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In vitro Propagation of *Euphorbia pulcherrima* Willd. Through Somatic Embryogenesis

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Abstract

Euphorbia pulcherrima Willd. is one of the most popular ornamental houseplants. A protocol was developed for multiplication of *E. pulcherrima* through somatic embryogenesis from nodal explants. The induction of somatic embryogenesis in red pigmented callus was achieved on MS supplemented with 2ip (9.8 µM) and NAA (2.69 µM). Reduced level of NAA (0.54 µM) in the same medium caused maturation of somatic embryoids. The embryoids germinated successfully, turned into plantlets and were established in field with a high survival rate.

Introduction

Euphorbia pulcherrima Willd. (Family - Euphorbiaceae) is one of the most popular houseplants seen during the Christmas time. It has brilliant colored bracts ranging from scarlet, crimson, yellow to red and white. The ability of these spectacular bracts to remain fresh and intact for three - four months adds to its demand as an ornamental. Though *E. pulcherrima* is native of Central America, this fast growing plant is one of the most common growing shrubs throughout the world. The latex of *E. pulcherrima* has been reported to be poisonous to livestock (Anonymous 1978). However, in veterinary medicine it is used to kill maggots in the wounds of livestock.

This shrub can be conventionally propagated by seeds and cuttings. But seeds lose their viability on storage and propagation through cuttings is seasonal. Besides cuttings take six - eight weeks to root (Sochacki and Chimid 1994). These methods of clonal propagation are not sufficient to meet the demand of this ornamental shrub during the Christmas period. Earlier, *in vitro*

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studies on *E. pulcherrima* have been reported (Nataraja et al. 1973, Langhe et al. 1974). However, for reduction in the cost of production and rapid multiplication, somatic embryogenesis is an alternative to multiplication through axillary and apical buds and needs to be tried for ornamental plants (Razdan 1993). Because of this potentiality, work was initiated to develop a protocol for multiplication of *E. pulcherrima* through somatic embryogenesis.

Materials and Methods

Young nodal explants were collected from plants growing in the Botanical Garden of the M.S. University of Baroda. They were washed under running tap water (30 min) in a two-way flask (Kannan and Jasrai 1996) and pretreated (2 h) on a gyratory shaker (100 rpm). The pretreated explants were washed thoroughly with distilled water and rinsed with 70% (v/v) ethanol (3 sec). Further, manipulations were done under aseptic conditions in the laminar flow hood (ADC/Klenzaid, India). The explants were disinfected (10 min) with $HgCl_2$ solution (0.05% w/v) containing Tween-20 (0.025 ml) and followed by a thorough rinsing with sterile distilled water (five times). Finally, the explants were inoculated on agar-based (0.75%, w/v) MS supplemented with various combiantions of auxins (NAA and 2, 4-D) and cytokinins (Kn and 2ip). The pH of all media was adjusted to 5.8 and dispensed (5 ml) in test tubes (25 ∇ 150 mm) before autoclaving at 121_C (20 min). The nodal explants (measuring 5 mm) were inoculated on media either in vertical or horizontal position. The cultures were incubated in a culture room at 25_C with 16 h photoperiod under a photon flux of 30 µ-mol $m^{-2}s^{-1}$ provided by cool fluorescent lamps (Philips, India).

The generated embryogenic callus was subcultured every two weeks interval for the maturation of embryos. The generated embryoids were transferred to MS with the same supplements. Well developed 5 - 7 cm tall plantlets were transferred to net pots (3.2 cm diam.) containing perlite and soil (1 : 3) in protrays for acclimatization. The plants were transferred to the greenhouse after two - four weeks of hardening. For further growth the plants were established in field.

Results and Discussion

The most important factors contributing the induction of embryogenic callus and plant regeneration through somatic embryos are : the explant type, medium formulation and growth regulators (Buyukalaca and Mairtuna 1996). Somatic embryogenesis for different plants have been achieved using a variety of media ranging from relatively dilute - White's medium (1963) to a more concentrated formulations of Gamborg et al. (1968), Schenk and Hildebrandt (1972) and MS. However, over 70% successful cases have used MS salts or its derivatives (Evans et al. 1981). Among

the plant growth regulators, generally auxin is known to be essential for the induction of somatic embryogenesis and 2,4-D is the most commonly used auxin (Ammirato 1983). Moreover, a combination of 2,4-D or NAA with cytokinin was reported to be essential for the induction of somatic embryos (Gingas and Lineherger 1986, William and Maheswaran 1986). Certain cells may need simple MS medium for the induction of somatic embryos and further development (Jasrai et al. 199). The results of the present study, show that MS medium supplemented with 2ip (9.8 µM) and NAA (2.69 µM) yields the maximum amount of embryogenic callus compared to the other MS media tried (Table 1). A pale-yellow callus was seen one week after inoculation. Also, cells committed to anthocyanin pigment production (data not shown) were seen on the periphery of the callus two weeks after culture.

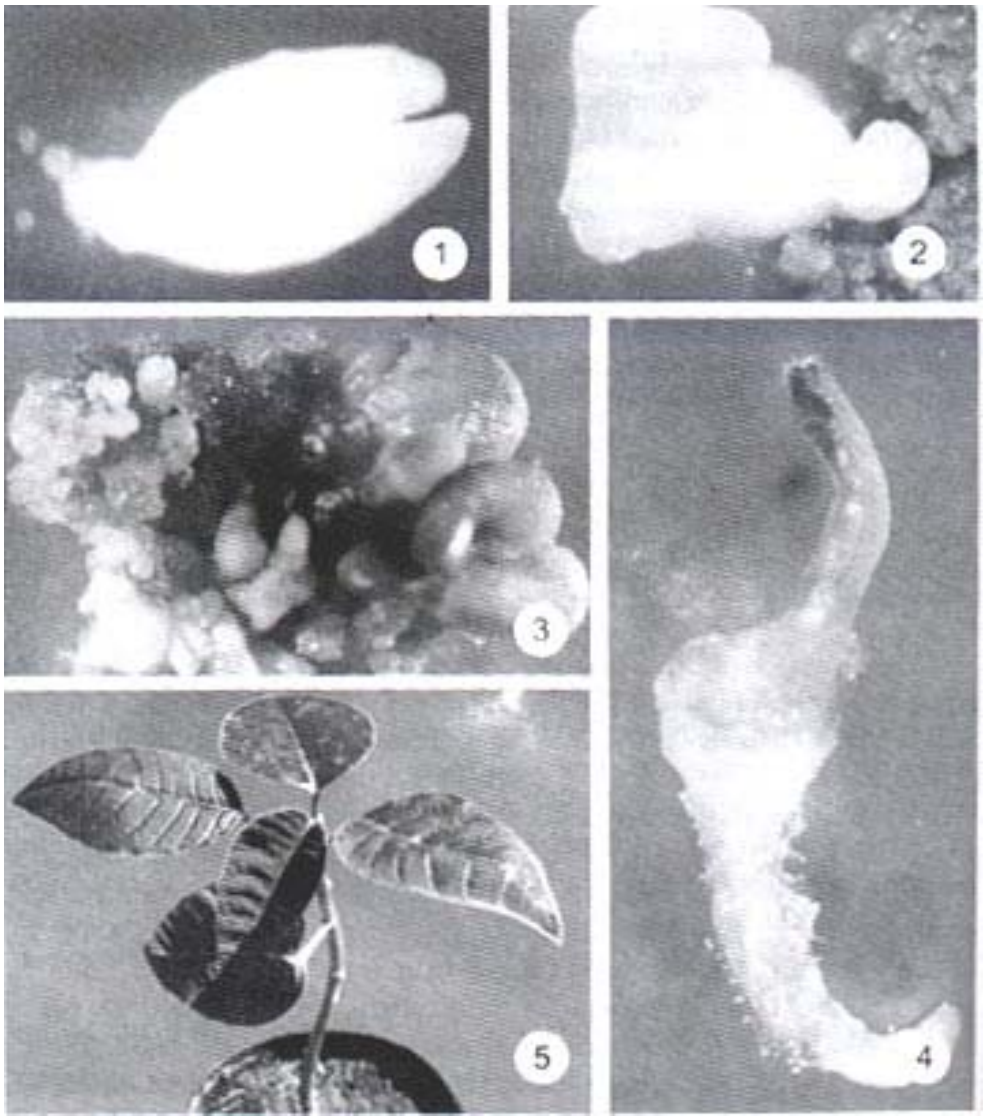
Table 1. Induction of embryogenic callus from the nodal explants of *Euphorbia pulcherrima* on MS containing various combinations of 2ip and NAA,

2ip (µM)	NAA (µM)	Callus (gm)* (Mean ± S.E.
4.9	2.69	-
9.8	2.69	0.20 ± 0.008
14.8	2.69	0.143 ± 0.036

- = no callus initiation, *= data collected after two weeks of inoculation.

The induction of callus was seen only at cut surfaces of the explants. The explants, which were placed horizontally on the medium showed stem necrosis and very poor survival. This was also observed earlier for *E. pulcherrima* (Riplay and Preece 1986). A vertical orientation in which the basal half of the stem was inserted into the medium demonstrated very reduced stem necrosis and better survival (100%). After four - five weeks of incubation on the callus maintenance medium (MS + 2ip (9.8 µM) + NAA (2.69 µM) the 1st and 2nd node derived callus showed induction of embryoids (Figs. 1 and 2). These clear differences observed among different calli from 1st and 2nd node for embryogenesis could be due to the differential gene expression. Clumps of somatic embryos were observed at the surface of a friable and red-pigmented callus only (Fig. 3). A white and compact callus failed to respond for somatic embryos even during further subcultures. Trnasfer to such pigmented embryogenic callus along with embryos on to the same medium demonstrated induction of further embryoids.

Incorporation of 2,4-D in place of NAA in the medium caused the formation of only non-embryogenic callus. On the other hand, reduction in the level of NAA (0.5 μM) in the MS medium containing 2ip (9.8 μM) caused simultaneous initiation and maturation of somatic embryos. Germination of such mature embryos was achieved (Fig. 4) on MS medium supplemented with 2ip (9.8 μM) and NAA (4.03 μM).



Figs. 1 - 5: Somatic embryogenesis and planlet formation in *Euphorbia pulcherrima* Willd. 1. Heart shaped embryo. 2. Torpedo shaped embryo. 3. Large number of somatic embryoids at various stages of development in embryogenic callus. 4. Generation of embryoid with shoot and root axis. 5. Acclimatized plantlet ready for transplantation in the field.

Transfer of embryogenic calli to the liquid medium with the same composition resulted in the senescence of embryos. Somatic embryos germinated on agar based MS basal medium and developed into complete plantlets. Earlier, Roy (2001) reported addition of different supplements such as coconut milk (15%), casein hydrolysate (100 mg/l) along with hormones for shoot multiplication and their growth from nodal explants. The shoots were then rooted to form complete plantelts. Since the present study involved plantlets production through somatic embryogenesis, the whole process was quite simple. The plantlets were transferred to net pots (3 cm diam.) in protrays containing perlite and soil (1 : 3). The protrays were covered with a clear polythene sheet to prevent desiccation of fragile plantlets. Plantlets were irrigated with one-fourth strength of MS without any supplements. A high humidity was maintained by a regular spray (at 1 h interval) of distilled water. After one month of hardening, the acclimated plantlets were transferred to the greenhouse and grown there for three weeks (Fig. 5).

Well developed plantlets were transplanted to field with 87% showing normal features without any morphological variation. The results described here, demonstrate successful and reproducible clonal propagation of *Euphorbia pulcherrima* through somatic embryogenesis.

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