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Research Article

Establishment of *in vitro* aseptic culture of *Eclipta alba* (L.) Hassk. - A traditional hair growth promoting plant

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Abstract

Eclipta alba was collected from natural habitat and established at LSS, Gandhigram Trust, Dindigul, Tamilnadu. Explants collected from the established plants were used for micropropagation under *in vitro* conditions. Explants of node and shoot tip can be used for initiation *in vitro* aseptic culture of *E. alba.* There was no much differentiation between nodal and shoot tip explants in BA treatment. However, a significant difference in the response to KN by nodal and shoot tip explants was noticed. Among the different concentrations of BA and KN studied, MS medium supplemented with BA 1.0 mg/l favored for shoot multiplication from node and shoot tip of *E. alba.* Among the three different auxins (IAA, NAA and IBA) studied for root initiation, the half strength MS medium supplemented with IBA 0.50 mg/l favored highest rooting of *in vitro* initiated shoot.

Keywords: *Eclipta alba*, micropropagation , nodal and shoot tip explants.

Introduction

There is a growing focus on the importance of medicinal plants and traditional health care systems in solving the health care problems of the world. Due to the indiscriminate harvesting, the wild populations of many medicinal plant species, forming the major resource base for the herbal industry, are facing a serious threat of extinction. India, one among the largest producers of medicinal herbs and appropriately called the botanical garden of the world, is in the 10th position of the world's plant diversity wealth, which is distributed across 16 agro-climatic zones. Today, medicinal plants are important to the global economy, as approximately 80% of traditional medicine preparations involve the use of plants or plant extracts (Dhyani and Kala, 2005). The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics has highlighted the need for conservation and propagation of medicinal plants. In India, about 1,000 plants have been used in the traditional systems of medicine, while tribals use more than 7,500 plant species for medicinal purposes. The major concern is that most of the medicinal plants are collected from the wild populations, and over 70% of the plant collection involves destructive harvesting mainly because of the use of plant parts like leaf, bark, wood and whole plants. Medicinal plants are of great concern to the re searches in the field of biotechnology not only for rapid propagation but also for production of valuable secondary metabolites.

Eclipta alba (L.) Hassk (Asteraceae) commonly known as False Daisy and "Karisalankanni" in Tamil, is a perennial herb usually found in moist ground in tropical and sub-tropical regions in India, Nepal, Sri Lanka, Indonesia, Philippines, Malaysia and some parts in Africa and South America. *E. alba* has been traditionally used to check hair loss and stimulate hair growth. The extracted juice if taken internally and applied to the scalp blackens the hair (Chopra *et al.*, 1955; Roy *et al.*2001,2007);Datta *et al.*, 2009,2010). Several researchers reported the biological activities of *E. alba* extract, antifungal (Venkatesan and Ravi, 2004), anti-bacterial (Sunita *et al.* 2010) analgesic

(Sawant *et al.*,, 2004), antihyperglycemic (Ananthi *et al.*,, 2003), hepatoprotectiv (Saxena and Singh,1993;Singh *et al.*,2001) and anti-cancer (Chaudhary et al.,2011). Wedelolactone and dimethylwedelolactone are the main active principles of *E. alba* (Wagner and Fessler *et al.*,1986; Nakaguchi *et al.*,2001;Syed *et al.*,2003). Four new taraxastane triterpene glycosides, namely eclalbasaponins VII-X along with eclalbasaponins I–VI were isolated from dried whole plants of *E. alba* (Yahara *et al.*, 1997).

It is important to develop an efficient micropropagation technique for *E. alba* to rapidly disseminate superior clones once they are identified. There are a few reports on *in vitro* propagation of *E. alba,* MS supplemented with Kinetin 0.05 mg/l produced maximum number of shoots (Borthakur *et al.*, 2000). Neeti and Kothari (2005) reported that the maximum shoot proliferation occurred when the explants were cultured on MS medium supplemented with BA 1 mg/l. The earlier reports available on *E. alba* demonstrated plant regeneration through axillary nodes, internodes and young leaves on media with very high concentrations of cytokinin (Baskaran and Jayabalan, 2005). In the present study, shoots were regenerated from excised shoot tips and nodal segments of *E. alba* on cytokinins supplemented medium.

Materials and Methods

Investigations on the *in vitro* studies on *Eclipta alba* were carried out at Plant tissue Culture laboratory, Gandhigram Trust, Dindigul, Tamilnadu. The chemicals used during the course of the study were of analytical grade. Inorganic salts were obtained from “Qualigens” and “S. D. fine chemicals”, (India). All vitamins, growth regulators, and Agar-agar were obtained from “Sigma Chemical Co.”, (USA) or “HiMedia Laboratories”, (India). Sucrose was obtained from “Qualigens” and “S. D. fine chemicals”, (India).

Preparation of plant tissue culture media and aseptic manipulation

The compositions of various media used for *in vitro* studies of *E. alba* are given in table 1. Double distilled water was used for the preparation of culture media. After addition of all constituents of media, the pH was adjusted to 5.8 using 0.1 N KOH or 0.1 N HCl. Gelling agents (agar-agar) was added as per requirement and the medium was steamed to melt the gelling agent. It was then dispensed into test tubes (20 ml per tube) or Erlenmeyer flasks (50 ml per 250 ml flask) or screw capped bottles (50 ml per 350 ml bottle) and was autoclaved at 121℃ at a pressure of 1.1 kg cm^{-2} for 20 min.

All aseptic manipulations like surface sterilization, preparation and inoculation of explants and subsequent subculturing were carried out in clean airflow chamber, which was also cleaned by spraying and swabbing with 70% ethanol and by switching on the UV-system for 20 minutes before commencement of inoculation. The forceps, scalpels, other tools and glassware were autoclaved at 121 ℃ for 20 minutes. During the course of inoculation and between each inoculation process the surgical blades and forceps used in the process of transfer of explants and plant materials were dipped in 70% alcohol for 15 to 20 seconds and flamed before use.

Explant collection

Explants such as nodes (2.0 to 2.5 cm), shoot tips were collected from healthy plants established in the campus. The explants were washed with running tap water for 30 min and washed with detergent solution (Lyzol 2%) for 5 min. Later they were dipped in 70% Alcohol for 30 seconds, washed with sterile distilled water, treated with 0.5% Sodium hypochlorite for 5 min, surface sterilized with $HgCl_2$ (0.1%) for 3 min, and finally washed with sterile distilled water 5 times for 15 minutes to remove all traces of $HgCl_2$.

Culture medium preparation

The nutrient media contained inorganic nutrients, carbon source and organic supplements in addition to vitamins and growth regulators. *In vitro* propagation of *E. alba* was evaluated in MS medium (Murashige and Skoog, 1962). Growth regulators such as BA, KN, IBA, IAA and NAA were supplemented to the media individually and in combination at different concentrations. The pH of the media was adjusted to 5.8 with 0.1N NaOH before autoclaving at 1.06 Kg cm^{-2} and 121 ℃ for 15 minutes. All culture media contained 3% sucrose (w/v) and for solid support Agar agar (Bacteriological grade, HiMedia, India) (Table 1).

Table 1. Composition of MS Media used in the *in vitro* studies of *E. alba*

MS Media compositions	Shoot initiation and multiplication	*In vitro* rooting
Macro and micro elements (g/l)		
NH_4NO_3	1 650	825
KNO_3	1 900	950
$CaCl_2.2H_2O$	440	220
$MgSO_4.7H_2O$	370	185
KH_2PO_4	170	85
KI	0.83	0.415
H_3BO_3	6.2	3.1
$MnSO_4.4H_2O$	22.3	11.15
$ZnSO_4.7H_2O$	8.6	4.3
$Na_2MoO_4.2H_2O$	0.25	0.125
$CuSO_4.5H_2O$	0.025	0.0125
$CoCl_2.6H_2O$	0.025	0.0125
Iron supplements(g/l)		
$FeSO_4.7H_2O$	27.8	13.9
$Na_2EDTA.2H_2O$	37.3	18.65
Organic elements(mg/l)		
Myo-inositol	100	50
Nicotinic acid	0.5	0.25
Pyridoxine-HCl	0.5	0.25
Thiamine-HCl	0.1	0.05
Glycine	2	1
#Sucrose (g/l)	30	15
Growth regulators(mg/l)		
BA	0-2	-
KN	0-2	-
NAA	0-2	0-2
IAA	0-2	0-2
IBA	-	0-2

#added freshly

Explant inoculation and maintenance

Explants were placed vertically in glass tubes (150 × 25 mm) containing 20 ml of culture medium and plugged tightly with non absorbent cotton and in bottles (300 ml) containing 50 ml of culture medium with a plastic cap. All cultures were maintained at 23 ± 1 ℃ with a relative humidity of 60-65%, 2000 – 3500 Lux light intensity (Cool white fluorescent lamps) and 16 hrs light and 8 hrs dark cycles. Explants were subcultured in every 4 weeks interval. Each data were scored after 21 days for multiple shoot induction and rooting.

Shoot and Root Initiation

Surface sterilized explants were inoculated on MS Medium (Murashige and Skoog, 1962) supplemented with different concentrations of BA (0.0, 1.0, 2.0 mg l^1) and KN (0.0, 1.0, 2.0 mg l^{-1}) alone for shoot initiation. Fully established shoots (~ 2.0 cm length) were inoculated on ½ strength MS media and supplemented with IBA, NAA and IAA at different concentrations (0.25, 0.5, 1.0mg l^{-1}) containing 0.8% agar for rooting.

Acclimatization

The rooted plants were removed from the culture medium and the roots were washed in sterile distilled water to remove all traces of gelling agent / vermiculite. Then the shoots were placed on vermiculite supports in paper cups containing MS basal salts solution (1/8 strength) devoid of sucrose and myo-inositol for 20 days.

Statistical analysis

All the results were subjected to analysis of variance (ANOVA) using SPSS version 14.0. Wherever necessary, treatment means were separated using Duncan's Multiple Range Test (DMRT) (Duncan, 1955).

Results and Discussion

The present study was conducted to investigate the micropropagation technique for producing more number of plants in a short duration. Effect of explant and concentrations of plant growth regulators were studied for efficient mass multiplication of *E. alba*. For the initiation of multiple shoots, use of different explants and growth regulators were studied. This was followed by rooting of *in vitro* raised shoots using different auxins in agar (Table 2 and 3).

The results obtained on the experiments conducted to evaluate the effect of BA on multiplication of *E. alba* are presented in table 2. As an initial step, the nodal explants were cultured on MS medium supplemented with different concentrations of BA.

Table 2. Effect of Butyric Acid and Kinetin on multiple shoot induction of *Eclipta alba*

Concentration (mg/l)	Shoot initiation (%)		Number of Shoots		Shoot length (cm)	
	Nodal explant	Shoot tip explant	Nodal explant	Shoot tip explant	Nodal explant	Shoot tip explant
BA (0.0)	24.1^{d}	22.1^{d}	1.1^{c}	1.0^{c}	1.3^{b}	1.2^{c}
BA(0.5)	84.1^{b}	83.3^{b}	2.5^{a}	2.5^{a}	2.3^{a}	2.6^{b}
BA(1.0)	86.3^{a}	86.6^{a}	2.6^{a}	2.6^{a}	2.2^{a}	2.9^{a}
BA(2.0)	66.6^{c}	63.3^{c}	2.1^{b}	2.1^{b}	2.1^{a}	2.6^{b}
KN (0.0)	22.1^{c}	22.1^{d}	1.1^{b}	1.0^{b}	1.3^{b}	1.2^{b}
KN(0.5)	51.3^{b}	78.3^{b}	2.1^{a}	2.1^{a}	2.3^{a}	2.1^{a}
KN(1.0)	63.3^{a}	82.6^{a}	2.3^{a}	2.3^{a}	2.3^{a}	2.3^{a}
KN(2.0)	52.1^{ab}	53.1^{c}	2.2^{a}	2.1^{a}	2.1^{a}	2.3^{a}

BA: Butyric acid, **KN:** Kinetin. Experiment conducted on MS basal medium Results were recorded after 21 days of culture. In each column, mean followed by the same letter were not significantly different ($p \leq 0.05$) according to DMRT.

Table 3. Effect of different form of auxins on root induction in *Eclipta alba*

Concentration (mg/l)	Rooting response (%)	No. of Roots	Root length (cm)	Shoot length (cm)
IBA (0.0)	43.1^{d}	2.1^{d}	1.6^{d}	3.1^{c}
IBA (0.25)	61.6^{c}	3.1^{c}	3.1^{c}	8.6^{b}
IBA (0.50)	83.3^{a}	3.6^{a}	5.3^{a}	9.6^{a}
IBA (1.00)	71.1^{b}	3.0^{b}	4.6^{b}	8.6^{b}
IAA (0.0)	43.1^{d}	2.1^{c}	1.6^{c}	3.1^{c}
IAA (0.25)	53.6^{c}	2.6^{a}	2.6^{b}	6.1^{b}
IAA (0.50)	73.1^{a}	2.6^{a}	3.3^{a}	6.6^{a}
IAA (1.00)	62.3^{b}	2.3^{b}	2.6^{b}	6.1^{b}
NAA (0.0)	43.1^{c}	2.1^{c}	1.6^{c}	3.1^{c}
NAA (0.25)	43.1^{c}	2.1^{c}	2.1^{b}	5.1^{b}
NAA (0.50)	71.6^{a}	2.3^{a}	2.6^{a}	5.6^{a}
NAA (1.00)	53.1^{b}	2.6^{b}	2.1^{b}	5.3^{b}

In vitro grown shoots (21 days old) were inoculated to ½ MS medium supplemented with respectful amendments. Results were recorded after 21 days of culture. In each column, mean ± SD followed by the same letter were not significantly different ($p \leq 0.05$) according to DMRT.

Among the various concentrations used BA 1.0 mg/l favored shoot multiplication (86.3% shoot initiation, 2.6 shoots per explants and 2.2cm shoot length) strongly followed by 0.5, 2.0 and control. Increase or decrease in BA concentration considerably affected shoot initiation, multiplication and length. Basal callus formation was also observed in medium supplemented with BA 2.0 mg/l. As an initial step, the nodal explants were cultured on MS medium supplemented with different concentrations of KN. Among the various concentrations used KN 1.0 mg/l favored shoot multiplication (63.3% shoot initiation, 2.3 shoots/explants, 2.3cm shoot length) strongly followed by 2.0, 0.5 mg/l and control. The effects on multiple shoot induction of the cytokinins (BA and KN) were studied on node explants. BA was more effective than the KN in shoot initiation, shoots per explant and shoot length.

Effect of BA on multiple shoot induction from shoot tip explants of *E. alba* was studied and results are presented in table 2. MS medium supplemented with different concentrations of BA. Among the various concentrations used BA 1.0 mg/l showed highest shoot initiation (86.6%), number of shoots per explant (2.6) and shoot length (2.9cm). The *in vitro* induced shoots in all the concentrations showed similar morphogenic response.

Effect of BA on multiple shoot induction from shoot tip explants of *E. alba* was studied and results are presented in table 2. The explants were cultured on MS medium supplemented with different concentrations of KN. Among the various concentrations used KN 1.0 mg/l favored the shoot initiation (82.6%), shoot multiplication (2.3 shoot per explants) and shoot length (2.3cm). Increase or decrease in KN concentration considerably affected shoot initiation, multiplication and length.

The few earlier reports available on *E. alba* demonstrated plant regeneration through axillary nodes, internodes and young leaves on media with very high concentrations of cytokinin (Baskaran and Jayabalan 2005). Shoot cultures are well established in a wide range of plant species and can be used for the clonal propagation. In the present study, shoots were regenerated from excised shoot tips and nodal segments of *E. alba* on cytokinins supplemented medium. The protocol reported here could be used for the establishment *in vitro* culture of *E. alba* as well as conservation of this valuable medicinal herb. However, this study also required further refinement of the protocol along with in depth studies for field establishment.

Healthy shoots were excised from the culture tubes and cultured on half - strength MS medium containing different concentrations of IAA, IBA and NAA for rooting. The rooting frequency, number of roots per shoot and length of roots were recorded after 21 days of culture.

The rooting response to different auxin treatments is shown in table 3. Limited rooting response was observed in auxin free half - strength MS medium. Half strength MS medium composition alone was found to be sufficient for satisfactory rooting of the shoots. Percentage of root induction and number of roots per shoot were noticeably influenced by the concentration and type of auxin used. IBA was found to be more effective for root induction than IAA and NAA.

IBA at 0.5 mg/l concentration produced maximum rooting (83.3%) with 3.6 roots per shoot, 5.3 cm root length and 9.6 cm shoot height within 21 days. IAA at 0.5 mg/l concentration produced 71.3% rooting with a maximum of 2.6 roots per shoot, 3.3 cm root length and 6.6 cm shoot length. NAA at 2.0 mg/l concentration showed 71.6% rooting with a maximum of 2.3 roots per shoot, 2.6 cm root length and 5.6cm shoot length. Therefore, IBA was considered to be the best for rooting the *in vitro* grown shoots of *E. alba*.

Different auxins (IAA, IBA and NAA) were supplemented at various concentrations to ½-strength MS medium to study the rooting response of *in vitro* raised plantlets, varied effects were observed. Among the three auxins studied, IBA at 0.5mg /l concentration was most effective in inducing roots. IBA has been used to promote rooting in a wide range of plant species including *E. alba* (Borthakur *et al.*, 2000; Husain and Anis, 2006).

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