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Research note

Micropropagation of *Eclipta alba* and *Eupatorium adenophorum* using a single-step nodal cutting technique

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Abstract

Protocols for the micropropagation of two traditional medicinal plants *Eclipta alba* (L.) and *Eupatorium adenophorum* (L.) from nodal segments were developed. Proliferated microshoots of *Eclipta alba* and *Eupatorium adenophorum* were obtained through axillary branching by culturing nodal segments in modified MS medium and half strength of MS, respectively, with minimal strength of nutritional support. Simultaneous rooting could also be induced in the same medium. Regenerated rooted plantlets were successfully acclimatized in soil where they grew normally without showing any morphological variation.

Abbreviations: BA – 6-benzylaminopurine

Two traditioual medicinal plants of compositae, grown in North Eastern region of India, *Eclipta alba* and *Eupatorium adenophorum* were investigated for their *in vitro* regeneration with an aim of obtaining minimal requirements of nutrient combination for optimal regeneration of whole plants.

Eclipta alba (L.) Hassk., a small, annual herb distributed in tropical and sub tropical region of the world is used as tonic, diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases (Anonymous, 1952). Wedelolactone and dimethyl wedelolactone are the main active principles of *E. alba*, both constituents containing anti hepatotoxic activity (Wagner et al., 1986; Franca et al., 1995). Four new taraxastane triterpene glycosides, namely eclalbasaponins VII-X along with eclalbasaponins I–VI were isolated from dried whole plants of *Eclipta alba* (Yahara et al., 1997). The root have 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* (Anonymous, 1952). The plant species is propagated by seeds. *In vitro* micropropagation of *Eclipta alba* from nodal segment explant was reported earlier by Franca et al. (1995).

Eupatorium adenophorum (L.) Spr., a herb found in higher elevations (1000–2000 m above mean sea level), is used in the treatment of stomach aches and to prevent bleeding (Uniyal, 1980). *E. adenophorum* is conventionally propagated by vegetative cuttings. There is limited information regarding seed propagation or micropropagation through tissue culture.

Shoot tips 1.0 cm in length with a single node were collected from the experimental garden of Regional Research Laboratory, Jorhat, India and the excised nodes were used as explants. Nodal segments were surface sterilized in a 0.1% mercuric chloride solution, washed 3 times with sterile water and then explants of 0.3–0.4 cm were placed in culture medium. The cultures were incubated at 23 ± 2 °C with a 16-h photoperiod under fluorescent light (40 μmol m^{-2} s^{-1}). To identify the most suitable medium combination for *in vitro* growth, nodal explants were inoculated first in MS medium (Murashige and Skoog, 1962) containing 3% sucrose (w/v), and 0.8% (w/v) agar with either kinetin (medium A) or BA (medium B) 0.05 mg l^{-1}.

Table 1. Effect of various nutrient media composition on shoot multiplication of *Eclipta alba*

Medium	Nutrient composition (mg l^{-1})	No. of multiple shoots/ explant	Shoot length (cm)	No. of nodes	No. of leaves	No. of roots	Root length (cm)	Flowers
A	MS + kinetin 0.05	16.0±3.5	19.2±1.0	36.0±2.6	74.1±5.4	18.6±2.0	4.8±0.4	7.6±1.4
B	MS + BA 0.05	6.0±1.8	14.2±4.9	19.3±4.3	40.5±8.2	30.2±3.5	2.2±0.7	3.5±0.7
C	MS + thiamine HCl 10 (as only vitamin)	14.6±0.9	10.5±2.6	14.6±5.7	40.1±5.8	12.5±3.3	3.1±1.1	0.8±0.8
D	Half strength of MS	15.1±2.8	19.5±5.1	22.5±5.6	44.0±11.3	21.6±6.1	4.5±2.1	3.6±1.0
E	Quarter strength of MS	3.3±1.5	11.6±1.0	9.5±5.0	30.7±8.5	12.8±2.7	3.0±0.4	1.2±1.0
F	Quarter strength of MS + kinetin 0.05	5.1±2.0	10.4±1.0	15.5±3.4	42.8±7.3	15.8±4.7	4.6±0.4	1.1±1.1

Values are means ± S.E. for 20 replicates.

Table 2. Effect of various nutrient media composition on shoot multiplication of *Eupatorium adenophorum*

Medium	Nutrient composition (mg l^{-1})	No. of multiple shoots/ explant	Shoot length (cm)	No. of nodes	No. of leaves	No. of roots	Root length (cm)	Flowers
A	MS + kinetin 0.05	4.1±1.2	10.2±1.1	20.5±8.2	48.0±13.4	12.5±4.2	13.1±1.7	–
B	MS + BA 0.05	2.2±1.0	9.5±1.0	14.0±2.2	30.6±3.4	9.8±1.4	12.8±1.9	–
C	MS + thiamine HCl 10 (as only vitamin)	3.6±1.0	8.7±1.6	17.±3.9	42.2±6.9	8.3±0.9	9.08±1.3	–
D	Half strength of MS	3.2±0.8	13.8±0.9	17.3±2.9	37.2±7.2	10.5±4.3	12.1±3.5	–
E	Quarter strength of MS	3.2±1.2	6.9±0.8	12.7±4.1	30.2±6.1	10.0±2.2	8.6±3.1	–
F	Quarter strength of MS + kinetin 0.05	4.0±1.8	8.0±2.7	20.3±5.6	44.0±6.1	8.8±3.1	10.1±0.6	–

Values are means ± S.E. for 20 replicates.

In one set of experiments thiamine HCl at 10 mg l^{-1} (medium C) was added to the MS basal medium in place of MS standard three vitamins. Thereafter based on results obtained, the composition of the MS basal medium was reduced to half strength (medium D) or quarter strength tested alone (medium E) and included kinetin 0.05 mg l^{-1} (medium F). 80 ml of medium were placed in each 250 ml Erlenmeyer flask. The pH of the medium was adjusted to 5.8 before autoclaving. Different nutrient combinations tested in this study are presented in the Tables 1 and 2. Each treatment had 20 replicates and all the experiments were repeated twice. The number of shoot initiation, shoot length, leaves, root induction, root length, nodes and flowers were recorded fortnightly and results were tabulated from 45 days old culture. Subculturing was not carried out for both species. However the nodes of a few regenerated shoots were used as microcuttings and cultured in the same media for mass micropropagation. Rooted plants were kept up to 10–12 weeks in the same nutrient medium and then plantlets were removed very carefully from the nutrient medium and washed thoroughly in tap water to remove agar from the roots. Later rooted plantlets were transferred to liquid MS mineral salt solution (half strength) for one month and then to quarter strength for another one month allowing further growth and elongation of shoots in growth chamber at 25±2 °C under 16-h daily illumination with cool fluorescent tubes (40 μmol m^{-2} s^{-1}). Survival percentage was recorded during this process. Thereafter based on hardening, plantlets were transferred to soil containing 1:1 mixture of autoclaved

Figures 1–4. Multiple shoot formation from nodal segment of *E. alba* (*1*) and *E. adenophorum* (*2*) Plants after 45 days of growth in pots *E. alba* (*3*) and *E. adenophorum* (*4*).

sand and soil. Plantlets were covered with glass beakers for 2 weeks to maintain high humidity. They were watered every other day with tap water and kept in a greenhouse at 25±2 °C. Based on new shoot growth, the covers were removed gradually.

Nodal explants of both *Eclipta alba* and *Eupatorium adenophorum* had initiated growth of axillary shoots within one week of culture. Within 15–20 days multiple shoot formation was observed from almost all the treatments without callus formation. However multiplication rate and shoot growth varied according to the medium composition. The composition and strength of the basal medium played an important role in shoot multiplication, elongation and root induction in both the species (Tables 1 and 2). The multiplication rate of the shoots obtained from node explant culture for mass micropropagation was found to be dependent on the number of the subsequent cultures which did not decline during the experimentation period of two years.

In *E. alba* the highest frequency of shoot production and root growth were recorded in MS medium supplemented with 0.05 mg l^{-1} kinetin. However, satisfactory multiple shoots was also recorded with BA 0.05 mg l^{-1} supplemented medium. The result was similar to the earlier investigator (Franca et al., 1995) though they had not obtained simultaneous rooting. In this investigation MS medium only with modification of its vitamin level (medium C) showed optimal response towards multiplication and growth of the shoots with simultaneous rooting (Figure 1). Half strength MS medium composition alone was also found to be sufficient for satisfactory shoot multiplication with simultaneous rooting of the shoots. But maintaining the culture in this medium for 6 weeks resulted gradual browning and defoliation of leaves of the shoot. The medium was also found suitable for the *in vitro* flowering. Maximum flowering was reported in kinetin supplemented MS basal medium. *In vitro* flowering was not reported earlier in the attempt made for micropropagation of *E. alba* (Franca et al., 1995). A reduction in concentration of nutrient media (one fourth strength of MS) was not suitable for healthy shoot and leaf growth when supplemented either alone or in combination with kinetin.

In the case of *Eupatorium adenophorum* half strength MS medium alone was found to be sufficient for both satisfactory shooting and simultaneous rooting of the shoots (Figure 2). However incorporation of lower concentration of kinetin 0.05 mg l^{-1} to MS basal medium resulted in maximum shoot multiplication and root elongation. Reduced level of nutrient composition, i.e. one fourth strength of MS nutrient medium, retarded shoot growth accompanied with smaller leafy structures. Here *in vitro* flowering could not be initiated in any concentration of nutrient media studied.

Though maximum shoot multiplication and growth were recorded in kinetin incorporated media for both species, considering all the characteristic for whole plant regeneration with minimal nutritional support, media C and D were selected for *E. alba* and *E. adenophorum* respectively.

As no additional step was adopted for rooting of the shoots for both the species, rooted shoots were directly removed from the culture vessels and transferred to MS inorganic salts at half strength, then to one fourth strength, after 10–12 weeks of culture periods. During this culture period shoot elongation and multiplication of new shoot buds were also recorded at regular interval for both *E. alba* and *E. adenophorum*. Survival percentage recorded was maximum, i.e. about 100% from *E. adenophorum* and 50% in case of *E. alba*. Proliferated plantlets were then transferred to potted soil composed of a 1:1 mixture of autoclaved garden soil and sand (Figures 3 and 4).

We have developed culture methods for multiple shoot induction and plant regeneration of two different medicinal plant species having pharmaceutical uses. Protocols described here provide a rapid and quick single step whole plant regeneration system which could be used for the large scale micropropagation and conservation of these two species.

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