Original Research Article

PLANT REGENERATION STUDIES IN EUPHORBIA FUSIFORMIS THROUGH SOMATIC EMBRYOGENESIS.

ABSTRACT

Euphorbia fusiformis is a rare medicinal plant. The genus Euphorbia belongs to the family Euphorbiaceae. *E. fusiformis* is commercially useful for production of latex and has many medicinal values. Based on the importance of the plant, it is selected for plant tissue culture work. The present research work is to establish somatic embryogenesis from explants of leaf. It is first attempt of the regeneration studies in *E. fusiformis* through somatic embryogenesis. For plantlet regeneration studies MS medium supplemented with α-Naphthalene Acetic Acid (NAA) 2.0 mg/l and 2, 4-Dichloro Phenoxy acetic acid (2, 4- D) 2.0 mg/l were used.

KEY WORDS: *Euphorbia fusiformis*, Somatic embryogenesis, α- Naphthalene Acetic Acid

INTRODUCTION

The genus Euphorbia belongs to the family Euphorbiaceae. It is one of the largest families of flowering plants comprising of five subfamilies, 49 tribes, 317 genera and about 8,000 species [1]. Members of the Euphorbiaceae have been popular for traditional ~~&~~ medicin~~es~~al ~~herbs~~. Euphorbiaceae members [2] are important for producing very useful substances & 33 species belonging to 17 genera of Euphorbiaceae were reported to be used in herbal medicine. Similar reports have been cited for the ancient Yucatan herbal system ~~applying~~ utilizing different Euphorbiaceae members. Major components of Euphorbia latex are sterols, terpenoids, vitamins and insecticides and anti-~~-~~cancer drugs as [3], [4] ~~published~~ reported ~~on~~ on chemical constituents and economic important plants of Euphorbiaceae. The experimental material chosen is a succulent herb with fusiform cylindrical rootstock, where 5 to 8 roots are spread out in different directions, apex is branched and produces 2 to 3 growing points below the soil level. *E. fusiformis* locally called as 'Ban-muli' is of high medicinal value used to treat various diseases like rheumatism, paralysis, liver

disorders, arthritis, and abdominal disorders. Embryos ~~were~~ are normally produced *in vivo* following the union of female and male gametes resulting in a bipolar structure that includes~~d~~ both a shoot and a root ~~meristem~~ apex [5]. Somatic embryogenesis was first reported in 1958 [6], [7]. Since then information on somatic embryogenesis has accumulated greatly. Through this research, it is hoped that the in vitro plantlets, callus cultures somatic embryogenesis and histological studies of *E. fusiformis* Buch.-Ham. can be used as source for the production of useful phytochemical compounds.

METHODOLOGY

The plant material was collected from a famous forest area near Pakala Lake in Warangal District, of Telangana State, India. [Plate I Fig. a & b]. The plants were sprayed with the fungicide and insecticide 2-3 week prior to initiation and over head watering was strictly avoided. Freshly grown leaves, were selected as an explant source (Figure Ia). Leaf explants were washed in running tap water for 10 minutes to remove the dust or sand particles. The leaves were surface sterilized by using 0.5% of Sodium hypochlorite for 20 minutes. Few drops of Tween-20 were also added as a surfactant. After 20 minutes the plant material was washed three times with sterile distilled water to remove the traces of bleach with gentle shaking under sterile conditions. To avoid the latex the explants of leaf were pretreated with Ascorbic acid before inoculation for 15 min following the sterilization with mercuric chloride (0.05%) for 3 to 5 min and washed several times with sterile, distilled water and then were inoculated on culture tubes containing culture medium.

CULTURE MEDIA

The surface sterilized explants were then aseptically inoculated on sterile MS medium consisting of salts and vitamins of nutrient medium, Commercial grade sucrose, (3%) were used as sole carbon source separately. The medium was gelled with 0.8% agar. Phytohormones like auxin (NAA, 2, 4-D; (0.5-3.0) alone was used. The pH of the medium in all cases was adjusted to 5.8 before autoclaving at a pressure 1.06 kg/cm3.

CULTURE CONDITIONS

The cultures were incubated at 25±2°C temperature under cool, white fluorescent light (2000-3000 lux) and 55±5% relative humidity. 16/8 photo and dark period were maintained in growth chamber, respectively. 20 cultures were raised for each treatment and all experiments were repeated at least thrice. Data on embryogenic callus induction, multiple shoot stimulation and rooting were statistically analyzed and then mean was compared at the 0.05 level of significance. Observations were recorded periodically.

PROLIFERATION OF SOMATIC EMBRYOS

Stock callus maintained after subculturing on MS medium with 2, 4-D got converted into yellowish green nodulated callus. Further, after 5-6 weeks of sub culturing of this nodulated callus on the manipulated MS medium fortified with 2, 4-D + BAP proliferated and passed through all the typical stages of embryo development. [Plate III, Fig. a & b]. These stages were clearly observed in anatomical study of this embryogenic calli. Mature somatic embryos were then transferred to MS medium supplemented with BAP only for shoot induction and further development. The Shoots emerged from somatic embryos were then transferred to rooting medium (MS+IAA/IBA/ alone) and then allowed to mature.

RESULTS

Among the various concentrations of 2, 4-D and NAA tested individually, the percentage of somatic embryo formation was found to be higher at (2.0 mg/L NAA) in leaf explants. There was generally an increased tendency of somatic embryo formation with the increasing concentration of NAA up to (2.0 mg/L) 2, 4-D/NAA induced the formation of somatic embryos. Above (2.0 mg/l) 2, 4-D/NAA (Table.1) concentration the somatic embryo formation reduced. This might be due to altered hormonal levels in the medium which are critical for embryo formation. In the present investigation mature leaf explants showed maximum percentage of somatic embryogenesis and high frequency of somatic embryo induction/ explants (20.0 ± 0.33) [Plate II a]. The calli developed from mature leaf explants containing globular

embryos were transferred to maturation medium containing. MS medium supplemented with 2.0 mg/L 2, 4-D + 0.5 mg/L BAP respectively. Germination of somatic embryos was not observed when six week old somatic embryos of globular and torpedo development stages were transformed ???into the same MS medium. Hence the somatic embryos with various developmental stages (Globular, torpedo and cotyledonary) were further sub cultured on fresh MS medium containing various concentrations of BAP (0.5–5.0 mg/l) alone for germination of somatic embryos induced from leaf explants. Of these media tested MS + 3.0 mg/l BAP proved to be best for somatic embryo germination and plantlet formation was observed after 6 weeks of culture. (Table-2) [Plate III Fig. b].

DISCUSSION

The results obtained on somatic embryogenesis relating to 2, 4-D and BAP is in coincidence with the earlier workers. NAA in combination with BAP was found to be suitable for induction of somatic embryos in different plant species such as *Pinus tadea, Pinellia tripartite, Gossipium hirsutum, Solanum melongena* [8], [9] & [10]. In addition to the hormonal regume (2, 4-D & BAP) and (NAA & BAB) casein hydrolysate was effective in promoting motivation of somatic embryos, as reported by earlier workers in number of species of plants.

After taking microphotographs,[NPC1] it is confirmed that there was an increase in formation of embryos with an increase in duration of incubation period and 70-75% of cultures induced root formation supplemented with MS basal medium however NAA induced only root ~~premordia~~primordia, where plants could not survive. The plantlets regenerated through somatic embryogenesis were transferred from lab to field where success rate of survivability observed was around 85%.

CONCLUSION

Callusing efficiency from leaf explants in *E. fusiformis* Butch-Ham is studied and the protocols have been established. Among the auxins tested. 2, 4-D induced the high quantity of callus in hypocotyls and leaf explants. Leaf explants were proved to be

better in inducing high yield of callus of *E. fusiformis* Butch-Ham and Somatic embryo formation was achieved on 2, 4-D/NAA from leaf explants of *E. fusiformis* Butch-Ham.

Somatic embryogenesis was also observed on MS medium fortified with different concentration of auxin 2.4-D and NAA in leaf explants of *E. fusiformis* Butch-Ham. Somatic embryos were induced in all the concentrations of 2, 4-D/NAA in leaf explants. Less number of somatic embryos was observed at low concentrations of 2, 4-D/NAA, gradually increased in leaf explants respectively. Maximum percentage of somatic embryogenesis and high frequency of somatic embryo formation were found on MS medium fortified with NAA. Later the somatic embryos appeared to progress through globular and heart stages. For further germination NAA + BAP hormonal combination proved to be better in leaf explants of *E. fusiformis* Butch-Ham. The species *E. fusiformis* Butch-Ham. has great importance in commercial value, for rapid multiplication and its usage, synthetic seeds can also be developed using these somatic embryos.

Plate I. *Euphorbia fusiformis* collected from Pakal Forest
a) Digging of *Euphorbia fusiformis*
b) Explant of *Euphorbia fusiformis* with rootstock

Plate II Somatic embryogenesis of *Euphorbia fusiformis* under stereomicroscope
a & b) Different stages of somatic embryogenesis in *Euphorbia fusiformis*
c) Globular and torpedo stages of somatic embryogenesis of *E. fusiformis*

Plate III Germination of somatic embryos, regeneration & hardenining of *E. fusiformis*
a) Initiation of somatic embryogenesis in *Euphorbia fusiformis*
b) Germination of somatic embryos in *Euphorbia fusiformis*
c) Formation of rooted regerating plant in *Euphorbia fusiformis*
d) Hardening of regenerating plant in *Euphorbia fusiformis*

PLATE-I

PLATE-II

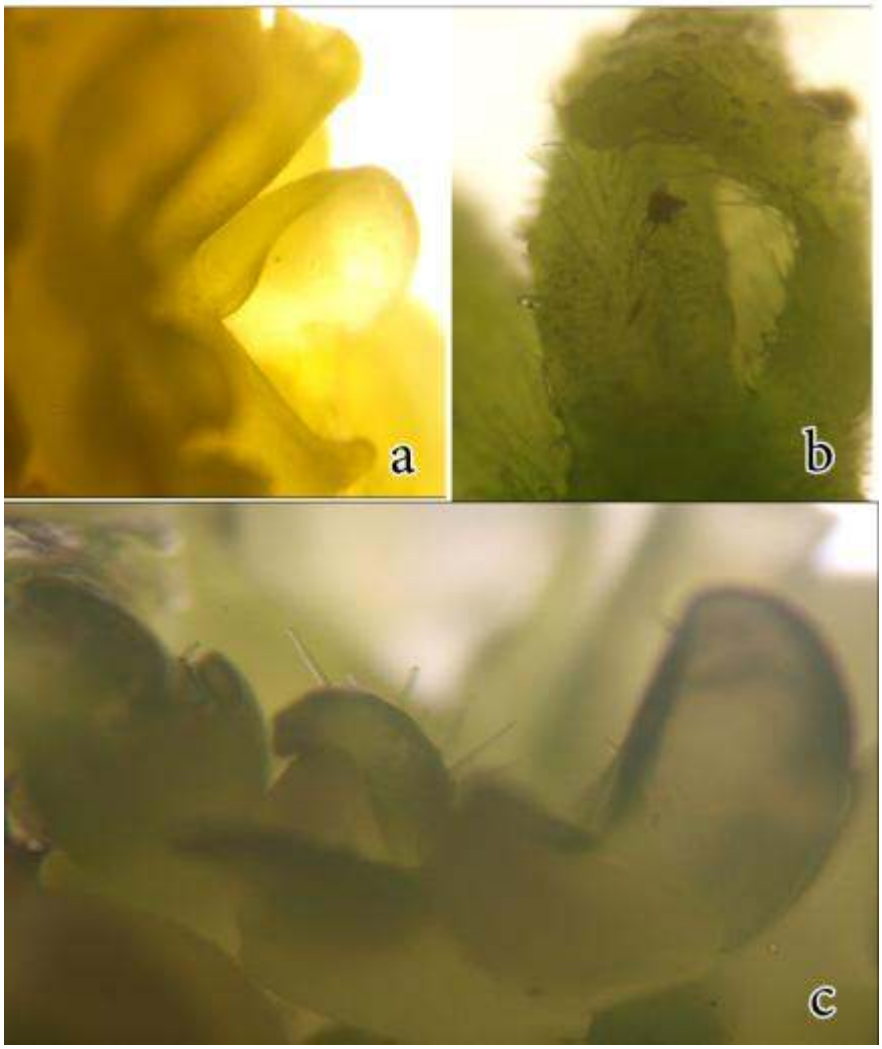

PLATE-III

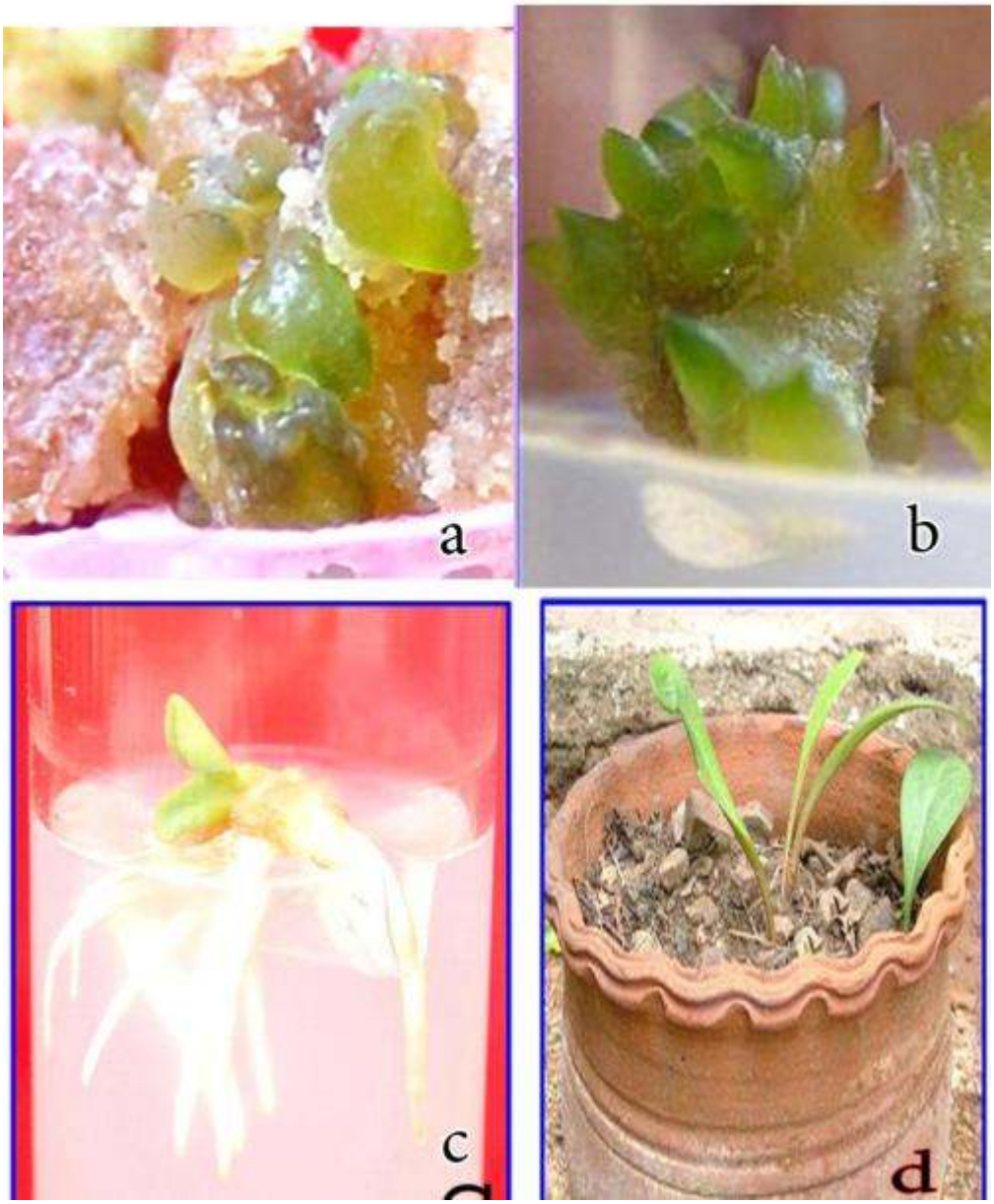

Table-1: Induction of somatic embryogenic callus from the leaf explants of *Euphorbia fusiformis* on MS medium supplemented with various concentration of 2, 4-D and NAA

Plant Growth regulator (mg/l)	% of cultures responding	% of response for somatic embryo formation	Average number of somatic embryos/ explants ±(S.E.)*
2, 4-D			
0.5	**70**	**50**	**8.3 ± 0.35**
1.0	**75**	**52**	**10.8 ± 0.36**
1.5	**80**	**66**	**12.0 ± 0.25**
2.0	**86**	**65**	**18.0 ± 0.25**
2.5	**65**	**70**	**15.0 ± 0.35**
3.0	**60**	**86**	**7.0 ± 0.36**
NAA			
0.5	**74**	**80**	**10.0 ± 0.45**
1.0	**76**	**65**	**12.0 ± 0.32**
1.5	**82**	**62**	**16.0 ± 0.42**
2.0	**90**	**60**	**20.0 ± 0.33**
2.5	**68**	**58**	**16.0 ± 0.23**
3.0	**64**	**50**	**0.8 ± 0.23**

*** Mean ± Standard Error**

Table-2: Effect of 0.5 mg/l IAA in combination with various concentration of BAP on the conversion of Somatic embryoids into Plantlets in *Euphorbia fusiformis*

Growth regulators	% of cultures responding	Germination of frequency (S.E)*
IAA + BAP		
0.5 + 0.5	60	10.0 ± 0.32
0.5 + 1.0	62	16.0 ± 0.46
0.5 + 1.5	64	18.0 ± 0.37
0.5 + 2.0	68	20.0 ± 0.43
0.5 + 2.5	70	22.0 ± 0.32
0.5 + 3.0	75	30.0 ± 0.32
0.5 + 3.5	68	26.0 ± 0.37
0.5 + 4.0	66	22.0 ± 0.36
0.5 + 4.5	55	18.0 ± 0.27
0.5 + 5.0	50	10.0 ± 0.37

***Mean ± Standard Error.**

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