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MICROPROPAGATION OF *ECLIPTA ALBA* (L.) HASSK. AN IMPORTANT MEDICINAL PLANT OF TRADITIONAL MEDICINE

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ABSTRACT

Eclipta alba was successfully micropropagated using nodal segments as explants. Multiple shoots were obtained by culturing the explants on MS medium supplemented with various concentrations of cytokinins *viz*., BAP and Kn alone or in combination with auxins (IAA/NAA). Rooting was induced by transferring the obtained multiple shoots to the medium supplemented with various auxins (IAA/NAA/IBA) at different concentrations. The effect of strength of MS medium on root induction was also observed. The *in vitro* developed rooted plantlets were acclimatized successfully and transferred to soil.

Keywords: Eclipta alba, In vitro, Micropropagation

INTRODUCTION

Eclipta alba (L.) Hassk. (Asteraceae) is a small, branched annual herb with white flower heads. The plant is commonly used in hair oil all over India for healthy black and long hair. The plant has a reputation as an anti-aging agent in Ayurvedic medicine. The main active principles of *E. alba* are wedelolactone and demethylwedelolactone, both of which possess anti hepatotoxic activity (Wagner *et al*., 1986 and Franca *et al*., 1995). In recent years, the harvest of medicinal plants on a mass scale from their natural habitats is leading to a depletion of plant resources. Therefore, the conservation of these valuable plants is necessary. *In vitro* culture techniques offer a viable tool for mass multiplication and germplasm conservation of important medicinal plants like *E.alba*. Therefore it is important to develop an efficient micropropagation technique for *Eclipta alba* for rapid multiplication and secondary metabolite production. There have been few reports to date on micropropagation in the genus using nodal explants (Franca *et al*., 1995; Zafar and Sagar, 1999 and Borthakur *et al*., 2000). The purpose of this study was to develop an *in vitro* propagation method from nodal explant of *E. alba*, an economically important species.

MATERIALS AND METHODS

The MS (Murashige and Skoog, 1962) medium fortified with 3% sucrose, 0.8% agar-agar (used to solidify the medium). The pH of the medium was adjusted to 5.8 by adding 1N NaOH/ 1N HCl and then autoclaved at121^{0}C for 20 minutes. Collection of healthy and profusely growing *Eclipta alba* plants was done from National Institute of Ayurveda and Amber hills area, Jaipur. Nodal segments were used for this purpose. The explants were washed under running tap water for few minutes 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 in laminar air flow cabinet under aseptic conditions. 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. Then the explants were cultured on agar solidified MS medium supplemented with different concentrations of cytokinins BAP/ Kn (0-3.0 mg/l) alone or in combination with auxins NAA/ IAA (0-3.0 mg/l). 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. Shoots obtained were transferred on rooting media like full strength MS, half strength MS, one fourth strength MS medium fortified with auxins IAA/IBA/NAA (0-5.0 mg/l). The rooted plantlets were taken out from the culture vessels and rinsed with sterilized distilled water to remove the adhering agar. After washing, plantlets were transferred to plastic pots containing a mixture of sterilized vermiculite and soil (1:3). For 2 weeks, they were placed in growth chamber and covered with inverted plastic pots to maintain humidity. After 2-3 weeks, these pots were uncovered and then they were exposed to partial and then complete direct sun light. Finally, these hardened plants were transferred to the fresh pots containing the normal garden soil for their further growth and development.

RESULT AND DISCUSSION

During the present study, various concentrations of BAP and Kn were used to obtain multiplication of shoots from nodal explant. Results proved that BAP was effective in multiple shoot formation. Data showed that MS media supplemented with BAP (1 mg/l) was optimum for shoot production where 15-17 shoots were produced. . Similarly, Baskaran and Jayabalan (2005) also showed BAP was better for shoot regeneration as compared to Kn. Similar results were shown in *Aconitum balfourii* Stapf. (Pandey *et al*., 2004) and *Chrysanthemum morifolium* Ramat (Shatnawia *et al*., 2010). However, Borthakur *et* al. (2000) in *Eclipta alba*, showed Kn enhanced the number as well as the length of shoots in comparison to BAP. During the present study, there was a decrease in the number of shoots as well as shoot length as the concentration of cytokinin was increased. Hu and Wang (1983) also reported reduction of number of shoots at higher concentration of cytokinin. Combination of auxins (NAA/IAA) with optimized concentration of cytokinin was also evaluated for multiple shoot regeneration (Table-1). It was observed that BAP (1 mg/l) with NAA (0.1 mg/l) proved to be most effective for shoot induction with 90 % shoot induction where highest numbers of shoots 17-20 were produced (Fig.-1). Similar results in *Eclipta alba* (Hassan *et al.*, 2008), *Solanum aculeatissimum* Jacq. (Ghimire *et al*., 2012).

During the present investigation, maximum rooting was induced on half strength of MS medium supplemented with IBA (0.5 mg/l) showing healthy roots with maximum number of roots (18±1.47) and root length (10-12 cm), 90 % root induction (Table-2; Fig.-2). Similar results in *E.alba* have also been reported by Baskaran and Jayabalan (2005) and Husain and Anis (2006). Rooting on IBA was also observed by several workers in various medicinal plant species e.g. *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Pentanema indicum* Ling (Sivanesan and Jeong, 2007), *Azadiracha indica* A. Juss (Shahin-uz zaman *et al*., 2008) and *Pistacia vera* L. (Tilkat *et al*., 2009). Contrary to this other auxins has been reported for rooting in *Thymus vulgaris* (Ozudogru *et al*., 2011). The plants were then successfully transferred to the field. The acclimatized plantlets were established in the field with approximately 35 % survival. Similar procedure was also used in *Jatropha curcas* L. (Rajore *et al*., 2002), *Psoralea corylifolia* L. (Baskaran and Jayabalan, 2009), *Citrullus colocynthis* (Linn.) Schrad. (Meena *et al*., 2010).

CONCLUSION

In conclusion, a culture method for multiple shoot induction and plant regeneration was developed. MS medium supplemented with combination of BAP and NAA was most successful for multiple shoot

proliferation. Protocols described here provide a rapid and quick system for whole plant regeneration which could be used for the large scale micropropagation of the plant.

Table - 1

Effect of various auxins in combination with cytokinin(BAP-1.0 mg/l) on multiple shoot regeneration in Eclipta alba (L.) Hassk.

Treatment	Total explant taken	Explant showing shooting	Per cent response	No. of shoots per explants Mean±SD	Average shoot length Mean±SD
NAA					
0	15	Nil	Nil	Nil	Nil
0.1	15	13.5	90	18.2 ± 1.55	4.7 ± 1.06
0.5	15	11.5	76	11.8 ± 1.76	4.0 ± 1.48
1	15	10	66	7.1 ± 1.69	3.4 ± 1.34
1.5	15	9	60	6.5 ± 1.41	3.1 ± 1.48
2.0-3.0	15	6	40	4.6 ± 1.34	2.5 ± 1.83
IAA					
0	15	Nil	Nil	Nil	Nil
0.1	15	12	80	11.6 ± 1.55	4.3 ± 1.76
0.5	15	10.5	70	7.5 ± 1.76	3.6 ± 1.27
1	15	8	53	6.1 ± 1.69	3.2 ± 1.83
1.5	15	7	46	4.8 ± 1.27	2.7 ± 1.69
2.0-3.0	15	5.5	36	3.4 ± 1.55	2.1 ± 1.55

Figure 1

***Effect of NAA in combination with BAP (1.0 mg/ l) on multiple shoot regeneration in Eclipta alba (L.)* Hassk.**

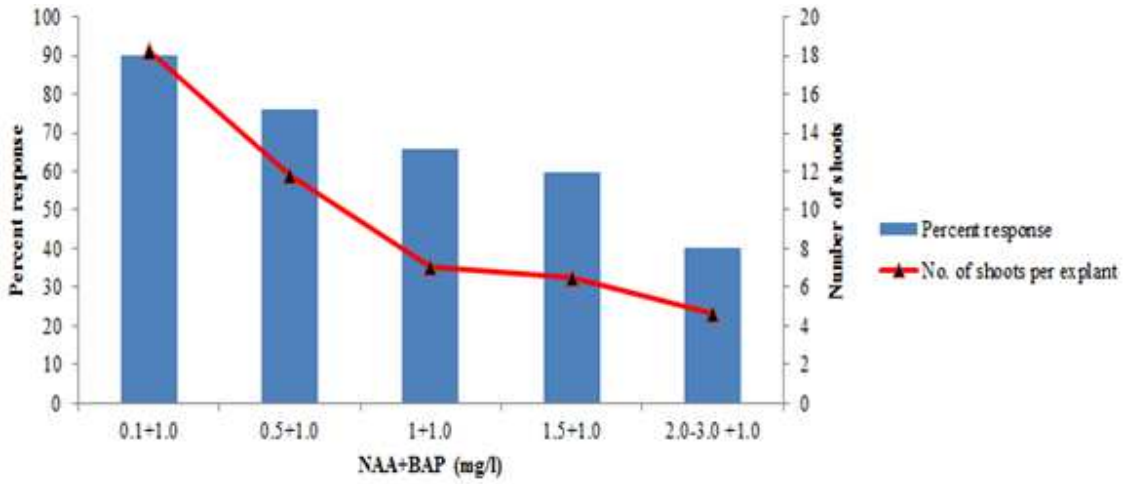
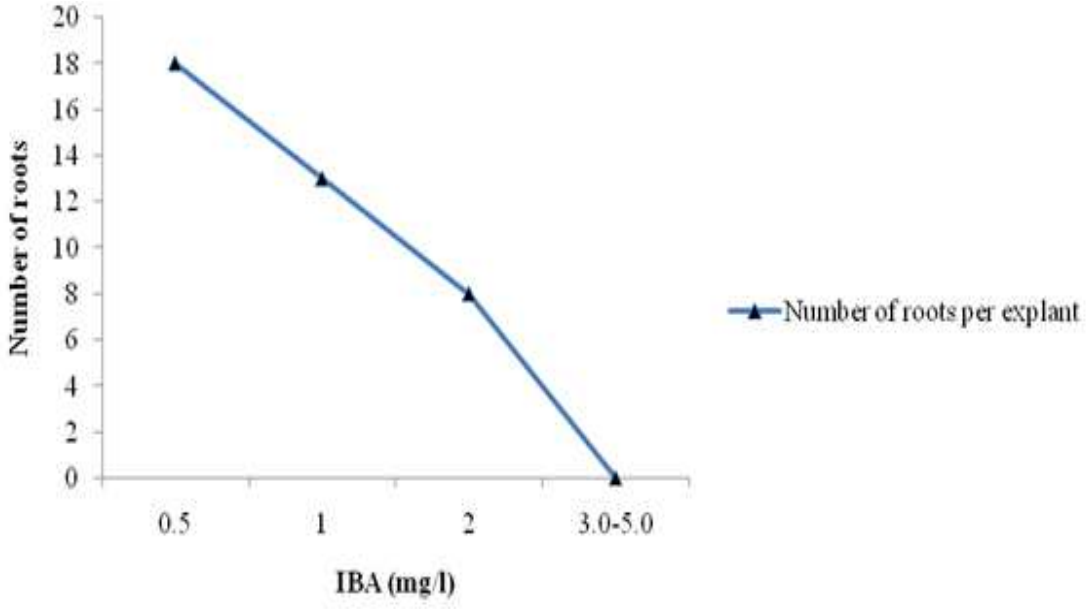


Table - 2
***Effect of various auxins on rooting in Eclipta alba (L.)* Hassk.**

Auxin concentration (mg/l)	Number of roots per explant	Remarks
IAA		
0	Nil	
0.5	4±0.56	Few roots were observed
1.0	6±0.70	
2.0	3±0.30	
3.0-5.0	Nil	
IBA		
0	Nil	
0.5	18±1.47	Well developed root system, roots were healthy, white in colour, fast growing
1.0	13±0.89	
2.0	8±0.20	
3.0-5.0	Nil	
NAA		
0	Nil	
0.5	5±1.12	Very small response was observed
1.0	3±0.17	
2	1±0.33	
3.0-5.0	Nil	

Figure 2
***Effect of IBA on rooting in Eclipta alba (L.)* Hassk.**



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