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Micropropagation of *Eclipta alba* (Linn.) Hassk- a Valuable Medicinal Herb

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Abstract

A protocol was established for mass propagation of a valuable medicinal herb, *Eclipta alba* (Linn.) Hassk (Asteraceae) through *in vitro* culture. Apical and axillary buds of young sprouts from selected plants were used as explants. Best shoot induction was observed on MS basal medium supplemented with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA, in which 94% of the explants produced 18 shoots per culture. Repeated subcultures in the same medium, resulted rapid shoot multiplication with 26 shoots per culture. *In vitro* raised shoots rooted on half strength MS medium with 1.0 mgl^{-1} IBA +1.0 mgl^{-1} NAA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 80%.

Key words: *Eclipta alba*, Medicinal plant, Shoot proliferation, Micropropagation, Acclimatization

Introduction

Eclipta alba (Linn.) Hassk. (Fam. Asteraceae), a common herbaceous multi-branched annual weed of moist places with long lanceolate leaves, hirsute stem and white flowers in axillary heads, grows all over the Bangladesh (Ghani 1998). It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases. The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus. *Eclipta alba* is widely used in India as a cholagogne and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder. The roots have emetic and purgative properties and it is applied externally as an antiseptic to ulcer and wounds in cattle. The shoot extract shows anti microbial activity against *Staphylococcus aureus* and *Escherichia coli* (Anonymous 1952).

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Decoction of the plant is used to invigorate the liver, to prevent premature graying of the hair and to staunch bleeding, especially from the uterus (Chevallier and Andrew 1996). From the whole plant of *Eclipta alba*, a new triterpene saponin, namely eclalbatin, together with alpha-amyrin, ursolic acid and oleanolic acid have been isolated (Upadhyay *et al.* 2001). In Ayurveda a large number of indigenous drugs have been mentioned possessing analgesic properties. The total ethanol extract of *Eclipta alba* have been shown to posses analgesic properties (Sawant *et al.* 2004).

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni 1998; Prakash *et al.* 1999). Commercial exploitation and elimination of natural habits consequent to urbanization has led to gradual extinction of several medicinal plants. Micropropagation is an effective approach to conserve such germplasm. Further, genetic improvement is another approach to augment drug-yielding capacity of the plant (Tejavathi and Shailaja 1999). Therefore it is important to develop an efficient micropropagation technique for *Eclipta alba* to rapidly disseminate superior clones once they are identified. There have been few reports to date on micropropagation of *Eclipta alba* using nodal explants (Franca *et al.* 1995; Borthakur *et al.* 2000). However, in Bangladesh, there is no report on the establishment of a micropropagation protocol for *Eclipta alba.* The present study was therefore undertaken to develop a protocol for mass clonal propagation of this important medicinal herb through *in vitro* culture.

Materials and Methods

The experiment was conducted at Biological Research Division in Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Eclipta alba* was collected from Bank Colony at Savar, Dhaka and BCSIR Campus, Dhaka and used as source of explants. Shoot tips and stem nodes with a single axillary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five rinses with sterile distilled water in front of a laminar air flow cabinet. The surface-sterilized explants were sized to 1-1.5 cm length containing a single node with an axillary bud or a shoot tip with an apical bud. The explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration.

MS (Murashige and Skoog 1962) basal medium was used for shoot proliferation and

adventitious shoot regeneration and half strength MS was used for *in vitro* rooting. All media were supplemented with 30 gl^{-1} sucrose, 7 gl^{-1} agar (Difco) and dispensed into 15x150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 121^{O}C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2^{O}C under a fluorescent light.

Shoot proliferation from shoot tips and nodal explants was obtained in two separate sets of experiments. In the first experiment 0-2.5 mgl^{-1} BAP and 0-2.5 mgl^{-1} Kn were incorporated into MS to select the best cytokinin for the response of shoot induction. In the second set, combination of BAP-NAA (0-2.5 mgl^{-1}) and BAP-IAA (0-2.5 mgl^{-1}) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded after every week of inoculation.

For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted onto half strength MS with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were taken out from the culture tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with soil and compost (1: 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2°C under a 12 h photoperiod for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tips and nodal explants of *Eclipta alba* were cultured on MS media supplemented with various concentration of BAP alone and BAP with NAA or IAA for shoot regeneration. The explants were found to be swollen and they produced four to five shoots within three-four weeks after inoculation (Fig 1a) on MS containing BAP alone but the number of shoots increased up to 18 when the explants were cultured in MS with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA (Table I, Fig 1b). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from nodal explants. Combinations of BAP with IAA were not found suitable for shoot induction (Table I). Newly initiated shoots were separated and sub cultured repeatedly in fresh MS with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA, where the number of shoots increased up to 26 per culture (Fig 1c). Dhaka and Kothari (2005) reported that maximum shoot proliferation in *Eclipta alba* occurred when the explants were cultured on MS medium supplemented with 1.0 mgl^{-1} benzylaminopurine (BAP) and the shoot buds were further multiplied and maintained on medium containing 0.5 mgl^{-1} BAP and 0.5 mgl^{-1} GA_3. A similar phenomenon was observed in Eclipta alba by other researchers (Gawde and Paratkar 2004;

Table I. Effect of growth regulators in MS on morphogenic response of *Eclipta alba* shoot tips and nodal segments.

Growth regulators(mgl^{-1})			Shoot tips		Nodal segments	
BAP	NAA	IAA	Explants forming shoots (%)	Mean No. of shoot/explant	Explants forming shoots (%)	Mean No. of shoot/explant
0.0	0.0	0.0	-	-	-	-
0.5	0.0	0.0	73(0.8)	04(0.1)	76(2.3)	05(0.1)
0.5	0.0	0.1	83(0.8)	16(0.8)	88(1.8)	18(1.4)
1.0	0.0	0.2	68(6.6)	06(1.4)	72(0.8)	08(0.5)
1.5	0.0	0.5	71(1.4)	14(0.5)	73(0.1)	16(0.8)
2.0	0.0	0.5	76(0.8)	09(1.0)	79(0.5)	13(0.3)
0.5	0.1	0.0	94(0.5)	23(0.3)	96(0.7)	26(1.4)
1.0	0.2	0.0	81(1.0)	08(1.4)	84(0.1)	12(0.1)
1.5	0.5	0.0	78(1.8)	19(0.8)	81(1.4)	22(1.0)
2.0	0.5	0.0	72(1.4)	14(0.1)	76(0.8)	16(0.8)

Results are mean ± SE of three experiments with 15 replications.

Baskaran and Jayabalan 2005; Husain and Anis 2006; Han *et al.* 2007).

Ninety five percent regenerated shoots rooted (Fig 1d) when cultured individually on root induction medium consisted of half-strength MS salts with 1.0 mgl^{-1} IBA + 1.0 mgl^{-1} NAA (Table II). Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand 1998; Ajithkumar and Seeni 1998; Rai 2002; Baskaran and Jayabalan 2005; Sivakumar and Krishnamurthy 2000; Hassan and Roy 2005; Rahman *et al.* 2006; Baksha *et al.* 2007).

After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 80 percent of the transplanted plants of *Eclipta alba* survived if the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30±2^{o}C) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig 1e).

After three weeks, plants were transferred to an open place and gradually acclimated to outdoor conditions, where 80 percent plants survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation for

Fig. 1. *In vitro* regeneration of *Eclipta alba* from shoot tip and nodal explants.

(a) Induction of shoots in four weeks of culture on MS + 0.5 mgl^{-1} BAP + 0.1 $mg1^{-1}$ NAA

(b) Development and multiplication of shoots on MS + 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA after eight weeks of culture.

(c) Development and multiplication of shoots on MS + 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA after twelve weeks of culture.

(d) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 1.0 mgl^{-1} IBA + 1.0 mgl^{-1} NAA in third weeks.

(e) Acclimatized regenerated plants of two months old.

Table II. Effect of auxin(s) on root induction in regenerated shoots of *Eclipta alba* on half strength MS.

Auxins(mgl^{-1})	Shoots rooted(±SE) (%)	Days required for root induction (±SE)
IBA 0.5	82(0.8)	26(0.1)
IBA 0.75	73(0.1)	20(0.3)
IBA 1.0	66(1.0)	24(0.8)
NAA 0.5	81(0.3)	24(0.5)
NAA 0.75	76(1.4)	25(1.0)
NAA 1.0	73(0.5)	21(0.1)
IBA 1.0 + NAA 0.5	82(0.8)	23(1.4)
IBA 0.5 + IAA 0.5	71(0.1)	21(0.7)
IBA 0.5+ NAA 0.5+ IAA 0.5	68(0.7)	19(0.3)
IBA 1.0+ NAA 1.0	92(0.3)	18(0.8)
IBA 1.0+ IAA 1.0	76(0.8)	25(0.3)
IBA 1.0+ NAA 1.0 + IAA 1.0	62(0.5)	23(0.5)

Data were recorded after four weeks of culture. Results are mean ± SE of 15 replications.

sustainable use in the industry. Moreover, by standardizing the protocols for clonal propagation of selected elite plants, it is possible to achieve a tenfold increase in the products per unit area of cultivation (Hassan and Roy 2005). Clonally propagated plants would also have identical phytochemical profiles (Roja and Heble 1993). Likewise it could be possible to propagate important medicinal plants for cultivation and sustainable use and consequently to conserve them from their extinction.

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