



## Original Research Article

**RAPID IN VITRO SHOOT MULTIPLICATION THROUGH HYPOCOTYL SEGMENT CULTURE IN CALOTROPIS GIGANTEA R. BR.**

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**Abstract:** Micro propagation is a handy technique for rapid multiplication of plants. Micro propagation allows the production of large number of plants from small pieces of the stock plant in relatively short period of time. The seedling organs such as epicotyl and hypocotyl are very good source of starting materials for rapid regenerations. In the present study either only 6-Benzylaminopurine (BAP) or in combination with indole-3-acetic acid (IAA) promoted multiplication of shoots in hypocotyl and epicotyl segments at 10 $\mu$ M concentration. Direct regeneration of shoots from cut ends of hypocotyls is greatly influenced by the hormonal balance and type. Only BAP could induce significant number of shoots with in a short time along with Indole-3-acetic acid (IAA) rather than with naphthalene acetic acid (NAA) or 2,4-Dichlorophenoxyacetic acid (2,4-D). Adventitious shoot regeneration was strongly influenced by the explant type, shoot production being highest when hypocotyls were placed on medium with 10 $\mu$ M BAP and in combination of IAA. Shoot elongation was also achieved on medium containing 10 $\mu$ M BAP and IAA. The Plant Growth Regulators (PGR) combinations like BAP and IAA were found to be very ideal growth promoting substances for inducing tissue culture regeneration in this species through seedling organs.

**Key Words:** Micro propagation, *Calotropis gigantea*, MS medium, Plant growth regulators.

## INTRODUCTION

*Calotropis gigantea* R.Br belonging to the family Asclepiadeae, (Apocynaceae) is an important medicinal plant and widely distributed in India (Agharkar, 1991). *Calotropis* is a genus of plants that produce milky sap hence commonly called giant milkweed. The intrinsic property of excised shoots and roots to regenerate a whole plant has been recognised (Street, 1997). Tissue culture technologies have had a major impact on the *ex situ* conservation of plant genetic resources. Micropropagation, using somatic embryo and shoot tip culture techniques, assists many crop plant improvement programmes and increasingly these methods are being used for the conservation of endangered plant species. In these studies 1-Naphthaleneacetic acid and 2, 4-D have induced laticifers in culture conditions at various concentrations. *In vitro* regeneration using mature plant parts were known from the reports of Roy and De (1986). Though, *C. gigantea* is considered to be highly important medicinal plant tissue culture studies are very limited (Bari *et al.*, 2008; Rathore *et al.*, 2010). The technology would be useful for propagation, extraction of secondary metabolites and for conservation of genetically rich populations of genetically rich populations of either taxonomically distinct form of *Calotropis gigantea*.

## MATERIALS AND METHODS

*Calotropis gigantea* plants were collected from wastelands of Tirunelveli district. *C. gigantea* seeds were collected and were allowed to germinate in pre sterilized petriplates containing BAP 10 $\mu$ M

concentration. Two petri plates were considered as control. One plate was kept under dark condition, and the other was maintained under light conditions.

Explants were obtained eight day old seedlings. Explants such as hypocotyls, stem and leaf explants were cultured on MS medium with 0.8% (W/V) agar. The medium was supplemented with auxins (NAA, IAA, 2, 4-D) and cytokinins (BAP and Zeatin) at 10 $\mu$ M concentration. Seedling organs were trimmed in to small pieces (1cm) Prior to culture the explants were surface sterilized in water for one hour followed by Sodium hypochlorite (0.01%) and mercuric chloride at 0.1% concentration. Finally the explants were thoroughly washed in sterilized distilled water to remove all traces of surfactants. Cultures were maintained under a 16-18hrs light/dark regime under 4000lux light intensity. The temperature of culture room was maintained at 25 $\pm$ 2 $^{\circ}$ C.

## RESULTS AND DISCUSSION

### Seed germination and culture of seedling organs

Fresh seeds were germinated in Petri dishes containing plain water in both light and dark conditions (Fig. B). Seeds were also grown *in vitro* on MS basal medium. Synchronous seed germination was observed in these two set ups. In cultured conditions delayed and non-synchronous germination results in different morphogenetic responses (Makunga, and Staden, 2008) However, light and *in vitro* grown seedlings provided sufficient hypocotyl and epicotyl explants. 1cm long hypo and epicotyls were cultured on MS

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medium supplemented with various PGR combinations including BAP 10 $\mu$ M, IAA 10 $\mu$ M and 2, 4-D 10 $\mu$ M. Hypocotyl explants responded quickly by producing direct shoots from the proximal region within 10 days of culture initiation (Fig. C, D, E, F).

### Shoot multiplication

The shoots initiated with leaf like structures and subsequently multiplied in large numbers. An average of 15 shoots could be produced by one hypocotyl explant (Fig. G, H). Shoot multiplication was enhanced by the combined effect of BAP and IAA at equal concentration (Fig. G, H, I). A maximum of 30 shoots and an average of 22 shoots multiplied within 40 days of subculture and in one cycle of growth conditions. Transfer of 3cm shoots to basal medium also resulted in shoot elongation and multiplication of shoots *in vitro*. Roots could be induced on medium containing 10 $\mu$ M BAP and IAA within 30 days of culture (Fig. I).

Tissue culture studies were earlier reported from *Calotropis* on *in vitro* differentiation of laticifer from seedling organ and callus cultures (Dhir, *et al.*, 1984; Dutta and De, 1986; Suri and Ramawat, 1995). In these studies NAA and 2,4-D have induced laticifers in culture conditions at various concentrations. *In vitro* regeneration using mature plant parts were known from the reports of Roy and De (1986). Similarly rapid shoot multiplication of *Holostemma annulare*-a member Asclepiadaceae has been reported (Sudha *et al.*, 1998). BA and NAA have induced multiple shoots from mature nodal explants. Entire plantlets were regenerated using NAA for root induction in *Holostemma*. In the present study BAP either singly or in combination with IAA promoted multiplication of shoots from hypocotyl and epicotyls segments at 10 $\mu$ M (Fig. E, F). Direct regeneration of shoots from cut ends of hypocotyls is highly influenced by the hormonal balance and type. Because only BAP could induce significant number of shoots within a short time along with IAA rather than with NAA, or 2,4-D. Adventitious shoot regeneration was strongly influenced by the explant type, shoot production being highest when hypocotyls were placed on medium with 10 $\mu$ M BAP and in combination of IAA. This result is in conformity with the reports of (Makunga, and Staden, 2008) in *Salvia* seedling organ culture. In many of the Asclepiadaceae members *in vitro* shoot multiplication was successfully achieved in medium containing Kinetin and IBA or Kin 2, 4-D (Parabia, 2007), however *C. giganteae* these combinations did not have any effect on regeneration.

In many cases of seedling organ cultures, shoot induction and subsequent shoot multiplication was readily accompanied by basal callus growth (Kone, *et al.*, 2007). Interestingly in this experiment such a

growth was observed only at the later stage of shoot multiplication where the compact callus appeared (Fig. G, H). Basal callus growth could be successfully controlled by regular subcultures. As the rate of multiplication was rapid at this dosage further higher or lower concentrations were not considered. Shoot elongation was also achieved on medium containing 10 $\mu$ M BAP and IAA (Fig. I). Using the above mentioned medium composition a rapid regeneration protocol for large scale propagation of *Calotropis* could be developed in subsequent months.



**Figure A:** Flowering Twig of *Calotropis giganteae* pink form

**Figure B:** Seed germination in Light

**Figure C, D, E, F:** Hypocotyl segments on MS medium + BAP IAA. Shoot bud initiation

**Figure G, H, I:** Hypocotyl segments on MS medium + BAP IAA. Shoot bud shoot multiplication.

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