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Research Article

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A Novel Protocol for Propagation of *Eclipta alba* (L) Hassak

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ABSTRACT

An efficient, rapid and large-scale in vitro clonal propagation of the valuable medicinal herb, Eclipta alba (Asteraceae) using seed was designed. The type of medium used, plant growth regulators markedly influenced in vitro propagation of E. alba. The in vitro plantlet production system was investigated on Murashige and Skoog (MS) medium with the synergistic combination of BAP (2 mg/L) and NAA(1 mg/L). This composition of the medium influenced rapid plant growth .Sub culturing of the cut segments of the grown plant on similar medium enabled continuous production of healthy shoots with similar frequency. Each segment of the plantlet proliferated and grew into healthy plants having perfect rooting and shooting. Micro propagated plants acclimatized in a mixture containing garden soil, and vermicompost (2:1).The established plant in the pot were uniform and identical to the donor plant with respect to growth characteristics. The method seems to be efficient which has been performed in two steps. The first step being cultivation of plant from seed which can be suitably used for genetic manipulation of the plant for useful purpose. The second step can be used for mass propagation. The method seems to be efficient as both the step can be performed on the same composition of the medium.

Keywords: *Eclipta alba, Seed, Plant as ex-plant, Propagation, Acclimatization.*

INTRODUCTION

Medicinal plants are valuable sources of medicine and many other pharmaceutical products. The conventional method used for propagation of these medicinally important plant is both time consuming, and also deficient in germination and growth. Also sometimes clonal uniformity is not maintained through seeds. The plants used in the phyto-pharmaceutical preparations are obtained mainly from the natural habitats. In recent years there has been an increase in the demand for the crude drugs, as a result of which the plants are being over exploited, threatening the survival of many rare species. Also, many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation, and indiscriminate collection. Advanced and sustainable methods of culturing plant cells and tissues have proved to be a determining factor in propagation of these potentially important plants. This would also provide new means for conserving and rapidly propagating valuable, rare, and endangered medicinal plants. Eclipta alba is an important medicinal plant[5,14,16]. It is known to contain various alkaloid[12] and chemical constituents[19] of diverse nature. Some of the chemical properties available in the plant has been used in hair growth[3,10,13] hepato protective role[9,18] neuro pharmacological role[17], role as anti oxidant[8] and as analgesic[15]. During present study *in vitro* propagation of *Eclipta alba* from seed has been described.

MATERIALS AND METHODS

Source of explant

Field grown plants of *Eclipta alba* was used as explants for *in vitro* cultivation. Various parts of the plant such as stem, node of the stem, leaf and seed was used as plant source.

Sterilization

The plant material was first washed in continuous running tap water for 2 hours and then in sterilized distilled water mixed with tween 20. Washing in tween 20 was performed with vigorous shaking to remove surface adhered contaminants. The plant sample was once again washed with sterilized distilled water mixed with Bavistin (01%).The plant sample was then washed three times in sterilized distilled water before transfer to laminar flow chamber for transfer to culture media. The plant sample was further disinfected with 0.1 %(w/v) mercuric chloride (HgCl2) for 5 min followed by thorough rinsing in autoclaved distilled water for at least 7 to 8 times. To avoid bacterial contamination the plant sample was treated with Mox solution (500 mg Mox dissolved in 100 ml of sterilized distilled water). The surface sterilized explants contained seeds, nodal segments having length of 0.5 to 1cm containing single node.

Culture medium and condition

MS medium was used for in-vitro culture of explants (Murashige & Skoog,1962). This medium was fortified with 3% sucrose, and 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 C for 20 minutes. α – Naphthalene acetic acid- NAA (1 mg/L) and 6-Benzylaminopurine - BAP (2 mg/L) were used to observe its effect on seed germination shoot initiation and root initiation. The cultures were maintained at 25 ± 1°C under 16 h photo-period provided by white fluorescent tubes.

RESULTS AND DISCUSSION

Review of literature on micro propagation of E. alba[1,2,4,6,7,14,16] suggest that this plant can be propagated on MS medium quite easily. There is report of its propagation from every part of the plant but there is scanty report of its propagation directly from the seed. Hence, propagation was undertaken directly from the seed.

Seed as ex plant

In the first instance matured seed of *Eclipta alba* was surface sterilized as described and transferred to culture media at desired temperature and exposure to light. After 7 days the seed appeared to germinate and acquired the shape of a newly germinated plant. After 25 days of incubation in a similar condition the growing plant gained a height of 2 cm along with 6 leaves attached to it. With lapse of time the plant acquired a luxuriant growth and also exhibited increase in the number of leaves and branching. The size of the leaves also increased. This has been sequentially shown (Fig 1, Fig 2 and Fig 3).

Fig.1- Germinating seed

Fig. 2- Growth after25 days

Fig. 3- Growth after 40 days

The fate of the growing ex plants has also been shown in Table 1.

Table-1. Fate of germinating seed of *Eclipta alba*

DAYS	SIZE OF EXPLANT(CM)	NO OF LEAVES	REMARKS
INOCULATED	EXPLANT	--------	---------
AFTER 18 DAYS	MINUTE STRUCTURE	----------	SEED GERMINATED
AFTER 25 DAYS	1.2	06	ATTAINED HEIGHT
AFTER 40 DAYS	2	12	MARKED BY BRANCHING

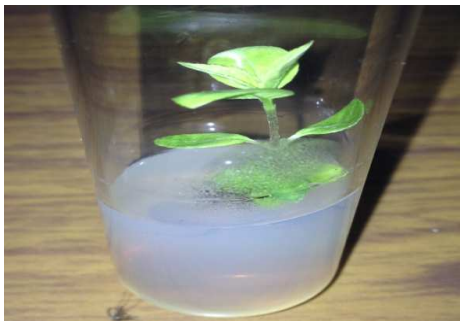
The growth of the plant became restricted after 40 days of the growth, so the plant was sub cultured on fresh culture media to allow further growth and development. The entire plant was gently removed from the culture tube along with the agar media. It was then washed with sterilized distilled water to remove agar. Subsequent upon this the plant was cut into small workable pieces .During cutting it was kept in mind that the structure of the explants should contain those plant part which is generally used for growing explants (Figure 4 ,5,6 &7).

Sub culturing the growing plant

The plantlet was transferred on freshly prepared MS media supplemented with BAP (2 mg/L) and NAA (1 mg/L).

Fig. 4-Subculture-Tip **Fig. 5-Subculture-Branch**

Fig.-6 and Fig. 7 -- Growth after 20 days



The transferred plantlet exhibited continuous growth and after 38 days of incubation tuft of rootlets also appeared (Fig 8 and Fig 9).

Fig.-8 and Fig. 9 -- Growth after 60 days

After 70 days of growth the upper leaves of the growing plant started showing yellowing of the leaves. But after 75 days the leaves again became green to become normal in morphological characteristics. This has been shown in Fig 10. The developed regeneration protocol enables a large scale commercial production and a possible system towards the genetic improvement of this crop.

Fig. 10-Full grown plant (75 days)

As the plant attained normal morphological growth in the culture tube, it became essential to transfer it to a plastic pot in order to acclimatize the plant to the soil. During present investigation, shooting and rooting was induced on MS medium supplemented with BAP (2 mg/L) and NAA (1 mg/L) showing healthy roots with maximum number of roots being 23 and root length (10-12 cm).Shoot length also increased at an interval of 10 days by 1 cm .Similar results in *E.alba* have also been observed[16] using different combination of BAP and NAA. Sharma A. et al. have suggested induction in shooting at a concentration of 1 mg/L of BAP and 0.1 mg/ml of NAA. A similar observation has also been suggested by [7]. [16] have reported rooting in *E. alba* using 0.5 mg/L of IBA. So, Sharma A. et al. used different combination for shooting and rooting but present finding seems to be an improvement over previous report of [16] and [7] as the same combination of medium initiated seed germination, root and shoot development and also propagation through nodal segment.[6] in a similar kind of experiment carried out on *Eclipta alba* to facilitate shortening of time for micro-propagation successfully used 6-benzyladenine(BA) and observed initiation of multi-shooting in the plant. In this study multi shooting could be observed in *Eclipta alba* using the same combination of media which has been used for rooting and shooting.

ACCLIMATIZATION AND TRANSFER OF PLANTLETS TO THE SOIL

Plantlets with well-developed roots were removed from the culture medium and after washing the roots gently under running tap water, plantlets were transferred to plastic pots containing autoclaved garden soil, vermicompost (2:1). All were irrigated with 1/8 MS basal salt solution . The potted plantlets were covered with porous polyethylene sheets for maintaining high humidity and were maintained under the culture room conditions. The transferred plant remained green and healthy four 4 days after which it started showing decay from the top. After 7 days however, fresh leaves appeared from the lower part of the plant. This suggested acclimatization of the transferred plantlet to the soil (Fig 11, Fig 12 and Fig 13).

Fig. 11- Fresh sub cultured plant in soil after 79 days

Fig. 12- Fresh sub cultured plant in soil after 85 days

Fig. 13-Acclimatized plant after 90 days

CONCLUSION

Present study revealed that the in vitro plantlet production system was effective in *Eclipta alba* on MS medium supplemented with the combination of NAA (1 mg/L) and BAP (2 mg/L).This composition of the medium was sufficient enough to encourage germination of the *Eclipta* seed and also favoured normal growth of the plant in the culture tube, as luxuriant growth of both shoot and root was noticed. This took almost 40 days to yield this result. A temperature of 25 ± 1°C was strictly maintained along with intermittent exposure of the growing plant to16 h photo-period provided by white fluorescent tubes. The growing plant was gently removed from the tube was cut into workable pieces and re- inoculated on the same medium as described above. The explants started growing to a limit of perfection. This is because every ex plants after 60 days turned into a miniature plant having developed shoot and root suitable for hardening. In conclusion it may be stated that the protocol presented in this study yields efficient shoot and root regeneration both from seed and cotyledonary nodes. These results will encourage large scale micro propagation of this important medicinal plant. The protocol reported here could also be used for conserving the plant and for mass scale clone propagation.

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