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IN VITRO RHIZOGENESIS FROM LEAF AND STEM CALLUS OF *HELIOTROPIUM INDICUM*, L.-MEDICINAL HERB.

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ABSTRACT: *Heliotropium indicum, L*. is one of the most common medicinal plants used by diverse cultures and tribal groups. *In vitro* callogenesis and rhizogenesis is achieved from different explants in stem and leaf of *Heliotropium indicum*, L Explants were incubated on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of auxins like 2,4-D, NAA and IBA at 1.0mg/L-3mg/L and cytokinins like Kn and BAP at 2mg/L. In the present study on *Heliotropium indicum,* L indicated that there is a strong ability for *in vitro* rhizogenesis than caulogenesis.This ethnomedicinal herb is known to have unique medicinal properties. The roots are bitter, astringent, aphrodisiac, expectorant, febrifuge and ophthalmic and are useful in cough, bronchitis, fever, ringworm. Ethnolic crude extracts of roots of *Heliotropium indicum* L., showed the presence of alkaloids, steroids, and carbohydrates. This provides a basis for germplasm conservation and for further investigations of bioactive constituents of roots of this plant.
Key Words: Callogenesis, *Heliotropium indicum*, L., *In vitro,* Medicinal herb, rhizogenesis.

INTRODUCTION

Heliotropium indicum, L. is an important ethnomedicinal medicinal herb belongs to the family Boraginaceae. This herb is known to have high medicinal values specially the root. The roots are bitter, astringent, aphrodisiac, expectorant, febrifuge and ophthalmic and are useful in cough, bronchitis, fever, ringworm. Ethnolic crude extracts of roots of *Heliotropium indicum* L., showed the presence of alkaloids, steroids, and carbohydrates. It is important to note that in approximately 60% of the medicinal plants used in the traditional system of medicine (Ayurveda, Unani and Siddha) roots are the principle material for drug preparation(1). It is estimated that more than 90% of the plant species used by the pharmaceutical industry is collected from the wild and more than 70% of the plant drugs involved destructive harvesting (2) and very few are in cultivation. Root culture system offers unique opportunities for providing root drugs in the laboratory, without re-sorting to field cultivation. An alkaloid N- indicine oxide from *H. indicum* (3) was isolated and observed the significant anti-tumour activity of the compound. In present study the callus potent for high propensity of adventitious roots were induced from leaf and stem explants on MS with different combinations and concentrations of growth hormones.

MATERIALS AND METHODS

Young healthy leaf and stem explants of *Heliotropium indicum, L* collected from wild populations. Later selected explants were washed with 1% Laboline and kept under running tap water for 30 minutes and subsequently surface sterilized with 0.1% mercuric chloride for 2 minutes. The explants were washed with sterile distilled water 3-4 times. The sterilized explants were inoculated on MS (Murashige and Skoog 1962) basal media gelled with 0.8% agar (Himedia). The medium was supplemented with 3% (w/v) sucrose (Himedia) as carbon source. The medium was supplemented with different combinations and concentrations of growth regulators. Auxins namely NAA (1.0mg - 3.0mg/L), IBA (1.0mg-3.0mg/L), 2,4-D (1.0mg-3.0mg/L) and Cytokinins namely BAP 2.0 mg/L and KN 2.0mg/L. The pH of the medium was adjusted to 5.8. This media was autoclaved at a temperature of 121° C (15 psi) for 30 minutes. The percentage of callogenesis and rhizogenesis were recorded from each explant.

Statistical analysis

The percentage of explant response for degree of callus formation, colour of the callus, degree of root induction from different explant derived callus and number of roots from each explant callus were monitored as growth parameters. Statistical analysis was performed on the result of each experiment and data were compared using analysis of variables and least significant differences by SPSS version 14.

RESULTS AND DISCUSSION

In the present study, MS medium supplemented with number of combination with auxins 2, 4-D, NAA and IBA and Cytokinins KN and BAP were used for callus induction from stem and leaf explants. Slight and moderate callus were recorded (Fig C, 2 & 3.) with different colors like light green, white, light brown and brown. Explant culture *in vitro* may be involved in organogenesis and develop shoots or roots depending on the morphogenic potentiality of the cells. There are three distinct stages during organogenesis, namely dedifferentiation, induction of organogenesis pathway and development of organs (4). The present study showed the explant response to induce callus depends on different concentrations, type of plant growth regulators and type of explant used. The callus induction was better from stem explant than callus induced from leaf explants (Table 1). 2, 4-D proved to be very poor for callus induction where as, however it is in *Ionidium suffruticosum* (7) proved very effective callus inducing auxin. NAA and IBA were found to be more effective in callus induction than 2, 4-D in stem and leaf explant of *Heliotropium indicum* of present study. Better induction of callus was observed on MS medium supplemented with BAP 2mg/L and NAA 3.0 mg/L for stem explant is also observed in *Aristolochia* (8), but BAP 2mg/L and IBA 2.0mg/L were effective for induction of leaf explants of *Heliotropium indicum*. NAA was the good source of auxin for callus induction with both in kinetin or BAP for the both the explants of present study. It was observed that both the explants do not have the equal potential to regenerate roots. This also indicates they are differing in their endogenous content of chemicals. However, frequent rhizogenesis was noticed from the callus cultures of both explants. Good response for adventitious roots from the leaf callus with BAP 2.0mg/L + NAA 2.0mg/L and stem callus with BAP 2mg/L + NAA 3mg/L (Fig C, 4, 5 & 6.). Profuse rhizogenesis was also observed on MS medium with NAA + BAP in *Ocimum sanctum* (9). In many plant species it was shown that optimal root formation occurred in the presence of auxins and cytokinins (10) which also true in present study. An effect of various concentrations of auxins with BAP induces rooting (11) as in the present study. Therefore, it is indicated that cytokinins also have stimulating effects on root formation. Only root initiation was observed in earlier efforts as in *Glycine max* (12) and *Phaseolus vulgaris* (13). After few weeks of growth, root turns brown and flaccid. The loss of root differentiation ability is one of the drawbacks of maintaining normal root culture for long term as observed in *Duboisia* species (14) which is also true with the present study.

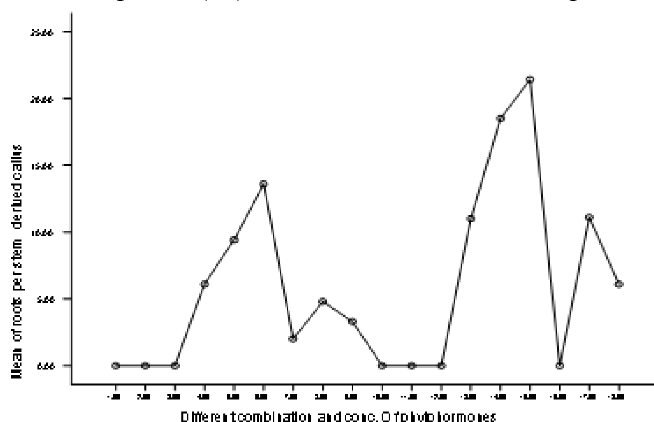

Fig A. Graph showing effect of different hormones on stem callus for *in vitro* rhizogenesis

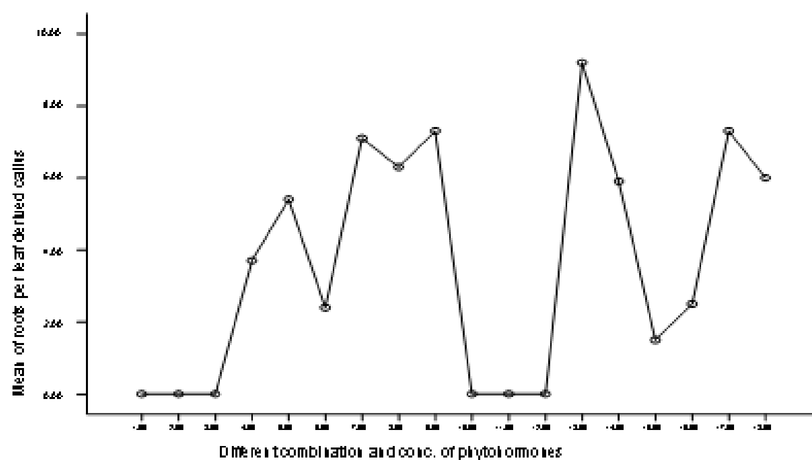

Fig B. Graph showing effect of different hormones on leaf callus for *in vitro* rhizogenesis

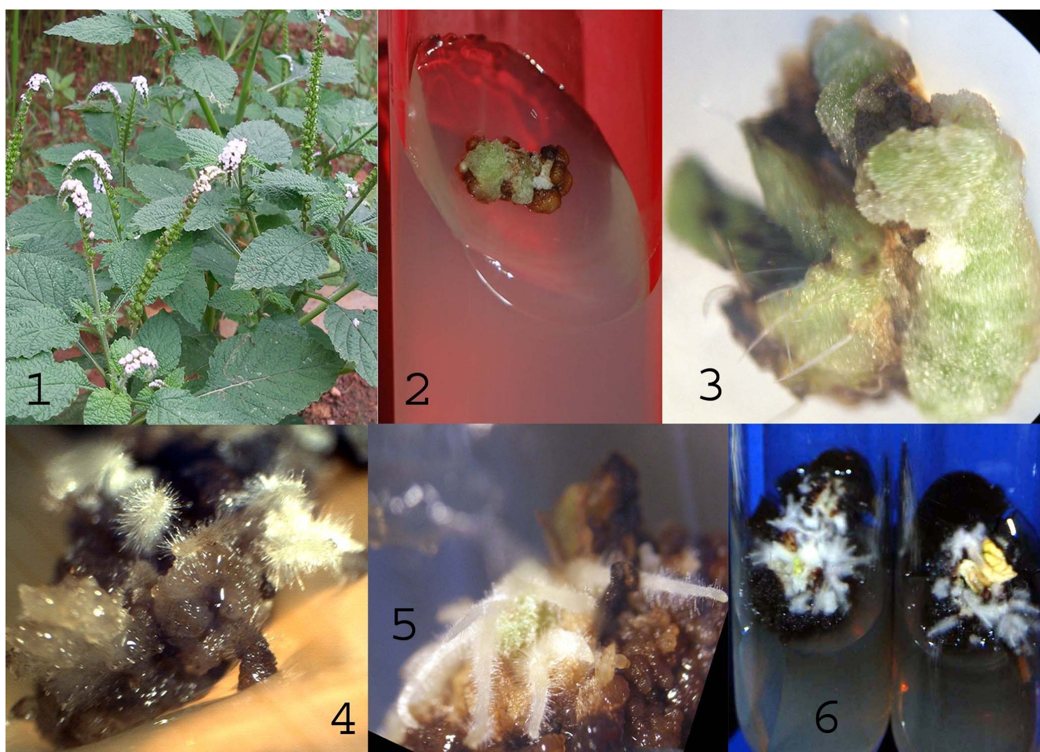

Fig C (1 -6) Photographs showing leaf callus, stem callus and *in vitro* roots induced on MS medium with different concentrations and combinations of BAP and Kinetin with IBA and NAA.

1. In vivo flowering shoot of *Heliotropium indicum*, L.
2. Callus induction from leaf explant.
3. Callus induction from stem explant.
4. Stereo microscopic photograph of roots induced from leaf callus.
5. Stereo microscopic photograph of roots induced from stem callus.
6. *In vitro* roots.

Table 1: Effect of KN, NAA, IBA AND 2, 4-D on *in vitro* callogenesis and rhizogenesis of stem and leaf explants with MS medium of *Heliotropium indicum*, L

Type of explant	*Growth regulators mg/l*	*% of explant induced callus*	*Callus colour*	*Degree of callus formation*	*Rhizogenesis (%)*	*Mean no of roots per explant*
Stem	KN+2,4-D					
	2.0+1.0	-	-	-	-	-
	2.0+2.0	10.2	B	+	-	-
	2.0+3.0	15..7	B	+	-	-
Stem	KN+NAA					
	2.0+1.0	25.5	LB	+	25	6.1±0.19
	2.0+2.0	36.0	LB	++	46	9.4±0.12
	2.0+3.0	45.1	W		52	13.6±1
Stem	KN+IBA					
	2.0+1.0	35.5	B	++	15	2.0±0.9
	2.0+2.0	50.0	LB	++	20	4.8±0.11
	2.0+3.0	55.0	LB	++	33	3.3±0.17
Stem	BAP +2,4-D					
	2.0+1.0	10.0	B	+	-	-
	2.0+2.0	12.2	B	+	-	-
	2.0+3.0	24.3	B	+	-	-
Stem	BAP +NAA					
	2.0+1.0	42.0	LG	+	47.6	11.0±0.9
	2.0+2.0	60.0	W	++	50.5	18.5±1
	2.0+3.0	65.0	LB	++	67.2	21.4±1
Stem	BAP +IBA					
	2.0+1.0	28.2	B	++	-	-
	2.0+2.0	58.0	B	++	58.2	13.1±0.9
	2.0+3.0	60.0	B	++	60.0	16.1±0.18
Leaf	KN +2,4-D					
	2.0+1.0	-	-	-	-	-
	2.0+2.0	10.0	B	+	-	-
	2.0+3.0	17.0	B	+	-	-
Leaf	KN +NAA					
	2.0+1.0	25.5	B	++	13.0	3.7±0.5
	2.0+2.0	39.5	LG	++	20.5	5.4±0.19
	2.0+3.0	28.2	W	+	15.0	2.5±0.13
Leaf	KN +IBA					
	2.0+1.0	20.0	W	+	20.7	7.1±0.5
	2.0+2.0	17.8	LB	++	27.0	6.3±0.5
	2.0+3.0	24.7	LB	+	33.5	7.3±0.5
Leaf	BAP +2,4-D					
	2.0+1.0	-	-	-	-	-
	2.0+2.0	-	B	+	-	-
	2.0+3.0	-	B	+	-	-
Leaf	BAP +NAA					
	2.0+1.0	16.4	B	+	32.0	1.5±0.13
	2.0+2.0	50.5	LB	++	41.2	9.2±1
	2.0+3.0	39.0	B	++	37.0	5.9±0.5
Leaf	BAP +IBA					
	2.0+1.0	23.1	W	++	10.0	2.5±0.16
	2.0+2.0	58.5	LB	++	35.0	7.3±0.5
	2.0+3.0	45.9	LB	++	33.8	6.0±0.5

B=brown, LB = light brown, W = white, LG = light green + = Slight callus, ++ = moderate callus, - = no callus

REFERENCES

1. Agarwal V S and Ghosh B. (1995). Drug plants of India (Root drugs), Kalyani Publishers, Ludhiana, India.

2. Ved D K, Mudappa A and Shankar, D. (1998).Regulating export of endangered medicinal plant species-need for scientific rigor. *Curr Sci*, 75: 341-341.
3. Kugelman M, Lui W-C, Axelrod M, McBride TJ, Rao KV. (1976) Indicine-N-oxide: the antitumor principle of *Heliotropium indicum*. *Lloydia,* 39: 125–128.
4. Bottino, P.J., Maire, C.E. and Goff, L.M. (1979). Tissue Culture and organogenesis in the winged bean. *Can Jour of Bot*, 57: 1773-1776.
5. Klerk, G J D, Arnholdt- Schmitt, B, Lieberei R and Neumnann K H. (1997) Regeneration of roots and embryos; Physiological, biochemical and molecular aspects. *Biol Plant*, 39: 53-66.
6. Arunkumar B Sonappanavar, M Jayaraj, Asha N Bagadekar and Anant V Bhandarkar (2009). *In vitro* propagation of *Ionidium suffruticosum* Ging. - A seasonal multipotent medicinal herb. *Plant tissue cult and biotech,* 19(2): 143-150.
7. N A Siddique, M H Kabir and M A Bari (2006). Comparative *in vitro* study of plant regeneration from nodal segment derived callus in *Aristolochia indica,* L. and *Hemidesmus indicus* (L) RBr. Endangered medicinal plants in Bangladesh. *Jour of Plant Science,* 1(2): 106-118.
8. Anwer Shahzad, Siddiqui S A. *In vitro* organogenesis in *Ocimum sanctum* L. A multipurpose herb (2000). Phytomorphology, 50 (1), 27-35.
9. Dudits, D., Nemeth, G. and Haydu, Z. (1975). Study of callus growth and organ formation in wheat *Triticum aestivum* tissue culture. *Can. Jour. Bot*. 53: 957-963.
10. Evan, D. A., Sharp, W.R. and Paddock, E. F. (1976). Variation in callus proliferation and root morphogenesis in leaf tissue cultures of *Glycine max* strain T 219. *Phytomorphology*, 26: 379-384.
11. Haddon, L. and Northcote, D.H. (1976). The influence of gibberellic acid and abscisic acid on cell and tissue differentiation of bean callus. Jour. *Cell Sci,* 20: 47-55.
12. Murashige T & Skoog F, (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum.* 15, 473-497.
13. Endo, T. and Yamada, Y. (1985). Alkaloid production in cultured roots of three species of *Duboisia. Phytochemistry*, 24, 1233-1236