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High frequency somatic embryogenesis and plant regeneration in nodal explant cultures of *Eclipta alba* L. Hassk

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ABSTRACT

Nodal explants excised from Eclipta alba L. plants maintained in vitro formed yellowish white, friable calluses after three weeks of culture on Murashige and Skoog (MS) medium supplemented with 10.75 µM α-napthaleneacetic acid (NAA) and 9.04 µM 2,4-dichlorophenoxyacetic acid (2,4-D). These calluses were subsequently transferred to MS basal medium where after an additional four weeks of culture, approximately 50% of the calluses could form somatic embryos. However, calluses formed on nodal explants that had been cultured on MS medium supplemented with indole-3-acetic acid (IAA) did not produce somatic embryos upon transfer to MS basal medium. Somatic embryos were developed into plantlets and subsequently grown to maturity. These results indicate that nodal explants have high competence for somatic embryogenesis in Eclipta alba .

Keywords: *Eclipta alba*. L. Hassk, Somatic embryogenesis, Plant regeneration, *in vitro* propagation.

INTRODUCTION

Eclipta alba is one of the most commercially important medicinal plants in the world. It is popularly used to prevent abortions, night blindness, hernias, bronchitis, leucoderma, vertigo, memory modulator and externally used for ulcers and as an antiseptic for wounds in cattle [1,2]. There are reports available on phytochemical analysis which indicates that this plant is rich in Wedelolactone, Dimethylwedelolactone, *β*-amyrin, stigmasterol and luteolin-*7*-glucoside containing anti-hepatotoxic activity [3-5]. It is also used as antihelmintic, expectorant, antipyretic, antiasthmatic, deobstruent in hepatic and spleen enlargement, in skin diseases and as

a substitute for Taraxacum, anti-oxidant, hepatoprotective, immunomodulatory and nootropic properties[6-10]. Due to the above mentioned medicinal properties, an efficient plant regeneration system are required for this species to propagate unique lines and to improve the quality based on somatic cell genetics and rDNA technology. Plant regeneration *via* organogenesis has been extensively studied in *Eclipta alba* using various explants [11]. However, somatic embryogenesis has only been studied in a limited way [12].

Somatic embryogenesis usually provides more advantages and benefits over organogenesis. Shoot and root development occurs simultaneously so that the procedure for plant regeneration is simple and a potentially greater multiplication of propagules can be achieved, especially when embryogenic cell suspension cultures are available. Somatic embryos of some plants like potato have been formed on internode derived calli, which has subsequently not completely developed into plants [13]. Somatic embryos have also been directly formed on leaf explants without intervening callus phase on a culture medium containing antibiotics at a low frequency [14] and on culture medium containing Indole acetic acid (IAA) and 6-benzylaminopurine (BAP) at higher frequency with subsequent development into plants [12]**.** However, these studies failed to obtain highly competent calluses for the production of somatic embryos. Highly competent calluses are required to establish embryogenic cell suspension cultures. In the present investigation, we studied about high frequency somatic embryogenesis and plant regeneration from highly competent node derived *Eclipta alba*.

MATERIALS AND METHODS

Plant materials:

Approximately 0.5 cm long shoot tips of *Eclipta alba* L. were cultured on Murashige and Skoog (MS) medium supplemented with 4.43 μM BAP in 500 ml culture bottles containing 100 ml of medium. Each bottle contained five shoot tips. After three weeks of culture under light (approximately 30 μmol m^{-2} s^{-1} from cool-white fluorescent lamps with a 16-h photoperiod) at 25^{o}C, multiplied axillary shoots were separated into single shoots followed by subculturing for multiplication or transfer to basal medium for regeneration into whole plants. Nodes of regenerated plant were cut into 0.5-1 cm long explants for embryogenic callus formation.

Culture media and conditions:

The culture medium used throughout the experiments consisted of MS inorganic salts, 100 mgl^{-1} myo-inositol, 0.4 mg l^{-1} thiamine HCl, 3% (*w/v*) sucrose, 0.4% (*w/v*) Gelrite. The pH of all of the media was adjusted to 5.8 before autoclaving. Twenty-five milliliters of medium were dispensed into each plastic Petri dish (87-15 mm). Unless mentioned otherwise, all cultures were incubated at 25^{o}C in the dark.

Induction of embryogenic calluses:

To investigate the effect of growth regulators on embryogenic callus formation, nodal explants were placed on MS medium supplemented with 2 to 21 μM 2,4-D or NAA at a sole growth regulator. In addition, nodal explants were placed on MS medium supplemented with 2.21, 4.43, 6.65, 8.87, 11.09, 13.31, 15.53 and 17.75 μM BAP in combination with 5.37 or 10.75 μM NAA. Each treatment consisted of 10 explants per dish with three replicates. After three weeks of

culture, data for the mean frequency of nodal explants producing yellow calluses were recorded. Calluses that formed on nodal explants were transferred to MS basal medium without removal of initial node explants under light, as described above. Each treatment consisted of ten explants per dish with three replicates. After an additional four weeks of culture, data for the mean percentage of nodal explants producing somatic embryos were collected.

Plant regeneration:
Plantlets developed from somatic embryos were cultured on MS basal medium under light, as described above. Approximately 2-3 cm long plantlets were cultured on half strength MS medium in 500 ml cultured bottles containing 100 ml of medium. Plantlets were subjected to acclimatization, transplanted to potting soil, and maintained in a growth chamber (25^oC, approximately 30 μmol l m^{-2} s^{-1} from cool fluorescent lamps with a 16 h photoperiod).

RESULTS AND DISCUSSION

Nodal explants cultured on medium supplemented with various growth regulators solely or in combination formed yellowish white friable calluses on the surfaces after three weeks of culture (Plate. 1). Node explants formed calluses at a frequency of 96% on medium containing 5.37 μM NAA as the sole growth regulator (Fig.1). The frequency of callus formation increased with increasing concentration of NAA up to 5.37 μM. Node explants formed few calluses on medium containing 8.09 μM 2,4-D and less as the sole growth regulator. However, nodal explants formed calluses at a frequency of 90% on medium containing 9.04 μM 2,4-D. Nodal explants turned brownish on medium containing BAP as a sole growth regulator, scarcely forming calluses. However, explants formed calluses on medium containing 6.65 μM BAP in combination with NAA at a frequency greater than 52.8% and reached the highest frequency of 94.6% when they were cultured on medium containing 10.75 μM NAA and 5.37 μM NAA.

Calluses formed somatic embryos:
After four weeks of culture on MS basal medium, calluses formed on nodal explants that has been cultured on medium containing either 2,4-D, NAA or BAP as a sole growth regulator did not form somatic embryos. However, calluses formed on nodal explant that has been cultured on medium containing BAP in combination with NAA formed globular to heart-shaped somatic embryos which subsequently developed into torpedo-shaped to cotyledonary somatic embryos (Plate.2). The highest frequency (50%) of somatic embryo formation was obtained from calluses on nodal explants that had been cultured on medium containing 13.31 μM BAP in combination with 5.37 μM NAA (Fig.2). Over the range of BAP tested in this study, calluses formed on node explants that had been cultured on medium containing 10.75 μM NAA produced somatic embryos at higher frequencies than that of cultured on medium containing 5.37 μM NAA. Somatic embryos were developed into plantlets at a frequency of approximately 80% (Plate.3). Plantlets transplanted into potting soil (Plate.4) were maintained in a growth chamber where they were grown to maturity in normal appearance.

Somatic embryogenesis in nodal explant cultures:
In this study, we have demonstrated that *Eclipta alba* nodal explants have a high competence for somatic embryogenesis. We established a somatic embryogenesis system that was different from other systems. In many somatic embryogenesis systems, embryogenic calluses are

morphologically different from non-embryogenic. In this study, at best 50% of the initial callus formed on node explants produced somatic embryos upon transfer to MS basal medium, indicating that all the calluses formed on root explants were not embryogenic. However, there was no morphological distinction between embryogenic and non-embryogenic calluses.

Fig. 1: The frequency of callus formation on nodal explants cultured on MS medium supplemented with various concentrations of 2,4-D (diamond), NAA (filled square), and BAP (triangle) as a sole growth regulator and BAP in combination with 5.37 *µ*M (cross) and 10.75 *µ*M (star) NAA in *Eclipta alba.* Data were collected after three weeks of culture. Each treatment consisted of 10 explants per dish with three replicates. Vertical bars represent SE of the mean.

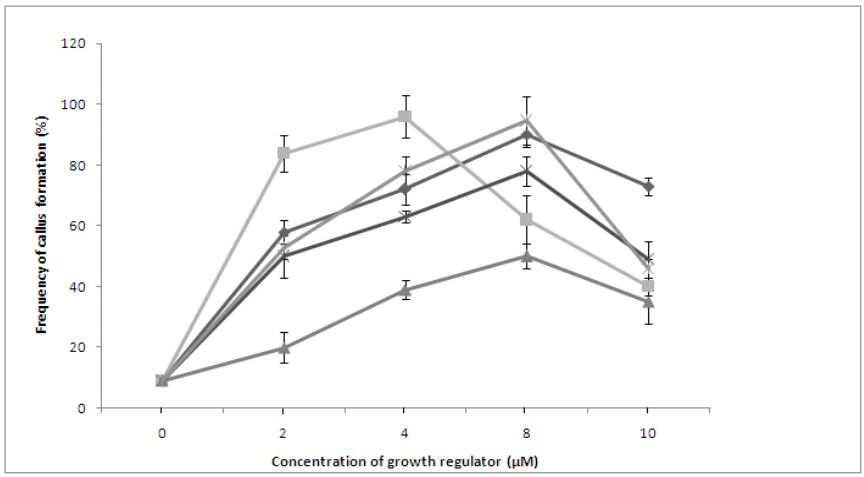
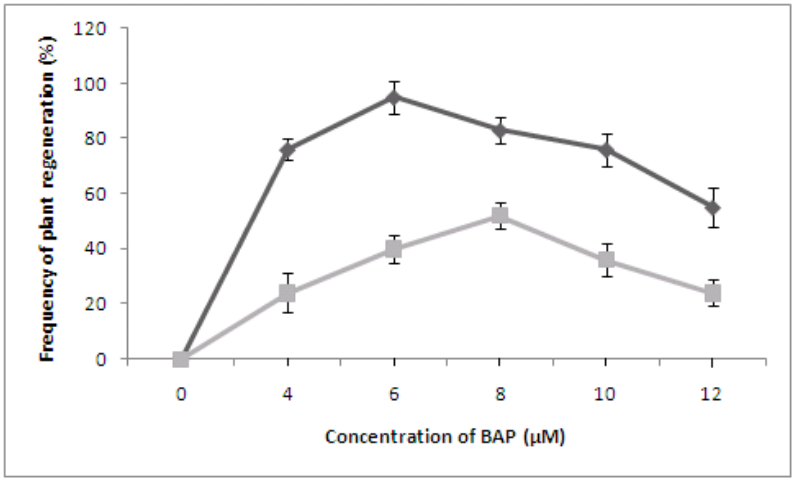


Fig. 2: The frequency of somatic embryo formation on calli derived from nodal explants that had been cultured on MS medium supplemented with various concentrations of BAP in combination with 5.37 *µ*M (triangle) and 10.75 *µ*M (diamond) NAA in *Eclipta alba.* Data were collected after an additional four weeks of culture. Each treatment consisted of 10 explants per dish with three replicates. Vertical bars represent the SE of the mean.



All the initial callus formed on node explants were yellow and friable. In addition, 2,4-D is used

most often as the sole growth regulator for producing embryogenic calluses in many species. However, both 2,4-D, NAA and BAP as the sole growth regulators haven't lead to competent callus formation for the production of somatic embryos.

Plate-1. Yellowish white friable callus

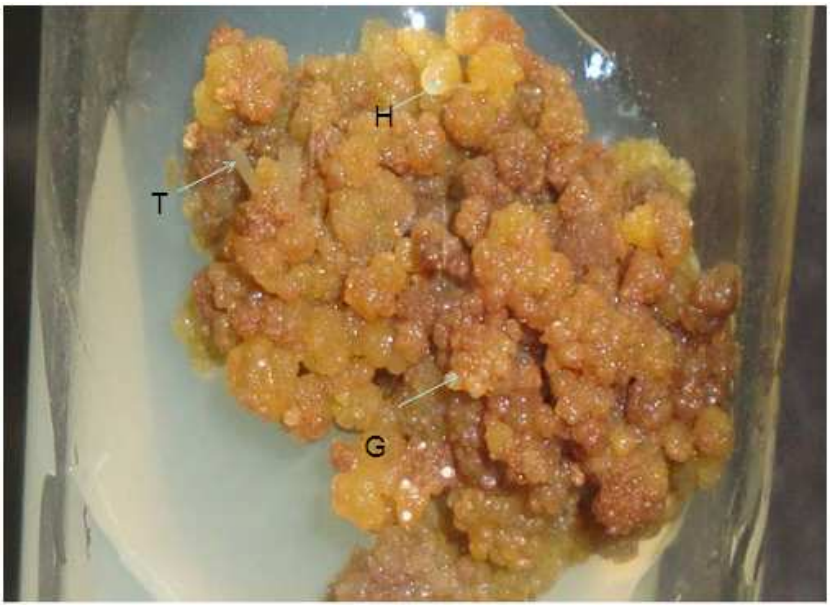


Plate-2 Somatic embryogenesis showing different stages. T-Torpedo, G-Globular and H-Heart shape

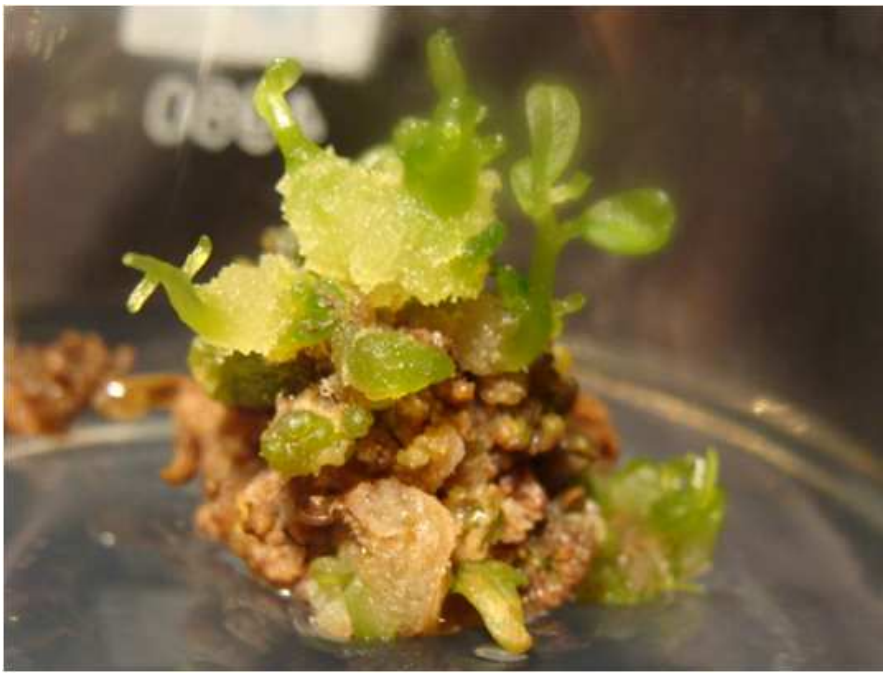


Plate-3 Regeneration from somatic embryoides

Plate-4 Transferred to pots

Competent calluses were formed on nodal explants cultured on medium containing BAP in combination with NAA.

Nodal explants have been considered as competent explants for plant regeneration *in vitro*. However, somatic embryos have been formed directly from nodal explants in a few species, including Teasle gourd [15]. Recently, plant regeneration from node derived callus through somatic embryogenesis was reported in *Clematis* [16] and *Trachyspermum ammi* [17].

CONCLUSION

In previous studies, results had shown low frequency of competent calluses for the production of somatic embryos in *Eclipta alba*. We are able to obtain highly competent node derived calluses for somatic embryogenesis. We are now trying to develop cell suspension cultures with these calluses that will enable multiplication of unique *Eclipta* lines on commercial scale in addition to biotransformation.

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