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

1. 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 & medicinal herbs. Genus *Euphorbia* and indeed family Euphorbiaceae was named in honour of a Greek physician King Juba II of Mauritania. Since time immemorial, many Euphorbiaceae members [2] are important for producing very useful substances & 33 species belonging to 17 genera of Euphorbiaceae were used in herbal medicine. Similar reports have been cited for the ancient Yucatan herbal system applying different Euphorbiaceae members. Major components of *Euphorbia* latex are sterols, terpenoids vitamins and insecticides and anti cancer drugs [3], [4] published on chemical constituents and economic important plants of Euphorbiaceae. The present species choosen for research *E.fusiformis* is a succulent herb, rootstock cylindrical, fusiform, buried in the ground, 12 to 85 cm long (sometimes even 100 cm long) and 3 to 5.5 cm in diameter, with 5 to 8 roots emanating in all directions over the rootstock, which is sometimes branched near the apex, with

2 to 3 growing points produced below soil level. The species epithet refers to its fusiform root. Locally it is called “Ban-Muli” by the tribal people. It is said to be of medicinal value, its latex being used as an antidote for snake and scorpion bites. The tuber pulp is used as a cure for arthritic pains. *E. fusiformis* root powder in experimental animals, which may be helpful as diuresis therapy in urinary stones. The ethnobotanical value of this plant is due to its action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis, liver disorders and diarrhea [5]. The tuberous roots of this plant were used by *Bhagats* (Tribal physicians) of Dangs forest for the treatment of various abdominal disorders, especially for tumors of abdomen, and urinary stones. Somatic embryogenesis offers an alternative and efficient protocol for plant regeneration. The technique of somatic embryogenesis has also contributed information for the genetic, morphological and physiological manipulation.Embryos were normally produced *in vivo* following the union of female and male gametes resulting in a bipolar structure that included both a shoot and a root meristem apex [6]. Somatic embryogenesis was first reported in 1958 [7], [8] 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 *Euphorbia fusiformis* Buch.-Ham. can be used as source for the production of useful phytochemical compounds.

2. METHODOLOGY

The plant material was collected from a famous forest area near Pakhala 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.

2.1. 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.

2.2. 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 atleast 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.

2.3. 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.

3. 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 increased tendency of somatic embryo formation with the increasing concentration of NAA upto (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. Individual embryos enlarged into distinct bipolar structures and passed through each of the typical developmental stages (Globular, torpedo) [Plate II Fig. c] after 6 weeks of culture when these embryos with different developmental stages were transferred to the some medium further germination of embryos was not observed. 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].

4. DISCUSSION

Reports of earlier scientists [9] on somatic embryogenesis in a diverse group of plants viz., Hevea, *Saccharum officinarum* L. and *Vigna aconitifolia* (Jacq.) Marechal. etc. on the same hormonal regime (2,4-D and BAP) supported the results obtained by the author. In contrast to the above results, NAA in combination with BAP have been found beneficial for the induction of somatic embryos in different plant species i.e. *Pinus tadea, Pinellia tripartite, Gossipium hirsutum, Solanum melongena* etc. [10], [11] & [12]. Further, embryogenic callus upon regular sub culturing on the same medium and hormonal regime along with caseine hydrolysate (10.0 mg/L) passed through various stages of embryo development culminating into maturation of embryos and leading to germination after three weeks of incubation. The promotory effect of caseine hydrolysate on maturation of somatic embryos was also reported by earlier workers in a number of plant species.

However, embryo germination was also confirmed by taking microscopic photographs and the exposed view of the same. The percentage response of embryos forming shoots and their length increased with increase in incubation period on the same media regime. Individual tiny shoots were separated from the clump and transferred on MS basal medium containing (1.0 mg/L IBA, about 70-75% cultures induced roots [Plate III Fig. c]. However, other auxin like NAA only induced root primordia, which was not suitable for plant survival. Similar results were also obtained in *Vitex negundo* L.[13], *Withania somnifera* L. [14] and in *Phyllanthus urinaria* L.

[15]. However, in oppugnance to this, [16] reported optimum rooting on NAA (0.5 mg[illegible]) in *Saccharum officinarum* L. The plantlets regenerated through somatic embryos were taken out from culture vials, freed from agar and finally transferred to the field by the procedure mentioned in "Materials and Method". Plantlets have shown 55% survivability in natural environment.

This study confirmed the formation of somatic embryos in *Euphorbia fusiformis* Buch.-Ham using a protocol established for other genotypes [17]. Our study upholds these structures to be somatic embryos based on bipolarity, vascular continuity of the shoot and root meristems, vascular autonomy from the explant, and presence of epidermis, raphides, starch, and storage of protein. The latter four traits were previously reported for somatic and zygotic embryos of *Euphorbia fusiformis* Buch.-Ham. Observations in this study support the hypothesis that somatic embryos of *Euphorbia fusiformis* Buch.-Ham originate within the mesophyll via direct embryogenesis. There is also evidence for a proembryonic cell complex in the mesophyll potentially forming embryos. The presence of the pro-embryonic cell complex may account for reports of both isolated and tightly clustered somatic embryos within the same explant [18]. The results of this study are encouraging for regeneration of non chimeric transformed plants, provided gene transfer is targeted to the mesophyll and the transformed cells are those that regenerate. For absolute confirmation of somatic embryo ontogeny, a nondestructive methodology for the continuous study of embryogenesis is required.

5. HARDENING, AND TRANSFER OF PLANTLETS TO FIELD

The plantlets developed *in vitro* were taken out from the rooting medium and washed thoroughly but delicately to remove adhering agar. The plantlets were then transferred to pots containing a mixture of vermin compost and sterilized soil (1:3), and then these pots were incubated in growth chamber for their hardening and acclimatization for about 2-3 weeks. Potted plants were covered with inverted glass beakers to ensure high humidity and watered every day, while with few drops of half strength of MS salt solution twice a week. After 2-3 weeks, inverted glass beakers were removed in order to acclimatize plants to field conditions [illegible]Plate III Fig. [illegible]) Plants were then transferred to earthen pots containing garden soil and watered with tap water.

6. CONCLUSION

Callusing efficiency from leaf explants in *Euphorbia 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 *Euphorbia Fusiformis* Butch-Ham and Somatic embryo formation was achieved on 2,4-D/NAA from leaf explants of *Euphorbia 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 *Euphorbia 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 *Euphorbia fusiformis* Butch-Ham. The species *Euphorbia 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 sterio microscope

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 regerating plant in *Euphorbia fusiformis*

PLATE-I

PLATE-II

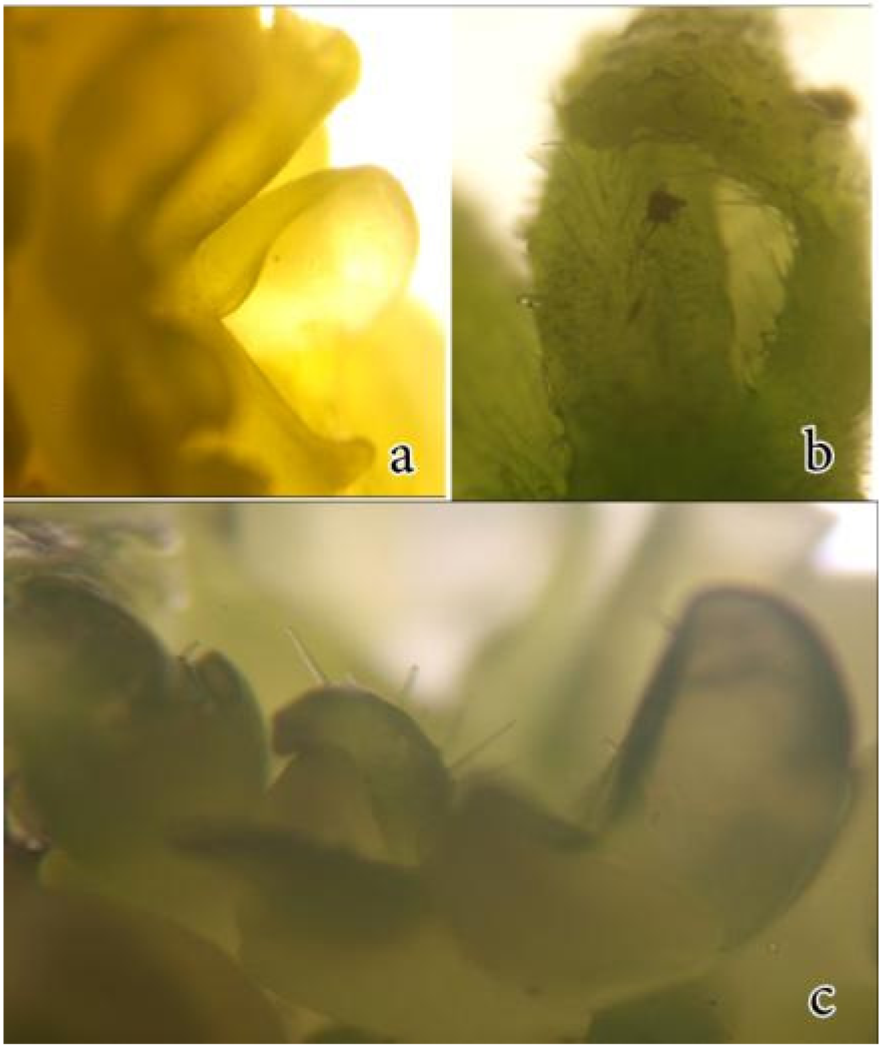

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PLATE-III

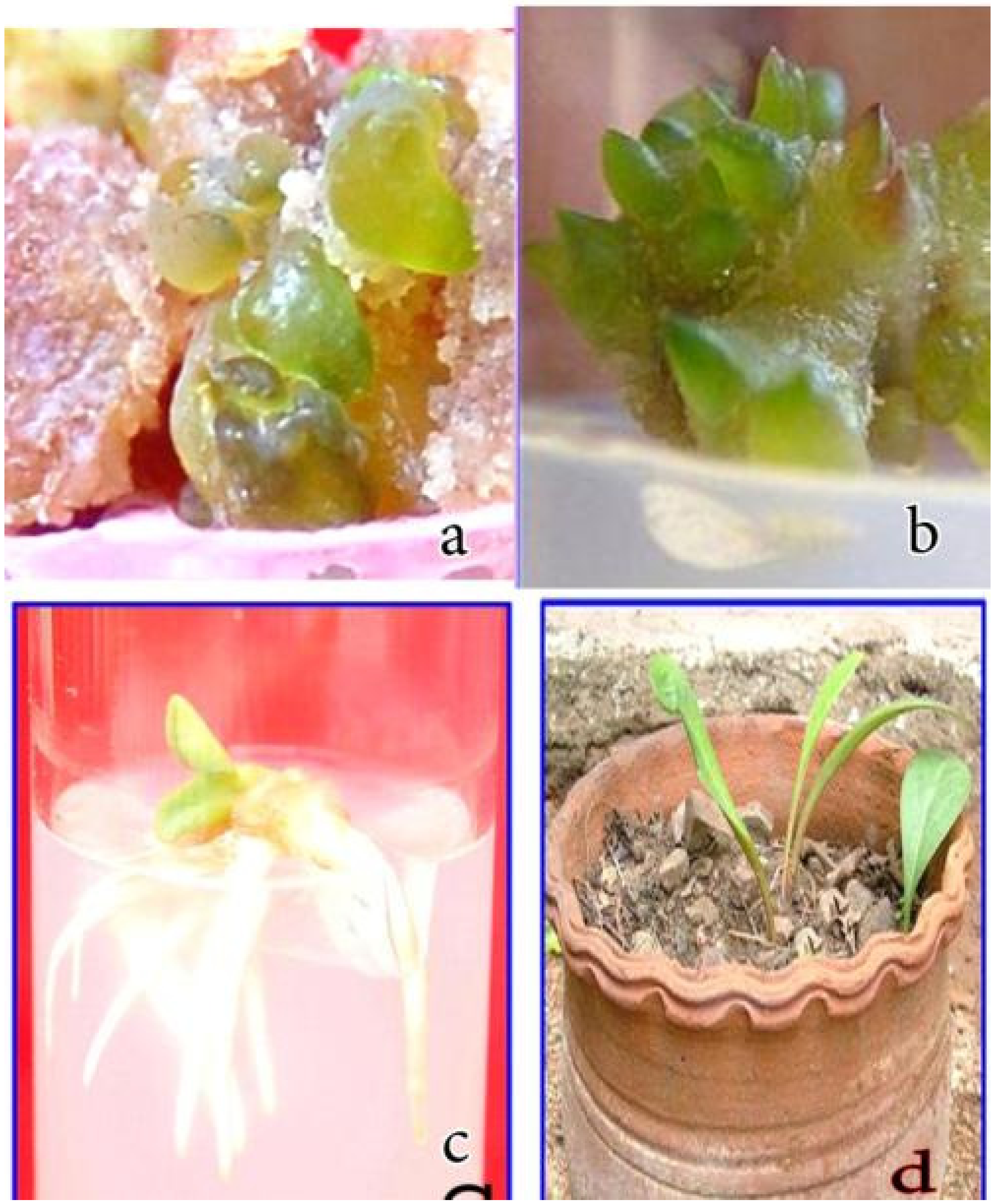

Table -1

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	% of cultures responding	% of response for somatic embryo	Average number of somatic

(mg/L)		formation	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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