Micropropagation of *Eclipta alba* Hassk.: An approach to shorten the protocol

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A successful attempt was made to shorten the protocol for micropropagation of the elite variety of *Eclipta alba*. Multiple shoots were obtained from nodal explants on MS medium supplemented with different concentrations (0.44-22.2 µM) of 6 benzyladenine (BA); the best response was obtained with 4.44 µM. Further, the shoot multiplication and simultaneous rooting was obtained with lowered BA concentration (0.44 µM). The *in vitro* plantlets were successfully acclimatized and transferred to the field.

**Keywords:** *Eclipta alba*, micropropagation, Knops hydroponic solution, vermicompost

**Introduction**

*Eclipta alba* Hassk. (Fig. 1a) syn. *E. prostrata* (Linn.); *E. erecta* (Linn.), belonging to the family Asteraceae, is a common herb found in the tropical and subtropical regions of the world during rainy season. It grows well in moist places and shows two types of habit, viz. erect and prostrate; hence, rightly called *E. erecta* and *E. prostrata*, respectively. The prostrate type shows profuse branching and is characterized by white ray florets.

*E. alba* is traditionally used in hair oil preparations, since it promotes hair growth and maintains hair black. Its aerial parts are administered in cases of jaundice1-4. The herb possesses hepatoprotective, anti-inflammatory, antibacterial and antifungal properties5-8. The anti-inflammatory and hepatoprotective properties are exhibited by the coumestan derivative, wedelolactone6.9. The herb is used in gastritis and respiratory disorders, like cough and asthma. It is antihypertensive and is also effective in conditions of anemia10,11.

The herb is a weed of paddy fields. It shows seasonal and varietal variations in the amount of active constituents12. However, little attention has been paid towards its systematic cultivation and propagation. Therefore, a protocol was established for the micropropagation of the elite variety. The present paper aims at shortening the culture period for micropropagation by identifying the optimal concentrations of plant growth regulators for rapid multiplication as well as simultaneous rooting of the cultures.

**Materials and Methods**

**Collection and Treatment**

The herb was collected from Kelkar Education Trust’s medicinal and aromatic plants garden at Mulund (W), Mumbai. Nodal segments (1 cm), initially washed with Teepol followed by Dettol and antifungal Dithane, were used as explants for tissue culture. They were kept under running tap water for 3 hrs. Further sterilization procedures were done in a laminar airflow cabinet, which consisted of 50% alcohol treatment for 30 sec followed by antifungal (Bavistin) wash for 5 min, and then treatment with 0.1% HgCl₂ for 4 min. Two washings of deionized water were given after each of the above-mentioned steps. Finally, nodal segments were treated with antibacterial chemical Ciplox for 4 min.

**Medium and Cultural Conditions**

The explants were placed on sterile blots and were inoculated in 25×100 mm glass culture tubes (Borosil, India) containing Murashige and Skoog (MS) medium with 3% sucrose and supplemented with various concentrations (0.44-22.2 µM) of 6 benzyladenine (BA) for multiple shoot initiation. The pH of the medium was adjusted to 5.7 before addition of agar. The medium was solidified with 0.8% agar and sterilized by autoclaving at 121°C and 15 lb pressure.

The cultures were incubated at 22±2°C, 16 hr photoperiod, provided by cool fluorescent tubes with 3000 lux light intensity. After a 15-day culture, the initiated multiple shoots were subcultured on MS medium, containing decreased concentrations of BA (0.44 µM), to obtain further proliferation, simultaneous rooting and elongation of the initiated shoots. The rooted shoots were transferred to different
media using two methods for hardening. In the first
method, *in vitro* plantlets were acclimatized directly
using a soil:vermicompost (1:1) mixture. In the other
method, soil, sand:soil and Knop’s hydroponics
solution were used. The hardened shoots were
transferred to greenhouse and then successfully
acclimatized to field conditions. Each culture
treatment consisted of 15 replicates and each
experiment was repeated thrice.

**Results and Discussion**

The multiple shoot induction from nodal explants
of prostrate form was reported in a couple of earlier
works\(^{13,14}\). In the present study, the erect form was
used. Multiple shoot initiation was obtained within 15
days of culturing. Table 1 gives the media containing
various concentrations of BA (0.44-22.22 µM) used
for shoot initiation. Not more than 1-2 shoots were
obtained with BA concentrations 0.44, 0.88, 2.22,
17.76 and 22.22 µM. Increase in the number of
shoots, i.e. 2-3 was observed with BA concentrations
8.88 and 13.32 µM. All these media show
simultaneous root initiation and thus inhibiting further
shoot multiplication. However, the shoots obtained in
these media, i.e. BA concentrations 0.44, 0.88, 2.22,
8.88, 13.32, 17.76 and 22.22 µM, show significant
increase in leaf size, internode elongation and stem
thickness.

The optimum concentration of BA for multiple
shoot induction was 4.44 µM. Ten to twelve shoots
were obtained within 2 weeks of culturing (Fig. 1b).
Simultaneous rooting was not observed and the
increase in leaf size, internode elongation and stem
thickening were also negligible (Fig. 2).

Supplementation of 1-naphthalene acetic acid
(NAA), simultaneously with shoot induction, further
enhanced the rooting but had no major role to play
with the multiple shoot induction. NAA (0.54-1.08
µM) did not show significant alteration in the multiple
shoot induction. However, with further increase in the

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**Table 1**

<table>
<thead>
<tr>
<th>BA Concentration (µM)</th>
<th>Shoots Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.44</td>
<td>1-2</td>
</tr>
<tr>
<td>0.88</td>
<td>1-2</td>
</tr>
<tr>
<td>2.22</td>
<td>1-2</td>
</tr>
<tr>
<td>8.88</td>
<td>2-3</td>
</tr>
<tr>
<td>13.32</td>
<td>2-3</td>
</tr>
<tr>
<td>17.76</td>
<td>2-3</td>
</tr>
<tr>
<td>22.22</td>
<td>2-3</td>
</tr>
</tbody>
</table>

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*Fig. 1—*a. *E. alba* habit; b. multiple shoot initiation from nodal segments; c & d. further shoot multiplication and simultaneous rooting (culture tube & flask); e. harvested *in vitro* plantlets; f. plantlets transferred to Knop hydroponics solution; g. primary hardening of *in vitro* plantlets in Knop hydroponics solution; & h. hardened plants.
concentration (2.7-5.37 µM) of NAA, problems like callusing at the nodal base or rooting were observed, which inhibited shoot formation.

These results clearly indicate the necessity of only BA and no other hormone. The concentration of BA required for the initiation of multiple shoots is considerably low. The time period required for the initiation of multiple shoots is also very low and consists of only 10-12 days in comparison to earlier protocols, which require minimum 30 days for multiple shoot initiation. These protocols make use of IAA-BA, BA, kinetin, or 2ip. 13, 14.

The multiple shoots, so obtained on MS medium supplemented with BA (4.44 µM), when subcultured on MS medium with lowered BA concentration (0.44 µM) showed further shoot multiplication and simultaneous rooting (Figs 1c & d). On an average, 35 rooted shoots were obtained. The medium also favoured stem thickening, elongation and leaf size increase. With further increase in the BA concentration (2.22 µM), the multiplication of shoots enhanced and about 50 shoots were obtained. However, these did not root simultaneously and remained short with notable increase in leaf size. Of all, the poorest response was obtained with BA (4.44 µM), where only 10-12 shoots were obtained with simultaneous rooting. The chlorophyll content in leaves decreased with increase in BA concentration, with very less chlorophyll in shoots grown in BA (4.44 µM) and, hence, the leaves appeared yellowish (Table 2; Fig. 3).

In general, a rooting response requires the addition of a synthetic auxin to the culture medium. In the present study, however, rooting was observed in the presence of low amount (0.44 µM) of cytokinin (BA). Moreover, shoots proliferated and became well elongated in the same medium. Shoot elongation, leaf size increase and internodal elongation were also observed in the same medium. The proliferation of in vitro multiple shoots and the simultaneous rooting and elongation in the same medium obviously excludes the additional rooting and shoot elongation steps, which are part of most tissue culture protocols. Within a period of 45 days, almost 35 plantlets were generated unlike that of the earlier protocols, which require 30 and 60 days of culturing. 13, 14. In addition to this, earlier protocols make use of high hormonal concentrations. The present protocol makes use of low amounts of only a single hormone, BA. It is also a two-step protocol to obtain rooted plantlets in 45 days. All this makes it a short and economic protocol for the micropropagation of E. alba.

Ready for hardening, the in vitro plantlets were transferred to small plastic pots, separately containing sterile soil and sand:soil (1:1) but their survival

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Table 1—Multiple shoot initiation from nodal explants of E. alba

<table>
<thead>
<tr>
<th>No.</th>
<th>Medium</th>
<th>Average number of shoots per explant (M ± SE)</th>
<th>Length of the shoots (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS + BA (0.44 µM)</td>
<td>1.4 ± 0.131</td>
<td>82.46 ± 2.22</td>
</tr>
<tr>
<td>2</td>
<td>MS + BA (0.88 µM)</td>
<td>1.47 ± 0.134</td>
<td>77.8 ± 2.52</td>
</tr>
<tr>
<td>3</td>
<td>MS + BA (2.22 µM)</td>
<td>2.66 ± 0.186</td>
<td>80.8 ± 2.28</td>
</tr>
<tr>
<td>4</td>
<td>MS + BA (4.44 µM)*</td>
<td>10.27 ± 0.418</td>
<td>50.47 ± 3.1</td>
</tr>
<tr>
<td>5</td>
<td>MS + BA (8.88 µM)</td>
<td>3.6 ± 0.310</td>
<td>67.93 ± 2.65</td>
</tr>
<tr>
<td>6</td>
<td>MS + BA (13.32 µM)</td>
<td>1.8 ± 0.222</td>
<td>65.8 ± 2.99</td>
</tr>
<tr>
<td>7</td>
<td>MS + BA (17.76 µM)</td>
<td>1.3 ± 0.127</td>
<td>68.13 ± 2.42</td>
</tr>
<tr>
<td>8</td>
<td>MS + BA (22.2 µM)</td>
<td>1.1 ± 0.103</td>
<td>66.4 ± 3.37</td>
</tr>
</tbody>
</table>

Cultural period: 15 days  No of replicates: 15
M ± SE: Mean ± Standard error, *Best Response

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Fig. 2—Multiple shoot initiation from nodal explants of E. alba with different concentrations of BA
percentage was very low in both the medium (Table 3). It may probably be due to rotting and desiccation of roots. This problem was overcome by using a soil:vermicompost (1:1) mixture that proved to be successful with a survival rate of 95% plantlets. This may be due to:

- The water holding capacity of soil:vermicompost lies between water logging and water drainage and hence proved to be the best for root survival.
- The pH of the soil, sand and vermicompost were 5.15, 6.37 and 6.53, respectively. Whereas, the pH of the soil: vermicompost was 5.64, which was in accordance with the culture medium. It again probably supports the better survival of the plantlets.

- Vermicompost not only improved the water holding capacity and pH but it also enhanced the porosity and thus provided better aeration too.

The shoots were acclimatized within 8 days and further transferred to field after a month.

Other than soil:vermicompost, a trial was done where the shoots before transfer to soil were put to an intermediary step of Knops hydroponics solution (Table 3). Knops medium is a sugar free medium with very low macro and microelements. The shoots were kept in it for 8 days under non-aseptic conditions (Figs 1e & f). No signs of contamination were seen probably because it was a sugar free medium. The plants showed primary hardening at this stage, which was indicated by the increase in leaf size, shoot height and root mass (Fig. 1g). The plants were then transferred to soil where they showed 100% survival.

The plants potted in small plastic pots were transferred to the hardening room maintained at a temperature 24±2°C. The pots were covered with polythene bags and the plants were frequently watered and misted, so as to keep high humidity. The plants hardened here within 8 days. This was indicated by the arrival of new apical leaves, the increase in the thickness of the stem and the increase in the size of leaf. The plants were then transferred to the greenhouse from the hardening room. They were repotted in large plastic pots. The conditions in the greenhouse were monitored to maintain a temperature of 28-29°C and 76% humidity.

The tissue cultured plants showed increase in vigour. The morphological characteristics, viz. root biomass, leaf size (both length and breadth), plant height and stem thickness, of these plants showed a threefold increase in comparison to the control plants.

### Table 2—Shoot multiplication and rooting of multiple shoots obtained with BA

<table>
<thead>
<tr>
<th>No.</th>
<th>Medium</th>
<th>Response</th>
<th>Final number of shoots (M± SE)</th>
<th>Leaf size(mm) (M± SE)</th>
<th>Increase in internodal length(mm) (M± SE)</th>
<th>Height of shoots (mm) (M± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS + BA (4.44µM)</td>
<td>Shoot multiplication + Rooting</td>
<td>11.2 ± 0.39</td>
<td>4.3 ± 0.36</td>
<td>5.1 ± 0.39</td>
<td>77.27 ± 1.99</td>
</tr>
<tr>
<td>2</td>
<td>MS + BA (2.22µM)</td>
<td>Shoot multiplication</td>
<td>51.5 ± 2.09</td>
<td>3.9 ± 0.23</td>
<td>2.3 ± 0.23</td>
<td>53.67 ± 1.57</td>
</tr>
<tr>
<td>3</td>
<td>MS + BA (0.44µM)</td>
<td>Shoot multiplication + Rooting</td>
<td>34.5 ± 1.26</td>
<td>10.3 ± 0.31</td>
<td>28.47 ± 0.9</td>
<td>95.73 ± 1.49</td>
</tr>
</tbody>
</table>

Cultural period: 30 days
Number of replicates: 15
M ± SE: Mean ± Standard error.

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Fig. 3—Shoot multiplication, elongation and leaf size increase of multiple shoots with different concentrations of BA

- Vermicompost not only improved the water holding capacity and pH but it also enhanced the porosity and thus provided better aeration too.
The present paper is a successful attempt to generate a quick, economical and reproducible protocol for the micropropagation of *E. alba*.

**Acknowledgement**

We thank Dr M R Kurup, Principal, V G Vaze College; Mr G D Kelkar, Chairman, Kelkar Education Trust; Dr M R Heble, Scientific Advisor, Kelkar Scientific Research Centre and Dr Sudha Sundaram, Deputy Director, Kelkar Scientific Research Centre for their advice, constant encouragement and support.

**References**

6. Wagner H *et al.*, Coumestans as the main active principles of liver drugs *Eclipta alba* and *Wedelia calendulaceae*, *Planta Med.*, 52 (1986a) 370-373.

<table>
<thead>
<tr>
<th>No.</th>
<th>Hardening medium</th>
<th>pH</th>
<th>Porosity</th>
<th>Water holding capacity</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soil</td>
<td>6.37</td>
<td>Highest</td>
<td>10%</td>
<td>4-5%</td>
</tr>
<tr>
<td>2</td>
<td>Sand : Soil</td>
<td>5.15</td>
<td>Least</td>
<td>19%</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>Soil : Vermicompost</td>
<td>5.64</td>
<td>In combination with soil enhances the soil porosity</td>
<td>15%</td>
<td>95%</td>
</tr>
<tr>
<td>4</td>
<td>Knops hydroponic solution</td>
<td>5.7</td>
<td>—</td>
<td>—</td>
<td>100%</td>
</tr>
</tbody>
</table>