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DIRECT ORGANOGENESIS FROM LEAF EXPLANTS OF *HELIOROPIUM ZEYLANICUM* (BURM.F.)

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ABSTRACT

Protocol for the micropropagation of traditional medicinal plant *Helioropium zeylanicum (Burm.F.)* Lam from leaf segments were developed. Proliferated micro shoots of *Helioropium zeylanicum* were leaf segment explants were inoculated on MS basal medium containing 0.8% agar, 3.0% sucrose and different concentration of cytokinim and combination with Auxin maximum number of multiple shoots was obtained on medium supplemented with BAP (3mg/l) and combination with NAA (0.05mg/l). The *in vitro* regenerated shoots were transferred to rooting medium fortified with different concentration of IBA (1-3 mg/l). Best rooting obtained with (2 mg/l) IBA. The rooted plantlets were successfully hardened and establishment in the soil where they grew normally without showing any morphological variation.

Keywords: *Heliotrpium zeylanicum*, Leaf explants, Boraginacece.

INTRODUCTION

Heliotropium zeylanicum (Burm.F.) Lam, a multipotent medicinal herb belonging to the family Boraginacece has a wide range of medicinal values. Tissue culture techniques are being used globally for the conservation of plants and also to produce secondary metabolites of medicinal importance. The present study was to establish protocol for plant regeneration and leaf in *Heliotropium zeylanicum. (Burm.F.) Lam*, additionally; the plants grow in the wild only for few months and not available throughout the year, hence studies offer availability of raw material throughout the year. It has been used traditionally, like it is useful for local applications for ulcers, sores, wound, skin infections, stings of insects. The plant is bitter, astringent, thermogenic, emollient, diuretic and vulnerary. Leaves and stems are used to treat Sores, ulcers, throat infections. Insect stings and snake bites. The leaves are bitter, in fever, ulcers, wounds, local inflammation and gonorrhea. The roots are useful, astringent, aphrodisiac, expectorant, febrifuge and ophthalmic [1,2]. Keeping all this in mind the present work is under taken to multiplication of this plant as to provide raw material for medicinal use by extracting drugs from secondary metabolites.

MATERIALS AND METHODS

Wild plants were collected and grown in the experimental Narthamalai, Pudukottai District for the resource of explant. Leaf were selected as explants for the direct organogenesis. Explants were washed in running tap water for 30 min and few drops of liquid detergent (1% labolene, Qualigens, India) for 15 min. Then after thoroughly washed explants were surface sterilized with 2% Carbendezim solution. They were then sterilized with 70% alcohol for 1min and followed by immersing in 0.1% of HgCl solution for 3-5 min. At the final step surface sterilized explants were rinsed thoroughly 4-5 times with autoclaved distilled water. The sterilized explants were inoculated on MS Murashige Skoog (1962) [3]. Basal media gelled with 0.8% agar (Himedia). The medium was supplemented with 3% (w/v) sucrose (Himedia) as carbon source. Various growth regulators, such as 6- benzyl adenine purine (BAP), Kinetin (KN), Indole-3-butyric acid (IBA) and2!-naphthalene acetic acid (NAA) were supplemented with MS medium at various concentrations /combinations. The pH of the medium was adjusted to 5.6 prior to autoclaving at 121° °-2 -1C for 30 min. All the cultures were incubated at 25±2C under 16hrs. photoperiod with light intensity of 60 µMol m s by white fluorescent tubes. The relative humidity was maintained at 55-60%.

Multiple shoot induction

The leaf were cultured on MS medium supplemented with cytokinins BAP (6-Benzyl adenine purine) at concentrations of (1 to 3mg/L), KN (Kinetin), Ads at concentrations of (1 to 4mg/L) and auxins IAA and NAA (Naphthalene acetic acid) at concentrations of

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(0.05mg/L).

Root induction

The well-developed multiple shoots were excised and were transferred to MS medium supplemented with IBA (Indole 3- butyric acid) at concentrations of (1 to 3mg/L), .5% activated charcoal

Statistical analysis

The culture tubes were observed periodically, results were recorded now and then in terms of number of culture responding, number of shoots. and length of the shoot. Each treatment consisted of 30 explants and the experiment was repeated thrice, observed response was calculated for frequency (mean ±SD).

RESULTS AND DISCUSSION

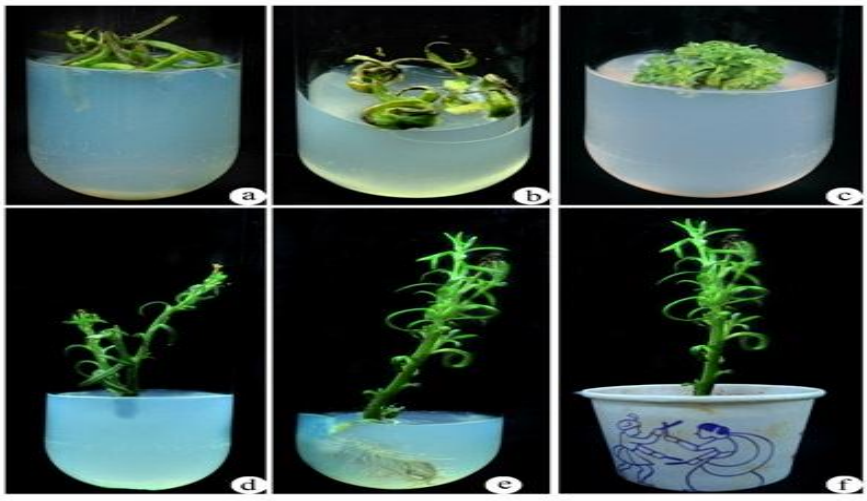
The leaf explants was grown on MS media containing BAP, Kn and AdS in different concentration ranging from 1 to 4mg/L (Table1,2,3,4, plate1). The PGRs were studied separately for their potential of shoot induction from the leaf explants. It is found that BAP in the concentration of 3mg/L had the highest response (93%) compared to Kn and Ads. Studies with *Tanacetum cinerariifolium* , *Solanum surattenese* [4], *Solanum nigrum* [5-7], *Garcinia indica* [8] and *Aquilaria malaccensis* [9] too had good shoot induction with BAP, but the percentage was comparatively lower than reported in this study. We also wish to note that TDZ was effective in direct shoot regeneration of leaf explants in *Gaultheria frantissima* [10]. It is noted that Kn had substantial growth response (73 to 88%) till concentration of 3mg/L but on higher concentration (4m/L) there was a drop in shoot induction (36.6%). Studies with *Stevia rebaudiana* [11] showed that BAP +Kn + IAA in combination had better shoot generation. Ads had a moderate shoot induction potential.

The induced shoots were further subjected to differentiation using PGRs in combinations (Table 2. Plate1) i.e. BAP, Kn and AdS with NAA and IAA. Comparatively it is found that BAP (3mg/L)+NAA (0.05mg/L) had highest response (95%) compared to other combination. Similar results were also seen in *Solanum surattenese* [4]. While studies with *Tanacetum cinerariifolium* [5], had similar results but shoots proliferate at lower concentration of BAP and higher concentration NAA. While on the other hand *Arachis hypogae* [12] showed better response in higher concentration of BAP and NAA. Contradictorily on the other had similar studies with *Solanum nigrum* [5] it was observed that BAP+IAA had better response. In this study AdS in combination with both NAA and IAA had the least response especially at 4mg/L.

The differentiated shoots were transferred to MS was media containing GA_3 in combination with BAP (0.05mg/L) for shoot elongation and the ideal concentration of $GA_3$1.5 mg/L (6..1±0.1cm) compared to other concentrations studied (Table 3 Plate1) later the elongated shoots were grown in MS media containing IBA, IAA and NAA alone in different concentration for root induction (Table 4, plate1) and it was found that IBA (2mg/L) had the highest response potential (8.3±9.0 roots/explants) than IAA or NAA. Similar results were obtained by Sidhar and Naidu (2012) [6] in *Solanum nigrum and* Preethi *et al*, (2011) [11] n *Stevia rebaudiana* using IBA respectively. On the other hand *Arachis hypogae* [12] and *Garcinia indica* [8] produced roots in MS media with BAP and NAA as supplement. Contradictorily *Tanacetum cinerariifolium* [5], produced more roots in B5 media with NAA as supplement compared to this study. The least response was seen in NAA (4 mg/L) with only around 1 root/explants. Moreover [5] too reported similar results with shoot and root development with the same combinations done in this study. On the other hand IAA alone was able to induce rooting in *Aquilaria malaccensis* [9].

Plate - 2

Direct organogenesis from leaf explants



a) Curving
b) Shoot induction
c)Multiple shoot formation
d) Shoot elongation
e) Rooted plantlet
f) Hardening

Table 1. Effects of different concentration of cytokinin shoot induction of *H. zeylanicum* (Burm.F) Lam.

PGR (mg/L)	Explants				
	Leaf				
	No of explants culture	No of explants responded	% of response	No of shoot bud /explants	Shoots length (±SD)
BAP					
1	150	120	80.0	5.6±0.40	2.6±0.47
2	150	130	86.6	6.6±0.40	3.6±0.40
3	150	140	**93.3**	**9.0±0.00**	**5.0±0.20**
4	150	111	74.0	4.6±0.47	1.6±0.40
Kn					
1	150	110	73.3	4.0±0.00	1.3±0.44
2	150	120	80.0	5.0±0.81	2.8±0.68
3	150	132	**88.0**	**5.3±0.40**	**3.3±0.42**
4	150	55	36.6	3.3±0.40	1.0±0.00
AdS					
1	150	100	66.6	4.6±0.4	1.3±0.22
2	150	112	74.6	5.3±0.4	1.6±0.44
3	150	115	**76.6**	**6.0±0.8**	**2.0±0.87**
4	150	108	72.2	4.3±0.1	0.6±0.44

Table 2. Effects of different cytokinin in combination with NAA& IAA on Shoot induction of *H. zeylanicum* (Burm.F) Lam.

PGR (mg/L)	Explants				
	Leaf				
BAP + NAA 0.05	No of explants culture	No of explants responded	% of response	No of shoot bud	Shoots of length (±SD)
1	150	125	83.3	4.3±0.4	3.3±0.47
2	150	132	88.0	4.6±0.4	3.6±0.4
3	150	143	**95.3**	**8.6±0.4**	**5.0**±0.8
4	150	118	78.6	2.0±0.0	1.4±0.3
BAP + IAA 0.05					
1	150	100	66.6	4.0±0.4	2.0±0.8
2	150	105	70.0	4.3±0.4	3.3±0.4
3	150	110	**73.3**	**5.0±0.0**	**3.6±0.4**
4	150	99	66.0	3.0±0.0	1.0±0.0
Kn + NAA 0.05					
1	150	90	60.0	3.3±0.4	2.3±0.94
2	150	100	66.6	3.6±0.47	2.6±1.04
3	150	120	80.0	**4.0±0.0**	**4.3±0.4**
4	150	80	53.3	2.0±0.0	1.4±0.4
Kn + IAA 0.05					
1	150	88	58.6	2.6±0.47	1.3±0.4
2	150	99	66.6	3.6±0.4	2.3±0.4
3	150	110	73.3	**4.0±0.81**	**4.0±0.0**
4	150	77	51.3	1.8±0.4	1.0±0.0
AdS +NAA 0.05					
1	150	31	20.6	1.3±0.4	2.0±0.8
2	150	35	23.3	1.6±0.4	3.6±0.4
3	150	45	30.0	**2.6±0.4**	**4.0±0.8**
4	150	28	18.6	1.2±0.0	1.1±0.0
AdS + IAA 0.05					
1	150	40	26.6	0.0±0.0	1.3±0.4
2	150	50	33.3	0.0±0.0	2.3±0.4
3	150	80	53.3	**1.6±0.8**	1.6±0.4
4	150	29	19.3	0.0±0.0	0.0±0.0

Table 3. Effects of different concentration of GA_3 with BAP 0.05 on Shoot elongation of and Auxins on root induction *H. zeylanicum* (Burm.f) Lam.

PGR (mg/L) Concentration		Leaf explants	
		Shoot elongation cm	Number of roots (Mean ±SD)
GA_3	BAP		
0.5	0.05	4.1±0.0	-
1.0	0.05	5.2±0.7	-
1.5	0.05	**6.1±0.1**	-
2.0	0.05	5.5±0.0	-
2.5	0.05	4.9±0.1	-
3.0	0.05	3.3±2.1	-
IBA			
1	-	-	7.6±0.5
2	-	-	**8.3±0.9**
3	-	-	6.0±0.0
4	-	-	4.3±0.5
IAA			
1	-	-	4.6±0.5
2	-	-	**5.0±0.0**
3	-		4.6±0.3
4	-	-	4.0±0.0
NAA			
1	-	-	3.3±0.3
2	-	-	**4.3±0.0**
3	-		2.4±0.4
4	-	-	1.3±0.3

CONCLUSION

Direct organogenesis leaf explants on MS medium supplemented with the combinations of auxins and cytokinins. Shoot induction and multiple shoot was high when leaf is used as an explant. Charcoal was added to the rooting media to minimize the inhibitory actions of phenolic compounds. *In virtro* plant was induced by culturing the leaf on MS medium with combinations of auxins and cytokinins. The in vitro grown rooted plantlets and later transferred for hardening in a plastic containers and their survival rate is up to 85%. The plantlets described in the present study may have practical values in wide hybridization and fertilization studies of *Heliotropium zeylanicum. (Burm.F.) Lam.*

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