the induction of callus from roots. MS medium supplemented with BA (3.0 mg/ l) and IAA (0.5mg/l) was the most suitable combination for callus regeneration. MS medium supplemented with 0.5mg/l IBA was most effective for rooting of shoots. Plantlets with fully expanded leaves and well-developed roots were hardened under controlled conditions for 20 days and eventually established in the field. Morphologically there was no detectable variation between *in vitro* raised and naturally grown plants.

Introduction

Heliotropium keralense is an important endemic medicinal plant of Kerala (Sasidharan and Sivarajan, 1966; Sivarajan and Balachandran 1994). The plant belongs to IUCN globally Endangered red list category (Nayar, 1996; Biswas 2006). The plant is useful in the treatment of worms, skin diseases, scorpion and snake poisoning, asthma, cough, anaemia, insanity and epilepsy (Sivarajan and Balachandran, 1994; Tiwari *et al*., 2001). Efficient micropropagation protocol of important red listed plants is an essential requirement for their conservation and mass production (Tyagi and Prakash, 2001). The present paper describes a protocol which can be used for rapid multiplication and genetic manipulation of *Heliotropium keralense* .

Materials and Methods

Roots of H. *keralense* were collected from plants growing in Calicut University campus. The explants were washed thoroughly under tap water, followed by treatment with 5% extran (v/v) (Merck) for 5 minutes and subsequently washed 3 times with sterile water. The explants were surface disinfected with 0.1% mercuric chloride solution for10 minutes and after decanting the sterilant, explants were washed with sterile double distilled water.

The explants were then cultured on nutrient medium under aseptic conditions.

For the induction of callus the explants after surface sterilization were cultured on semi-solid MS medium containing 3% sucrose and 0.8% agar, supplemented with varying concentrations and combinations of Benzyl Adenine (BA), Kinetin (Kn), 2-isopentyl-adenine (2 iP) and 2,4-Dichlorophenoxyacetic acid (2,4-D).For the regeneration of shoots calli were cultured on MS medium supplemented with varying concentrations and combinations of BA, Kn and IAA. For rooting *in vitro* raised shoots were excised and cultured on MS medium supplemented with varying concentrations of IAA, IBA and NAA.

The pH of medium was adjusted to 5.7 prior to autoclaving at a pressure of 15psi for 15 minutes. Cultures were incubated at 25 ± 2°C under 16 h photo periods at 1000 lux light.

Plantlets with well developed root and shoot systems were taken out from the culture tubes, and after thorough washing with sterilized water, were transferred to plastic cups containing sterile sand and soil (1:1) mixture. These were subjected to acclimatization by covering them with polythene bags and kept in green house at 90% relative humidity. Successfully hardened plantlets were eventually established in natural soil.

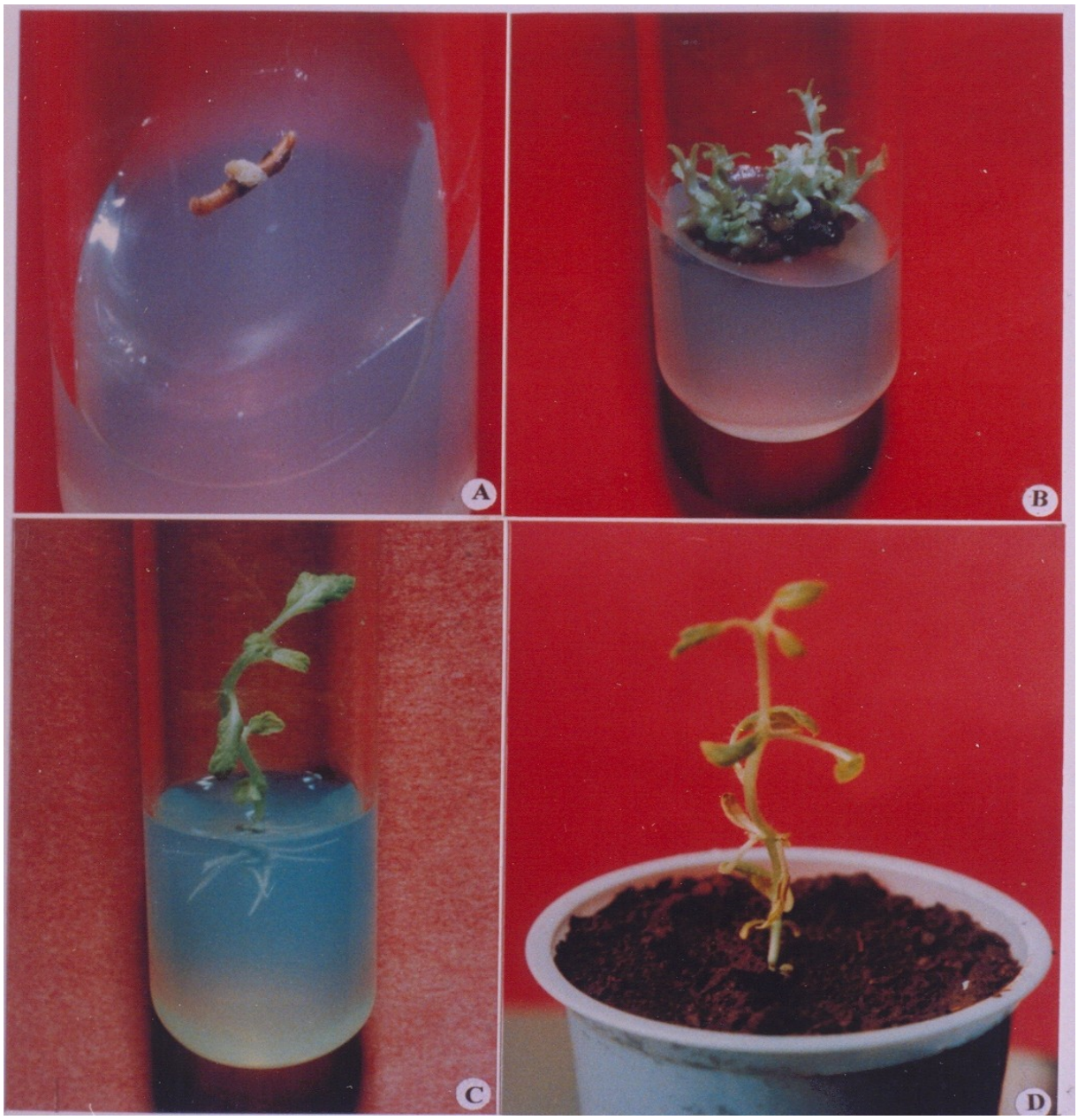
Result and Discussion

Callus formation observed from root explants of *Heliotropium keralense* in relation to growth regulators supplemented in MS medium is summarised in Table l.

Cytokinins when used alone were found to be ineffective in the induction of callus from root explants of *Heliotropium keralense* .However combination of auxins and cytokinins were found to be highly effective in callus induction from root explants (Figure 1 A) .Among the various combinations, MS medium supplemented with 0.5 mg/l 2,4-D and 3.0mg/l 2 iP was found highly effective. Enormous amount of calli were obtained from root explants on MS medium supplemented with 0.5 mg/l 2,4-D and 3.0mg/l 2 iP. The same was selected for regeneration studies . Murashige and Skoog's medium supplemented with BA (3.0 mg/l) produced highest number of shoots from callus .Of the two cytokinins tested only BA was found to be effective in shoot regeneration from calli (Figure 1 B).Weekly subculturing was necessary to retain morphogenic potential of calli. Calli which turned brown in the absence of weekly subculturing never showed regeneration. Among the various auxins IBA at (0.5 mg/l) in MS medium was found as most effective for rooting of *H. keralense* shoots (Figure 1 C; Table 2) and produced highest number of roots (25/shoot) with high frequency (90%). Plantlets with well developed shoot and root systems formed in cultures were taken out and acclimatized in natural soil (Figure 1 D). Out of the 420 plants transferred to the field 391 plants survived and developed into mature plants. Micropropagated plants were morphologically similar to normal field grown plants.

In the present studies nature and proliferation of callus formed from roots varied with the growth regulators supplemented in the nutrient media.

Figure.1 Various stages of micropropagation of *H.keralense* through indirect organogenesis from roots



(A) Callus induced on MS+2,4-D (0.5 *mg*/l) and 2 i P (4.0 mg/l) (B) Shoot regeneration from callus on MS+ BA (3.0 mg/ l) and IAA (0.5mg/l) (C)Roots induced on MS+0.5mg/l IBA (D)Hardened plantlet in a small pot.

Table.1 Effect of growth regulators on callus induction and shoot regeneration from root exptants of *Heliotropium keralense*

Growth	**Callus formed**	**shoots /g of callus**
regulators [1]		(formed from root explants on
(mg/ liter)		MS +2,4-D (0.5 *mg*/l) and 2 iP (4.0 mg/l))
BA		
1.0	--	4.4±0.28
2.0	--	7.6 ±0.42
3.0	--	9.8±0.17
4.0	--	9.4±0.24
5.0	--	7.6 ±0.42
Kn		
1.0	--	Proliferation only
2.0	**–**	Proliferation only
3.0	--	Proliferation only
4.0	--	Proliferation only
IAA + BA		
0.5 0.5	- -	2.7±0.41
0.5 1.0	--	5.2 ±0.12
0.5 2.0	--	7.8±0.57
0.5 3.0	**--**	**10.8±0.32**
0.5 4.0		10.6 ±0.63
2,4 - D + 2 i P		
0.5 0.5	+	Proliferation only
0.5 1.0	+	Proliferation only
0.5 2.0	++	Proliferation only
0.5 3.0	+++	Proliferation only
0.5 4.0	++++	Proliferation only
2,4 - D + BA		
0.5 0.5	+	Proliferation only
1.0 1.0	+	Proliferation only

Data from 20 replicates in two experiments (Mean±SE)
--No callus formation,+ = very slight, ++ = Little, +++ = Moderate, ++++ = Profuse
Growth Period 50 days

Table.2 Effect of auxins (IAA, IBA & NAA) on *in vitro* rooting of *H. keralense*

Growth regulators(mg/l)	% Response	No. of Roots
IAA		
0.1	60	6.3 ± 0.88
0.2	75	9.8 ± 0.18
0.5	80	14.6 ± 0.42
1.0	80	16.4 ± 0.16
2.0	70	9.9 + C ± 0.11
3.0	75	7.2 + C ± 0.27
IBA		
0.1	40	11.8 ± 0.56
0.2	65	16.7 ± 0.12
0.5	90	**22.1 ± 0.22**
1.0	90	20.4 ± C + 0.63
2.0	85	16.8 + C ± 0.47
3.0	90	13.2 + C ± 0.29
NAA		
0.1	90	4.1 ± 0.15
0.2	85	6.6 ± 0.74
0.5	80	4.3 + C ± 0.19
1.0	80	5.2 + C ± 0.37
2.0	85	3.7 + C ± 0.61
3.0	75	2.9 + C ± 0.23

Data from 20 replicates in two experiments (Mean ±SE), Growth period 50 days

This observation was in corroborative with the reports of Lin *et al*., 2000 and Koroch *et al.,* 2002.Effectiveness of auxin-cytokinin interaction for callus induction and proliferation was reported by Koroch *et al* ., 2002.This report was in consonant with the results obtained in the present experiments on *H.keralense*. Reddy *et al., (2001)*and Kroch *et al*., (2002) reported effectiveness of auxin cytokinin combination in callus regeneration of many medicinal plants. These reports were in consonant with the results of present experiments on *H.keralense.* Our results showed that BA was highly effective than Kn for shoot regeneration of *H. keralense.* The simulative effect of BA over Kn in shoot regeneration was reported earlier in many medicinal plants (Tiwari *et al*., 2001; Babu *et al*., 2003; Kiani *et.al* 2010;. Shekhawat *et al*.,2012 and Delgadillo-Díaz de León *et.al*.,2013).

Present studies also demonstrated efficacy of IBA for the rooting of *in vitro* raised shoots of *H. keralense.* Similar results showing efficacy of IBA in *in vitro* rooting was reported earlier in many plant species (Echeverrigaray *et al*., 2000; Fracaro & Echeverrigaray, 2001Das & Rout, 2002; Faisal and Anis, 2003 Andoh *et al.,* 2005; and Yu *et al.,* 2012).

The protocol described here would be useful for genetic manipulation and rapid multiplication of *H.keralense* .

References

Andoh,Y., S. Yahara and Yoshizawa, N. 2005. *In vitro* regeneration of the medicinal plant *Phellodendron amurense* Rupr. Through excised leaves. PL..Cell Tiss. Org. Cult. 80: 43-50.

Babu, K.N., A. Sajna, D. Minoo, C.Z. John, P.M. Mini. K.V. Tushar, Rema and Ravindran, P.N. 2003. Micropropagation of Camphor tree (*Cinnamomum camphora).* Pl. CeIl Tiss.Org. Cult. 74: 179-183.

Biswas, S., 2006. Indian biodiversity for the present millennium: global prospects and perspectives. Satish Serial Publishers,India. pp.25.

Das, G., and Rout, G.R. 2002. Direct plant regeneration from leaf explants of *Plumbago* PI. Cell Tiss, Org Cult. 68:311-314.

Delgadillo-Díaz de León, J., J.F. Morales-Domínguez, M.S. Santos-Díaz ,and Pérez-Molphe- Balch, E. 2013. *In Vitro* propagation of mexican oaks (*Quercus* spp.)propagación *in vitro* de encinos mexicanos (*quercus* spp.) Polibotanica 35:85-97.

Echeverrigaray, S., S. Bias, F. Fracaro and Sarafini, 2000. Clonal micropropagation of Roman Charnomile *(Anthemis nobilis* L.). 1. of Herbs, Spices. Med. plant. 7 (2): 35-41.

Faisal, M., and Anis, M. 2003. Rapid mass propagation of *Tylophora indica* Merill via leaf callus culture. PI.Cell.Tiss. Org. Cult.75: 125-129

Fracaro, F., and Echeverrigaray, S. 2001. Micropropagation *of Cunila galiodes,* a popular medicinal plant of South Brazil PI. CellTtiss. Org. Cult. 64: 1-4.

Kiani, M., H. Zarghami, A. Tehranifar and Memariani, M. 2010. Conservation of *Colutea gifana*, a rare and potential ornamental species, using *in vitro* method. J. Cell. Mol. Res. 2 (2): 81-85.

Koroch, A., H.R. Juliani, J. Kapteyn and Simon Jen. 2002. *In vitro* regeneration of *Echinacea* from leaf explants. Pl.cell Tiss.Org.Cult.69: 79-83.

Lin, H.S., C.V. Toorn, Raemakers kjjm, R.G.F. Visser, M.J. Deeju and

Jacobson,E.2000. Development of a plant regeneration system based on friable embryogenic callus in the ornamental *Alstroemeria*. Pl.Cell Rep.19:529-534.

Nayar, M. P., 1996. Hot spots of endemic plants of India, Nepal and Bhutan. Tropical Botanic Garden and Research Institute Publishers, Trivandrum, India. pp. 96.

Reddy, P.S., R. Rodrigues and Rajasekharan, R. 2001.Shoot organogenesis and mass propagation of Coleus forskohlii from leaf drived callus. Pl. Cell Tiss.Org .Cult. 66:183-188.

Sasidharan. N., and Sivarajan, V.V. 1966. Flowering plants of Thrissur forest (Western Ghats, Kerala, India) Scientific Publishers; Jodhpur, India. pp. 303-304

Shekhawat, M.S., 2012. Direct Shoots Regeneration from Nodal Meristems of *Brugmansia suaveolens* (Humb. & Bonpl. ex Willd.) Bercht. & J.Presl. thepharmajournal.com .1(9): 50-55.

Sivarajan, Y.V. and Balachandran, L.1994. Ayurvedic drugs and their plant resources. Oxford and IBH publishing Co. Ltd., New Delhi.

Tiwari, V., K.N. Tiwari and Singh, B.D. 2001. Comparative studies of cytokinins on in vitro propagation of *Becopa monnieri*.. PI. cell Tiss. Org. Cult. 66: 9-16.

Tyagi, R.K., and Prakash, S. 2001.Clonal propagation and in vitro conservation of Jojoba (*Simmondsia chinensis* (Link) Schneider).Indian J.Plant Genet.Resour.14:298-300.

Vincent, K.A., K.P. Martin and Hariharan, M. 1998. *In vitro* culture studies on *Kampferia galanga* L. In; Khan IA and K hanum A (Eds.). Role of Biotechnology in Medicinal and Aromatic Plants. Ukaaz. Publ. Hyderabad, India.

Yu X; Wu H, Jaime A; Teixeira da Silva and Shen M 2012. Multiple shoot induction and rooting of *Paeonia lactiflora* 'Da Fu Gui' . African. J. Biotechnol.11(41): 9776-9781.