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Research Article

IN VITRO STUDIES ON CALLUS INDUCTION OF *ECLIPTA ALBA* (L.) HASSK: AN IMPORTANT PLANT OF TRADITIONAL MEDICINE

Shikha Bhansali [1]* and Ashwani Kumar [2]
[1]S.S Jain Subodh Girls College, Sanganer Jaipur, India
[2]Department of Botany, University of Rajasthan, Jaipur, India
*Corresponding Author Email: shikha.bhansali27@gmail.com

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ABSTRACT

An efficient method was developed for rapid and large-scale *in vitro* clonal propagation of the valuable medicinal herb *Eclipta alba* (Asteraceae). Different explants were tried, and best results were obtained by axillary shoot proliferation in cotyledonary nodal segments. The explant type, medium composition, various carbon sources, plant growth regulators markedly influenced *in vitro* propagation of *Eclipta alba*. An *in vitro* plantlet production system has been investigated on Murashige and Skoog (MS) medium. Callus profuse growth was obtained on MS medium augmented with 2,4-D (1.0 mg/l) and BAP (0.5 mg/l). This combination was selected to maintain the callus. BAP (1.0 mg/l) and NAA (0.1 mg/l) showed maximum shooting (16-20). Rooting was highest on full strength. Micropropagated plants were transferred to a mixture of garden soil, farmyard soil, and sand (2:1:1). These plants grew normally without showing any morphological variation.

Keywords: *Eclipta alba,* Asteraceae, Callus culture, drugs, traditional medicine.

INTRODUCTION

Eclipta alba (L.) Hassk.(Asteraceae) (synonym: *Eclipta prostrata* L.), a small, annual herb with white flower heads distributed in tropical and sub-tropical region of the world. *E. alba* is used as tonic, diuretic, catarrhal jaundice and for skin diseases[1]. It is a source of coumestan-type compounds wedelolactone and dimethyl wedelolactone are the main active principles of *E. alba*, They are used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis[2]. *E. alba* is widely used in India as a deobstruent in hepatic enlargement, cholagogne, jaundice and other ailments of the liver and gall bladder[3]. *In vivo* tests proved that wedelolactone neutralizes the lethal and myotoxic activities of rattlesnake venom[4].

Researches reviewed its phytochemical and pharmacological profile[5]. Recent researches have focused on enhancing the production of wedelolactone through cell suspension culture (CSC) in *Eclipta* alba (L.) Hassk[6]. Tissue culture techniques have been widely applied for the improvement of field crops, forests, horticulture, medicinal plants and plantation crops. This technique can be used for the enhanced production of plant secondary metabolites[7,8,9].

MATERIAL AND METHODS

Plant Material

Samples of *Eclipta alba* were collected from National Institute of Ayurveda and Amber hills area, Jaipur. It was identified and voucher specimen was deposited to the Herbarium, Botany Department, University of Rajasthan (*Eclipta alba* - RUBL-20837).

Selection of Explants

Only young, healthy and disease free plants were selected to obtain maximum shoot proliferation. Physiological age, overall quality and period of the year for collection of explants was kept uniform as far as possible. This facilitated maximum number of clonal propagules under *in vitro* conditions. Various explants (nodal segments and leaves) collected from *Eclipta alba* were used for tissue culture work on Murashige and Skoog's medium [10].

Aseptic Technique

Aseptic technique involves exclusion of all types of invading micro-organisms throughout the experimental procedure. These sterilization methods were used during all experimental manipulations and inoculations. The aseptic techniques are:

Sterilization of Glassware and Metalware

Glassware

During the present work all the glassware like beakers, test tube, pipettes, funnels, petri dishes and conical flasks were washed initially with lab detergents (Labolene-glaxo) and then rinsed with dilute chromic acid and then thoroughly washed with tap water. Following this, glasswares were rinsed once with sterilized distilled water and then they were kept in oven for drying for 8 to 10 hr at 120^0C. Prior to use all the glassware was autoclaved at 15 psi for 20 min.

Metalware

During the experiments all the stainless steel surgical instruments like scissors, scalpels, needles and forceps were also

sterilized. These instruments were usually sterilized by flaming on spirit lamp after dipping them in 95% alcohol.

Sterilization of Media

Plant tissue culture media was sterilized by autoclaving at121^{0}C and 1.05 kg /cm^2 (15 psi) for 20 min. Following this the media was allowed to cool and solidify at room temperature.

Sterilization of Inoculation Chamber

Laminar air flow chamber was sterilized by using ultraviolet radiation before the inoculation.

Sterilization of Various Explants

The different explants of the selected plant *Eclipta alba* such as leaves and nodes were thoroughly washed for few minutes under running tap water and subsequently they were rinsed with 0.2% tween 20 (mild detergent), then immersed in 70% ethanol (v/v) for 20 seconds. For surface sterilization, the explants were dipped in 0.01% aqueous solution (w/v) of $HgCl_2$ for 1 min and then they were washed in sterilized distilled water for 3-4 times till the sterilents were removed completely. Inoculation on the nutrient media was carried after the surface disinfection of explants was done. Such explants were inoculated into MS basal medium supplemented with 3% sucrose (w/v) (Table 1 and 2). Medium was solidified with 0.7% Difco Bacto-agar. The pH adjusted to 5.7 before autoclaving at 105 kPa for 20 min.

ESTABLISHMENT AND MAINTENANCE OF CULTURES

After inoculation, all the cultures were maintained in growth chamber with regulated temperature (26±2^{0}C), relative humidity (55±5%) and light conditions (16/8 hr photoperiod). Constant light of 2000-3000 lux intensity was provided in culture shelves by cool-white fluorescent tubes.

They were sub-cultured at an interval of 4 weeks interval. Thirty-day-old nodal segments were inoculated for shoot proliferation into MS basal medium supplemented with various concentrations of growth regulators.

Table-1: Murashige and Skoog (1962) medium

Stock soln.	Constituents	Conc. in stock soln. g/l	Vol. of stock soln. in final medium mg/l	Final conc. In medium mg/l
A	NH_4NO_3	82.50	20	1650.00
B	KNO_3	95.00	20	1900.00
C	H_3BO_3	1.24		6.20
	KH_2PO_4	34.00	5	170.00
	KI	0.166		0.83
D	$Na_2NoO_4.2H_2O$	0.25	1	0.25
	$CoCl_2.2H_2O$	0.025		0.025
E	$CaCl_2.2H_2O$	88.00	5	440.00
F	$MgSO_4.7H_2O$	74.00		370.00
	$MnSO_4.4H_2O$	4.46	5	22.30
	$ZnSO_4.5H_2O$	1.72		8.60
G	$CuSO_4.7H_2O$	0.025		0.025
	$FeSO_4.7H_2O$	5.57	1	27.85
H	Thiamine HCl	0.10		0.10
	Nicotinic acid	0.50		0.50
	Pyridoxine HCl	0.50	1	0.50
	Glycine	2.00		2.00
I	Meso-inositol	10.00	10	100.00

Table-2: Preparation of stock solutions for growth regulators

Growth regulator	Amount	Solvent (1ml)	Final volume	Final concentration
Indole 3-acetic acid (IAA)	10 mg	1 N NaOH	10 ml	0.5 mg/ml
Indole 3-butyric acid (IBA)	10 mg	1 N NaOH	10 ml	0.5 mg/ml
A-Naphthalene acetic acid (NAA)	10 mg	1 N NaOH	10 ml	0.5 mg/ml
2,4-Dichlorophenoxyacetic acid (2,4-D)	10 mg	C_2H_5OH	10 ml	0.5 mg/ml
6-Benzyl-amino purine ((BAP)	10 mg	1 N HCl	10 ml	0.5 mg/ml
6-Furfuryl amino purine (Kn)	10 mg	1 N HCl	10 ml	0.5 mg/ml

RESULTS

Induction, Establishment of Callus

Growth Media

Various media were used to observe callus growth and MS media showed maximum callus growth (Table-3). Further, investigation was carried out on MS medium incorporating various concentrations of auxins and cytokinin.

Table-3: Callus induction in *Eclipta alba* (L.) Hassk on different media

Media	Response
MS	C^{+++}
B_5	C^{++}

C: Callusing response, C^-: No callusing response, C^+: Slighting callusing, C^{++}: Moderate callusing, C^{+++}: Maximum callusing

Effect of Sucrose Concentration

MS media was supplemented with different concentrations of sucrose (1-6%) and control without sucrose was supplemented with inert sugar alcohol mannitol. Maximum callus induction was observed at 3% sucrose, hence the same concentration was used for the present study (Table-4). Control without sucrose showed no growth of the explant.

Table-4: Evaluation of sucrose concentration on callus induction in *Eclipta alba* (L.) Hassk

Sucrose level (%)	Response of callus induction
Control (Mannitol 1%)	Nil
1%	C^{+}
2%	C^{++}
3%	C^{+++}
4%	C^{++}
5%	C^{+}
6%	C^{+}

C: Callusing response, C^{-}: No callusing response, C^{+}: Slighting callusing, C^{++}: Moderate callusing, C^{+++}: Maximum callusing

Effect of Light

During the present study, effect of light intensity was observed. The range of light intensities was set between 1000-5000 lux. The best responsive of callus growth was observed at 3000 lux light intensity (Table-5).

Table-5: Effect of light intensity on callus induction in *Eclipta alba* (L.) Hassk.

Light intensity (in lux)	Response of callus induction
1000	C^{-}
2000	C^{++}
3000	C^{+++}
4000	C^{+}
5000	C^{-}

C: Callusing response, C^{-}: No callusing response, C^{+}: Slighting callusing, C^{++}: Moderate callusing, C^{+++}: Maximum callusing

Effect of Growth Regulators

Effect of Various Auxins on Callus Induction

Various auxins (2,4-D/NAA/IAA), when incorporated individually in MS medium, induced callus formation (Table-4.4; Plate-4.1). 2,4-D (1.0 mg/l) was found to be the best for the production of friable, creamish callus with fast growth (Table-6; Plate-1-A). However, NAA (0.5 mg/l) produced green compact callus, which showed moderate growth (Table-6; Plate-1-B). The callus produced on IAA was yellowish green compact callus with moderate growth (Table-6; Plate-1-C). Control showed no result.

Effect of Cytokinins in Combination with 2,4-D

2,4-D (1.0 mg/l), which gave optimal growth, was combined with BAP/Kn (0-3.0 mg/l) individually and in combination of BAP (1.0 mg/l) and Kn (1.0 mg/l). Control showed no result. It was observed that a combination of 2,4-D (1.0 mg/l) and BAP (0.5 mg/l) was found to be the best for callus induction and its proliferation (Table-7; Plate-2-A). The callus so produced was healthy, nodular and green with rapid growth. However, Kn (0-3.0 mg/l) proved less effective as compared to BAP. It was observed that Kn (1.0 mg/l) along with 2,4-D (1.0 mg/L) gave moderate amount of light green compact callus (Table-7; Plate-2-B). The combination of 2,4-D (1.0 mg/l) along with Kn (1.0 mg/l) and BAP (1.0 mg/l) was found optimum for the production of compact greenish callus with moderate growth (Table-7; Plate-2-C).

Thus, the callus with profuse growth was obtaine d on MS medium augmented with 2,4-D (1.0 mg/l) and BAP (0.5 mg/l). This combination was selected for maintaining the stock callus cultures (Plate-3).

Table-6: Effect of various auxins on callus induction in *Eclipta alba* (L.) Hassk.

Auxin concentration (mg/l)	Response	Remarks
2,4-D		Creamish, friable callus with fast growth.
0	C^{-}	
0.5	C^{++}	
1.0	C^{+++}	
1.5	C^{++}	
2.0	C^{++}	
2.5	C^{+}	
3.0	C^{+}	
NAA		Green compact callus with moderate growth.
0	C^{-}	
0.5	C^{++}	
1.0	C^{+}	
1.5	C^{+}	
2.0	C^{-}	
2.5	C^{-}	
3.0	C^{-}	
IAA		Yellowish green compact callus with moderate growth.
0	C^{-}	
0.5	C^{++}	
1.0	C^{+}	
1.5	C^{-}	
2.0	C^{-}	
2.5	C^{-}	
3.0	C^{-}	

C: Callusing response, C^{-}: No callusing response, C^{+}: Slighting callusing, C^{++}: Moderate callusing, C^{+++}: Maximum callusing

PLATE-1

Effect of 2,4-D (1.0 mg/l)

Effect of NAA (0.5 mg/l)

Effect of IAA (0.5 mg/l)

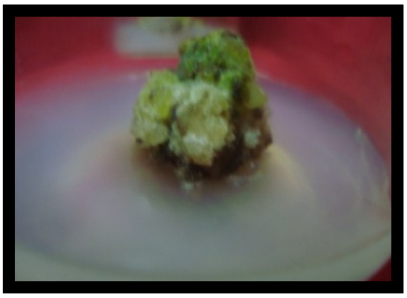
Effect of various auxins on callus induction in *Eclipta alba* (L.) Hassk from leaf explants

Table-7: Effect of cytokinins in combination with 2,4-D (1.0 mg/l) on callus induction in *Eclipta alba* (L.) Hassk.

Cytokinin concentration (mg/l)	Response	Remarks
BAP		Healthy profuse green nodular callus with rapid growth.
0	C^{-}	
0.5	C^{+++}	
1.0	C^{++}	
1.5	C^{++}	
2.0	C^{+}	
2.5	C^{+}	
3.0	C	
Kn		Light green compact callus.
0	C^{-}	
0.5	C^{+}	
1.0	C^{++}	
1.5	C^{+}	
2.0	C^{+}	
2.5	C	
3.0	C^{-}	
BAP + Kn		Compact greenish callus.
1.0 + 1.0	C^{++}	

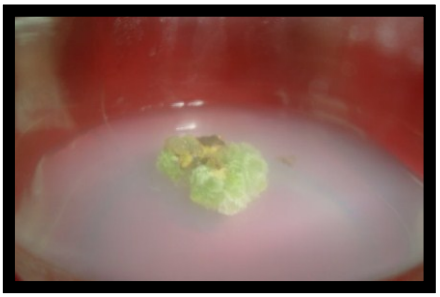
C: Callusing response, C^{-}: No callusing response, C^{+}: Slighting callusing, C^{++}: Moderate callusing, C^{+++}: Maximum callusing

PLATE-2



Effect of BAP (0.5 mg/l)

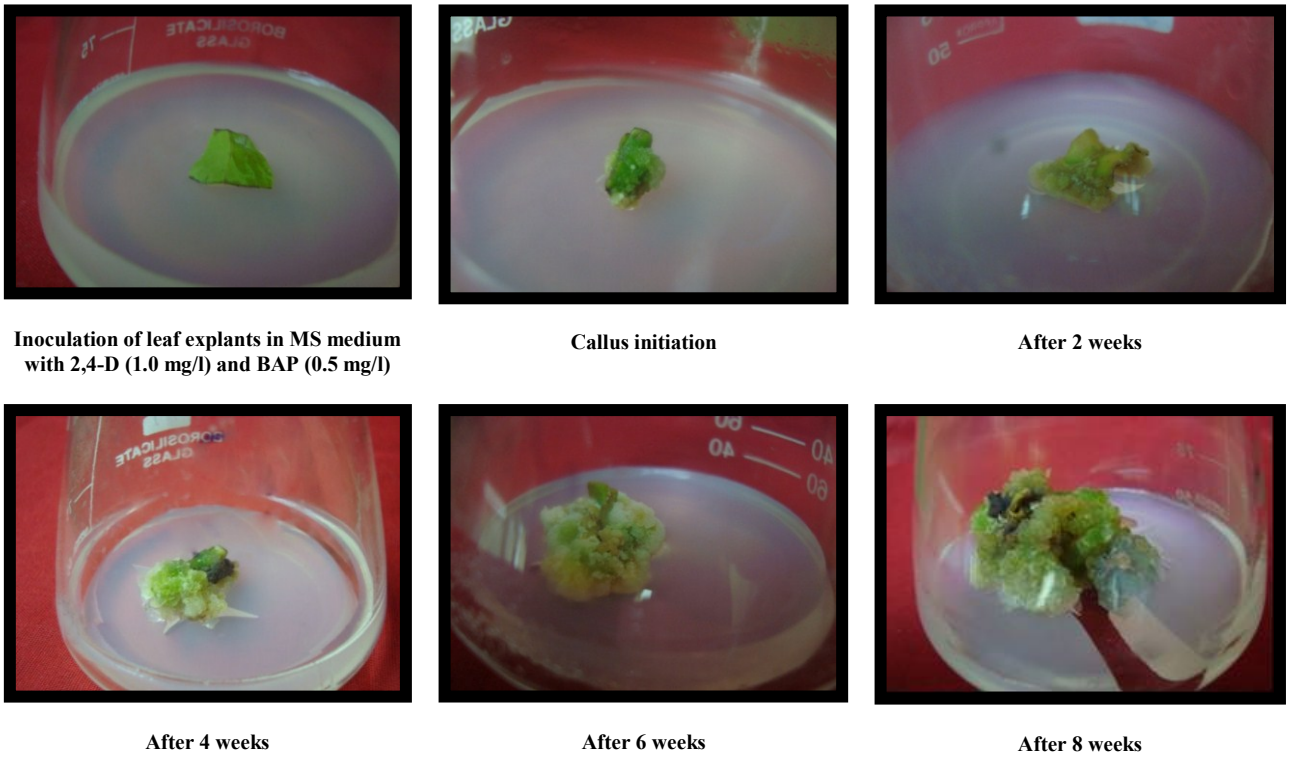
Effect of Kn (1.0 mg/l)



Effect of BAP+Kn (1.0 mg/l)

Effect of various cytokinins in combination with 2,4-D (1.0 mg/l) on callus development in *Eclipta alba* (L.) Hassk.

PLATE -3



Inoculation of leaf explants in MS medium with 2,4-D (1.0 mg/l) and BAP (0.5 mg/l)

Callus initiation

After 2 weeks

After 4 weeks

After 6 weeks

After 8 weeks

Various stages of callus development in *Eclipta alba* (L.) Hassk.

DISCUSSION

Callus induction can be achieved by proper manipulation of media and growth conditions[11]. Micropropagation is the application of tissue culture technique for the propagation of plants starting with very small parts grown aseptically in a test tube or other suitable containers[12]. Micropropagation is one of the key tools of plant biotechnology that has been extensively exploited to meet the growing demands for elite planting material in the current century. It has the advantage of rapid propagation of a superior plant while maintaining the genetic makeup and germplasm storage.

Antihepatotoxic activity in micropropagated plantlets of *Eclipta alba*[13]. Plant tissue culture is a faster method of reproduction[14] in comparison to propagation through seeds. Plants raised through seeds are heterozygous and show high variation in habit, yield and growth. Moreover, these plants may contain systemic fungi, viruses and bacteria which affect various characteristics of plants like quality and yield. In contrast, genetically pure elite populations can be developed through tissue culture techniques under *in vitro* conditions[15,16]. Plants raised through micropropagation are pathogen free and of uniform quality. It is a rapid method as plants can be made commercially available within 2 to 3 years from development rather than 5 to 10 years needed using conventional propagation. Also plants produced from tissue culture show improved quality and vigor and form superior seeds. Some of the techniques of tissue culture are applied directly in plant propagation and genetic improvement.

Wedelolactone and dimethyl wedelolactone are the main active principles of *E. alba*, both constituents containing anti hepatotoxic activity[13]. Four new taraxastane triterpene glycosides, namely eclalbasaponins VII-X along with eclalbasaponins I– VI were isolated from dried whole plants of *Eclipta alba*. The root has emetic and purgative properties and it is applied externally as an antiseptic to ulcers and wounds of cattle. The shoot extract shows antibiotic activity against *Staphylococcus ameus* and *Escherichia coli* [17].

CONCLUSION

In our study the type of explant and composition of medium and supply of growth regulators influenced the callus growth of *Eclipta alba.*

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