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DEVELOPMENT OF PROTOCOL FOR EFFICIENT CALLUSING IN *Heliotropium indicum* L. AN IMPORTANT MEDICINAL HERB

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Abstract

Tissue culture study was carried for the development of protocol for an efficient callusing in *Heliotropium indicum* L, an important medicinal herb from different explants (internodal + leaf) in MS medium supplemented with different growth regulators viz., 2,4-D, IBA(0.5-5mg/l) and BAP (0.5-1.0mg/l) concentrations either alone or in combinations. Internodal segment inoculated in MS basal medium supplemented with 1.5 mg/l 2, 4-D + 1.0 mg/l BAP gave the highest percentage of response for callusing which was 89.8 %. On this medium growth rate was also the best and the callus was white and compact. At this concentration and combination the leaf explants also revealed the highest percentage with respect to callusing, however, it was lower than the nodal explants. Next highest response for callusing in nodal explants was noted in MS + 1.5 mg/l 2, 4-D+ 0.5 mg/l BAP which was 82.6%.This was also true for the leaf explants. The unique medicinal property of *Heliotropium indicum* is due to the presence of various alkaloids, steroids and terpenoids etc. These chemicals can be extracted from the calli also. Efficient callus induction protocol can be exploited at commercial scale that will support uninterrupted supply of the chemicals at one hand while on the other hand it will support the conservation of species in its natural habitat.

Keywords: *Heliotropium indicum*, callus, nodal explants, natural habitat, Tissue culture, Conservation.

1. Introduction

Heliotropium indicum,Linn.is a wild herbaceous plants which belong to the family Boraginaceae and are found in barren lands during summer however scattered plants may be seen in the late September. This plant is characterized by the presence of deep green leaves with borage or rough surface, white flowers arranged on the curved inflorescence axis, which appears like an elephant trunk, the common name "Hathi Sur". This plant is highly valued in the folklore medicine and is believed to be used in treating Malaria, Abdominal Pain, fever, dermatitis, venereal diseases, insect bites, menstrual disorders, uriticaria and sore throat (Duttagupt and Dutta 1977). Decoction of different part is used for the treatment of kidney stone (Berhault 1974). The leaf paste is applied externally to cure rheumatism and skin infections (Nagarajuand and Rao , 1990; Barrett 1994). The tribals use the leaf paste over fresh cut and wounds and claim for its promising activity (Kumar et,al; 2007). Because, the plant contained several secondary metabolites such as volatile oil, Indicine_N-oxide, esters and terpenes, so it has potent wound healing ,anti-tumor and anti-leukemic activities (Mechan et,al., 2006; Yasukawa *et.al*., 2002; Kupchan *et.al*., 1976). Wound healing capability of extract of *H.indicum* has been reported by Sriniwas et,al., 2000; Dodehe *et.al*., 2011a). Decoction of leaves and young shoots is used for the treatment of ring_worm, gonorrhoea pharyngitis and tonsillitis. Root is used for the treatment of night blindness (Ghani, 1998).
Tissue culture technique is now being utilized for the mass multiplication and germplasm conservation of endangered and threatened medicinal plants (Ajithkumar and Seeni, 1998; Prakash *et.al*., 1999). Changes in the life style, conversion of forest cover into urban and industrial development, release of polluted water in the open fields, brutal collection of medicine plants from their wild population all have perturbed the natural habitat leading to gradual extinction of several species in general and the medicinal plants in particular. *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species (Lui and Li *et.al*., 2001; Wawrosch *et.al*., 2001, Martin 2002 and 2003; Azad *et.al*., 2005; Faisal *et.al*., 2003; Hussan and Roy, 2005). The induction of callus will provide us the raw material from which secondary metabolites can be extracted and thus plant shall be conserved in the natural habitat? Likewise *in vitro* callus induction techniques may be exploited at commercial scale. This will be an important source for the supply of the needed secondary metabolites which shall not be affected by climatic or biotic stress.

2. Materials and Methods

2.1 Culture medium

The nutrient culture medium consisting of MS salts and Vitamins + 3% Sucrose was supplemented with 0.5- 5.0 mg/l 2, 4-D, IBA and 0.5-1.0 mg/l either alone or in combination and gelled with 0.8% agar (Hi –Media). The pH of the medium was adjusted to 5.8 before addition of dissolved agar powder, and autoclaving. Above medium was dispersed in culture tubes and flasks 20 ml and 30ml. These culture medium containing tubes and flasks were plugged with cotton plugs and after wrapping with Aluminium foil were autoclaved at 121^0C at 15lb pressure for 18 minutes. These tubes and flasks were taken out and were stored in freeze after cooling.

2.2 Explants

Healthy young branch of *Heliotropium indicum* was collected from wild growing mature plants in the University campus of Muzaffarpur,Bihar,India. Leaves were separated and internodal segments were cut from the stem. Both explants were washed thoroughly under running tap water for 30 minutes followed by treatment with detergent teepol (5% W/V) for 5 minutes and were then surface sterilized with 70% alcohol for 30 sec and 0.1% W/V mercuric chloride solution for 4-5 minutes. Above explants were washed with sterile distilled water (3-4 washes, 5 min each), to remove even the trace of the chemical from the explants.

Above surface disinfected explants were inoculated in the culture medium in the aseptic condition of the Laminar flow chamber and cultures were incubated at 26± 1^0 C . The cultures were observed on alternate day and tubes showing any contamination were removed.

3. Result

Table-1: Showing impact of 2,4-D , IBA and BAP supplemented in MS basal medium on induction of callus in different explants (internodal segment) of *Heliotropium indicum.*

Explants	Growth Regulators Mg/l			% of explants showing callus induction + SE	Degree of callus formation	Status of callus colour and texture
Internodal segment	2,4-D	IBA	BAP			
	0.5	-	-	28.8 ±1.50	+	Greywhite,loose
	1.0	-	-	58.4± 1.54	+ +	White&compact
	1.5	-	-	78.6±1.56	+++	White&compact
	2.0	-	-	54.2±1.52	++	White&compact
	2.5	-	-	26.2±1.50	+	Grey &loose
	-	0.5	-	20.6±1.32	+	Grey &loose
	-	1.0	-	36.4±1.34	+	White&loose
	-	1.5	-	56.2±1.38	++	White&compact
	-	2.0	-	32.5±1.32	+	White&loose
	-	2.5	-	18.8±1.30	+	Grey &loose
	0.5	-	0.5	33.4±1.34	++	White&loose
	1.0	-	0.5	66.8±1.48	+ ++	White&compact
	1.5	-	0.5	82.6±1.52	++++	Grey &loose
	2.0	-	0.5	62.5±1.44	+++	White&compact
	2.5	-	0.5	32.2±1.34	++	Grey &loose
	-	0.5	0.5	26.8±1.32	+	Grey &loose
	-	1.0	0.5	38.2±1.34	+ +	White&loose
	-	1.5	0.5	60.4±1.38	+++	White&compact
	-	2.0	0.5	36.5±1.32	++	White&loose
	-	2.5	0.5	24.6±1.32	+	Grey &loose
	0.5	-	1.0	38.6±1.36	++	White&loose
	1.0	-	1.0	73.4±1.44	+ ++	White&compact
	1.5	-	1.0	89.8±1.46	++++	White&compact
	2.0	-	1.0	67.2±1.42	+++	White&compact
	2.5	-	1.0	37.5±1.36	++	White&loose
	-	0.5	1.0	32.4±1.36	++	White&loose
	-	1.0	1.0	46.6±1.40	+ ++	White&compact
	-	1.5	1.0	68.8±1.46	+++	White&compact
	-	2.0	1.0	45.2±1.40	++	White&compact
	-	2.5	1.0	26.6±1.32	+	Grey & loose

Table-2:Showing impact of 2,4-D , IBA and BAP supplemented in MS basal medium on induction of callus in different explants (Segment of leaf) of *Heliotropium indicum.*

Explants	Growth Regulators Mg/l			% of explants showing callus induction + SE	Degree of callus formation	Status of callus colour and texture
Segment of leaf	2,4-D	IBA	BAP			
	0.5	-	-	24.6±1.28	+	GL
	1.0	-	-	44.2±1.38	+ +	GC
	1.5	-	-	62.8±1.40	+++	WC
	2.0	-	-	38.4±1.38	++	WC
	2.5	-	-	18.6±1.22	+	GL
	-	0.5	-	17.8±1.24	+	W
	-	1.0	-	29.2±1.26	+	W
	-	1.5	-	40.6±1.26	++	WL
	-	2.0	-	27.5±1.24	+	W
	-	2.5	-	14.4±1.24	+	G
	0.5	-	0.5	26.8±1.34	++	W
	1.0	-	0.5	46.2±1.32	+ ++	WL
	1.5	-	0.5	68.4±1.36	++++	WC
	2.0	-	0.5	42.6±1.34	+++	WL
	2.5	-	0.5	22.5±1.32	++	W
	-	0.5	0.5	20.8±1.22	+	W
	-	1.0	0.5	34.6±1.24	+ +	WL
	-	1.5	0.5	44.2±1.24	+++	WL
	-	2.0	0.5	31.4±1.22	++	W
	-	2.5	0.5	18.6±1.22	+	W
	0.5	-	1.0	34.6±1.32	++	WL
	1.0	-	1.0	46.8±1.34	+ ++	WC
	1.5	-	1.0	70.4±1.38	++++	WC
	2.0	-	1.0	48.8±1.38	+++	WL
	2.5	-	1.0	28.6±1.32	++	WL
	-	0.5	1.0	26.6±1.28	++	W
	-	1.0	1.0	38.5±1.26	+ ++	WL
	-	1.5	1.0	54.8±1.28	+++	WC
	-	2.0	1.0	38.4±1.24	++	WL
	-	2.5	1.0	22.5±1.22	+	W

Key:

± = Standard Deviation
W= White Outgrowth
WL= White & Loose
WC=White & Compact
GL= Grey & Loose
WGC= White, Green& Compact

\+ = Very low density
++ = Low density
+++ = Moderate density
+++++ = High density

The induction of callus from internodal (Table 1) and leaf explants (Table 2) of *Heliotropium indicum* L occurred on MS basal medium supplemented with 2, 4-D, IBA and BAP. Here 2, 4-D promoted callus induction either alone or with BAP. This was also observed in case of IBA, within 5-6 weeks. It was noted that 2, 4 D alone at 1.5 mg/l concentration induced callusing in internodal explants which was 78.6% (Table 1 and Fig a & b) and 62.8% in leaf explants (Table 2 and Fig c, d & e). Similarly, 2,4-D at the same concentration induced callusing in leaf explants that was 62.8% and IBA induced callusing in leaf explants that was 40.6% only(Table 2). Percentage of callusing in internodal segment was 89.8(Table 1) and in leaf explants was 70.4% when 1.5 mg/l 2, 4-D + 1.0mg/l BAP. At the same combination and concentration IBA & BAP induced callusing in internodal and leaf explants that was 68.8 and 54.8% respectively. The rate of growth at these concentrations was the highest and the calli were white and compact. Both the lowest and the highest concentration had less inducing impact.

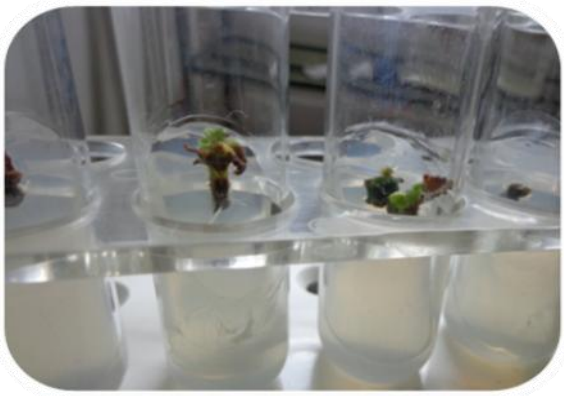

Figure A

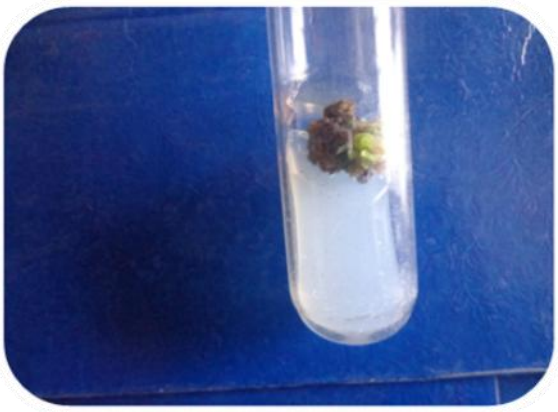

Figure B

Figure C

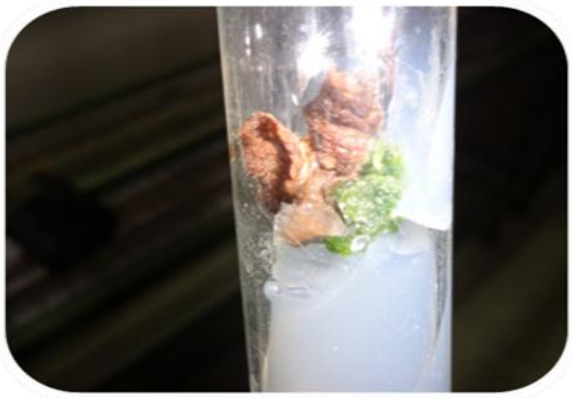

Figure D

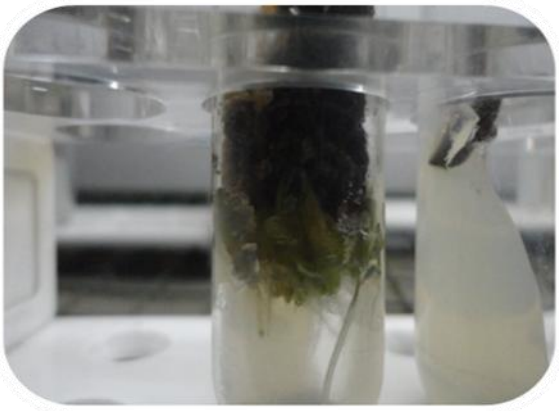

Figure E

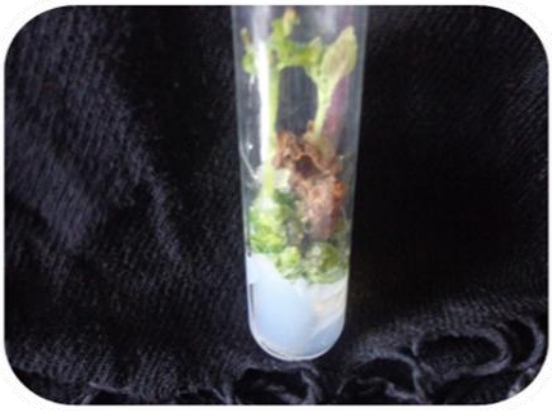

Figure F

Figure G

Figure H

4. Discussion

Hassan *et.al*.,(2010); reported micropropagation from nodal explants through axillary shoot proliferation in *H.indicum*. Hassan *et.al*.,(2010); also used apical buds for micropropagation. The present study explains induction of callus on internodal and leaf explants of *H.indicum*.MS medium supplemented with auxins 2, 4-D, IBA alone or with cytokinin BAP. From the table one and two it is evident that the explants revealed different response with respect to callus induction at different concentration & combination with 6-Benzyle amino purine the calli were grey, white, yellow or green. Similarly, there growth pattern was different. Explants cultured *in vitro* may be involved in organogenesis and develop shoots or roots depending on the morphogenetic potentiality of the cells (Fig F,G &H).There are three distinct stages during organogenesis namely, differentiation induction of organogenesis pathway and development of organs Bottino *et.al*., (1979). In the present study it has been observed that induction of callus depend on different concentration, kinds of the growth regulator and type of explants used from the tables 1&2. It is apparent that internodal explants revealed better callusing than callus induction from the leaf explants.IBA either alone or with BAP was found less effective with respect to induction of callus in both the internodal or leaf explants of *Heliotropium indicum* than 2,4-D in the present study. Better induction of callus was noted on MS medium supplemented with 2,4-D 1.5mg/l + 1.0 mg/l BAP for both the internodal or leaf explants. Thus, 2,4-D was best source of auxin for callus induction with BAP for both the explants used in the present study. The discrepancy among the explants with respect to induction of callus in both the explants in similar condition may be due to endogenous concentrations of the auxins and potential of the cells. This may be true for the growth rate and texture of the callus. Our observation corroborate with the findings of Bagadeker and Jaya Raj (2011).Dutitis et al; (1975).

5. Conclusion

Heliotropium indicum L.is an important medicinal herb. It grows in wild condition and is being utilized for the treatment of different diseases. Wound healing compounds have been extracted from the plants by Annelise *et.al*., 2011; Duttagupta and Dutta; 1977; Dodehe *et.al*., 2011b; Kumar *et.al*., (2007). Its extract has been used in several other experiments which also includes bacterial growth (Meehan *et.al*., 2006). Due to this the species is brutally exploited in its natural habitat. However, if the chemicals are extracted from the callus, the species shall be conserved in its natural population. Therefore the present finding may be utilized at commercial scale to protect the species.

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