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IN VITRO CALLUS INDUCTION AND SHOOT REGENERATION IN *ECLIPTA ALBA* (L.) HASSK

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ABSTRACT

Eclipta alba is a medicinal herb, also known as Bhringraj belonging to family Asteraceae. The plant has hepatoprotective, antimicrobial and hair growth promoting properties. The effect of various phytohormones on *in-vitro* callus induction and shoot regeneration in *Eclipta alba* from leaf explant was evaluated. The sterilized explants were inoculated on MS medium supplemented with different concentrations of auxins alone or in combination with cytokinins. Best result was observed on MS medium fortified with a combination of 2,4-D (1.0 mg/l) and BAP (0.5 mg/l). Shoot cultures were regenerated from the obtained callus on fresh MS medium containing BAP and NAA and its generation capacity, length and morphology were observed. Maximum number of shoots was obtained on a concentration of BAP (1.0 mg/l) and NAA (0.1 mg/l). *In vitro* generated callus and subsequent shoot proliferation can be used for large scale production of plant.

Keywords –*Eclipta alba*, MS medium, Plant hormones, callus cultures.

INTRODUCTION

Eclipta alba L. (Hassk) is a medicinal herb belonging to the family Asteraceae. It is commonly known as 'Bhringraj'. *Eclipta* grows in India in various areas. It is used for the treatment of several ailments. It is used to prevent premature greying of hair and as a dye for blackening the hair. It has also been used as a hepatoprotective, antimicrobial and for treatment of wounds and cuts. Various secondary metabolites present in the plant are responsible for its medicinal value. In recent years there has been a tremendous increase in the demand and consumption of herbal medicinal drugs as they have less side effects. There is an immense pressure on natural resources due to urbanization and industrialization, this coupled with the harvesting of plants as source of drug has threatened their survival thus there is a great necessity for large scale multiplication of the plant which is simple, rapid, genetically stable. Plant tissue culture offers a method for large scale multiplication of various medicinal herbs such as *Eclipta alba* and also secondary metabolite production. The present study was undertaken to study callus induction and subsequent shoot proliferation from leaf explant of *Eclipta alba* using various plant growth regulators.

MATERIALS AND METHODS

Eclipta alba plants were collected from National Institute of Ayurveda and Amber hills area, Jaipur. The leaves were used for callus induction. The leaf explants were thoroughly washed for few minutes under running tap water and subsequently they were rinsed with 0.2% tween 20 (mild detergent). Then they were rinsed several times using sterilized distilled water. Further sterilization was done under

aseptic conditions in laminar air flow cabinet.The explants were dipped in 70% alcohol for 3 minutes and again washed with sterilized distilled water. For surface sterilization, the explants were dipped in 0.1% aqueous solution (w/v) of $HgCl_2$ for 3 minutes then they were washed in sterilized distilled water for 3-4 times, till the sterilents were removed completely. The sterilized leaf explants were cultured on agar solidified MS medium fortified with 3% sucrose and supplemented with different concentrations of auxins, 2,4-D/ NAA/IAA (0-3.0 mg/l) alone or in combination with cytokinins, BAP/ Kn (0-3.0 mg/l). Callus obtained from leaf explants was transferred to MS medium supplemented with combinations of BAP (0-3.0 mg/l) and NAA (0.1 mg/l) for shooting. After the inoculation, all the cultures were maintained in growth chamber which with regulated temperature (26±2ºC), relative humidity (55±5%) and light conditions (16/8 hours photoperiod). 3000 lux intensity of constant light was provided in culture shelves by cool-white fluorescent tubes. Data was recorded after 4-6 weeks.

RESULTS AND DISCUSSION

Initially explants from leaf, were cultured on MS medium supplemented with different concentration of growth hormones, (auxins and cytokinins) alone or in combination for callusing. All the phytohormones produced callus singly or in combination with variable response. MS medium supplemented with 2, 4-D (1.0 mg/l) proved best for callus initiation which produced friable, creamish callus with rapid growth. In the present investigation, higher concentration of 2, 4-D was not used as high concentration of auxin can suppress morphogenesis. Similar results with 2, 4-D for callus induction were observed in other plants also like *Irvingia gabonensis* (Fotso *et al*., 2008), *Arnica montana* (Petrova *et al*., 2011) and *Ionidium suffruticosium* Ging. (Sonappanavar and Jayraj, 2011). The effect of various cytokinins along with optimized auxin was also taken into account for the successful stock callus induction. In *Eclipta alba*, callus developed from leaf explants on MS medium supplemented with 2,4-D (1.0 mg/l) and BAP (0.5 mg/l) gave best results. The callus produced was healthy, nodular and green with fast growth. Similar results in *Eclipta alba* was shown by Zafar and Sagar (1999). Some researchers observed similar results on 2,4-D and BAP supplemented media in *Tridex procumbens* L. (Wani *et al*., 2010) and *Gardenia latifolia* Ait. (Lakshmi and Reddy, 2012). However, Gupta *et al*. (2010) reported callus induction from leaf explant in *Stevia rebaudiana* on MS medium supplemented with NAA and 2,4-D. To induce shoot, the callus was grown in fresh MS medium containing different concentration plant growth regulators. The most suitable hormones concentration to induce explant regeneration was 1.0 mg/l BAP and 0.1 mg/l NAA where maximum number of shoot buds (16-20) were formed from callus within 5-6 weeks (Table-1; Fig.-1). Similar results were seen in *Casuarina cunninghamiana* Miq. (Jiang *et al*., 2012)..In *Saussurea obvallata,* callus differentiation was obtained on BA and NAA (Dhar and Joshi, 2005). However, contrary to this, IAA induced maximum shoots from callus in *Kosteletzkya pentacarpos* (L.) Ledeb. (Piovan *et al*., 2010).

CONCLUSION

From the current study, it is concluded that MS medium supplemented with growth regulator 2,4-D and BAP was successful for callus formation and NAA and BAP for multiple shoot proliferation of *Eclipta alba*. This study aims to develop a standard protocol to initiate multiple shoot culture at a standardized media and hormonal concentration of plant that maybe beneficial for *in vitro* large scale propagation of the plant.

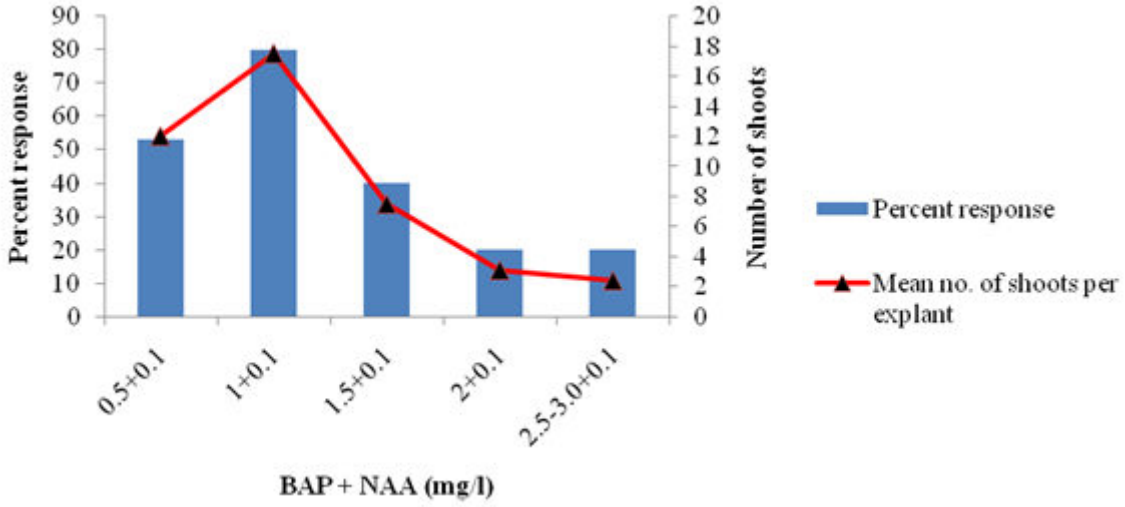
Table 1

Effect of combination of cytokinin (BAP) and auxin (NAA) on multiple shoot regeneration from callus of Eclipta alba (L.) Hassk.

Treatment ($mg.l^{-1}$)		Total explant taken	Explant showing multiple shoot regeneration	Percent response	No. of shoots per explant±SD	Average shoot length (cm)±SD
BAP	NAA					
0	0.1	15	0	0	0	0
0.5		15	8	53	12 ± 1.41	4.3 ± 1.41
1		15	12	80	17.5 ± 1.6	5.6 ± 2.1
1.5		15	6	40	7.5 ± 2.1	3.3 ± 1.06
2		15	3	20	3.1 ± 1.7	2.1 ± 1.76
2.5-3.0		15	3	20	2.4 ± 1.6	1.7 ± 1.8

Figure 1

***Effect of combination of cytokinin (BAP) and auxin (NAA) on multiple shoot regeneraton from callus of Eclipta alba (L.)* Hassk.**



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