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ORIGINAL RESEARCH

Micropropagation and induction of *in vitro* flowering in *Tridax procumbens* L.

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ABSTRACT
An efficient and reproducible protocol for *in vitro* regeneration and flowering of *Tridax procumbens* was established through nodal explants. Multiple shoot proliferation was observed on MS medium supplemented with 1.0 mg l^{-1} BAP. High frequency rhizogenesis was observed on half-strength MS medium augmented with 0.5 mg l^{-1} IBA. *In vitro* flowering was initiated on medium having BAP (1.0 mg/L) and adenine sulfate (100 mg/L). The present investigation describes hormonal regulation of morphogenesis *in vitro* in nodal explants of *T. procumbens*. The study has also laid a preliminary foundation for a further research to understand the complex mechanism of floral development.

KEY WORDS: *Micropropagation, Nodal explants, In vitro flowering, Tridax procumbens*

INTRODUCTION

In plant's life cycle transition from vegetative to reproductive phase is one of the cardinal events. Flowering is the most elusive and fascinating of all plant developmental processes. It is a complex process which is influenced by a multitude of environmental and physiological factors and its occurrence *in vitro* is of crucial importance as it provides a clear insight for studying flower initiation and development as well as provides an opportunity for conducting micro breeding. Both physiological and molecular studies have depicted the complexity of the mechanisms that control the transition from vegetative to reproductive growth (Levy and Dean, 1998). *In vitro* flowering is of great importance in plant breeding where pollen can be used from rare stock, thus provide a platform for recombination of genetic material via *in vitro* fertilization in otherwise non hybridizable lines. There are several reports on *in vitro* flowering of different plants, such as *Rosa indica* (Pratheesh and Kumar, 2012), *Cleome viscose* (Rathore and Shekhawat, 2013), *Ceropegia pusilla* (Kalimuthu and Prabakaran, 2013), *Blepharis maderaspatensis* (Drisyadas *et al.,* 2014), *Dendrobium huoshanense* (Lee and Chen, 2014), *Swertia chirayita* (Sharma *et al.,* 2014), *Lens culinaris* and *Vicia faba* (Mobini *et al.,* 2015).

Tridax procumbens L. (Asteraceae) commonly known as coat buttons, is an annual medicinal herb found throughout India. It is widely used for its pharmaceutical properties like immunomodulatory, antidiabetic, anti hepatotoxic and anti-oxidant, anti-inflammatory, analgesic, and insect and pest repellent (Vyas *et al.,* 2004; Jain, 2006; Reddipalli, 2008; Bhagwat *et al.,* 2010). To our best knowledge, this study is the first attempt to establish an efficient protocol for *in vitro* flowering and micropropagation of *T. procumbens*.

RESULTS AND DISCUSSION

In vitro establishment and multiplication

Development of single shoot per node was recorded on MS medium supplemented with BAP (0.1 mg l^{-1}). High frequency

multiple shoot bud formation was achieved when nodal explants were cultured on medium augmented with lower concentration of 1.0 mg l^{-1} BAP (Fig. 1 A). In the present study, an inhibitory effect of higher concentration of BAP on multiple shoot induction was observed in *T. procumbens*. Similar, adverse effect of higher concentrations of BAP on *in vitro* shoot proliferation has been reported in *Albizia chinesis* (Sinha *et al.,* 2000), *Pterocarpus marsupium* (Anis *et al.,* 2005), *Arachis hypogaea* (Banerjee *et al.,* 2007), *Doritis pulcherrima* (Mondal *et al*., 2013) and *Salvia splendens* (Sharma *et al.,* 2014). Other plant growth regulators, singly or in combinations could not trigger shoot proliferation in nodal explants of *T. procumbens.*

***In vitro* rhizogenesis**

Half/full-strength MS medium along with higher concentration of IBA (1.0 – 5.0 mg l^{-1}) induced low frequency of rhizogenesis *in vitro* (Fig. I B). High frequency root induction was induced on half-strength MS medium augmented with 0.5 mg l^{-1} IBA (Fig. I C). Similarly, the effect of IBA on rhizogenesis *in vitro* has been reported in *Plectranthus bourneae* (Thaniarasu *et al.,* 2015), *Passiflora foetida (*Shekhawat *et al.,* 2015)*, Morinda coreia (*Shekhawat *et al.,* 2015)*, Ceropegia evansii* (Chavan *et al.,* 2015).

***In vitro* flowering**

Flowering is considered to be a complex process regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare. In the present study, *in vitro* flowering was initiated on MS medium supplemented with BAP (1.0 mg/L) and adenine sulfate (100 mg/L) after 3–4 weeks of cultures. BAP is found to be playing an important role not only as a growth regulator but also as a factor regulating floral organ formation of regenerated plantlets (Mandal *et al.,* 2000). Other concentrations of BAP and adenine sulfate could not trigger floral development *in vitro*. There are several reports that indicate the beneficial effects of BAP on the induction of flowering *in vitro* (Anitha and Kumari, 2006; Saritha and Naidu, 2007; Jana and Shekhawat, 2011). The developed *in vitro* protocol can be successfully used for large-scale multiplication of *T. procumbens*. The study provides deeper insight into the complex mechanism of floral induction and has laid a preliminary foundation for a further research of *in vitro* flowering of *T. procumbens*.

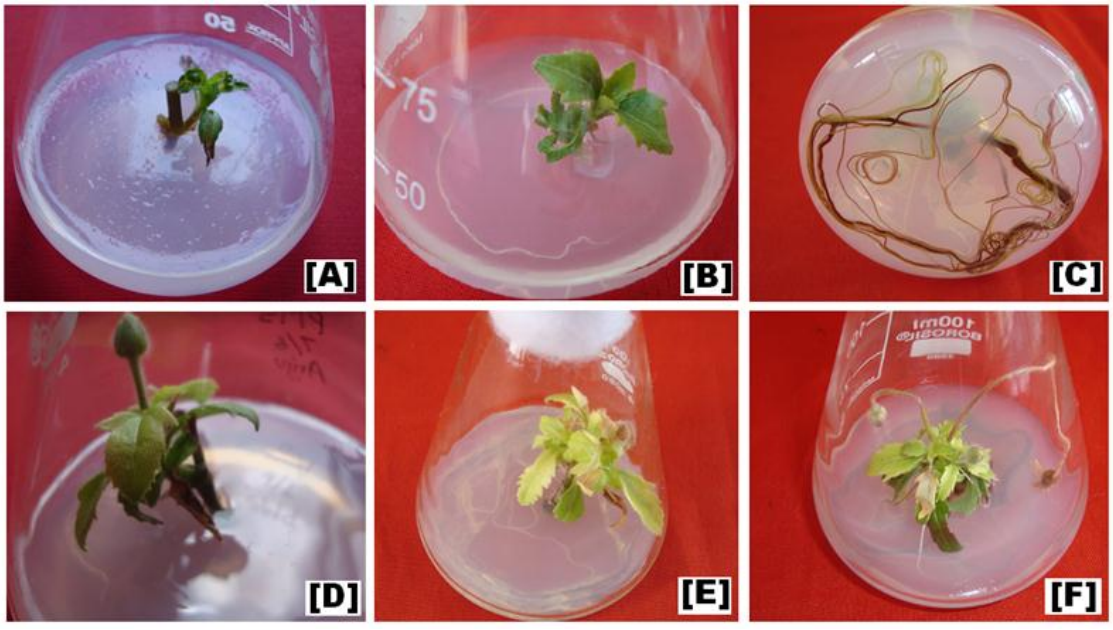

Figure.1: Nodal explant of *T. procumbens* showing single shoot proliferation (A), rhizogenesis (B, C) and *in vitro* flowering (D-F)

MATERIALS AND METHODS

Plant material and surface sterilization

Nodal explants were collected from healthy plants of *T. procumbens* growing in the Jhanana Nursery, Jaipur (India). The excised nodal explants were washed thoroughly under running tap water for 30 min to eliminate dust particles and then with 5% teepol for 8 -10 min and rinsed several times in sterile distilled water. Then, the explants were treated with an antifungal agent (Bavistin) for 1 hour and the again

rinsed three times with sterile distilled water. Thereafter, the explants were surface sterilized in a laminar flow cabinet with aqueous solution of 0.1% $HgCl_2$ for 3 min and finally washed with sterile distilled water for 2–3 timess.

Culture media and growth conditions

The sterilized nodal explants were cultured on MS Medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and various combinations/ concentrations of plant growth regulators to induce morphogenetic response. The pH of the media was adjusted 5.8 before autoclaving 121 ^oC for 15 min. All the cultures were maintained at 25 ± 2 ^oC and 65 - 70% relative humidity with photoperiod of 16-h using a photosynthetic photon flux density (PPFD) of 40 mmol m^2 s^1 provided by cool white fluorescent tubes (Philips, India).

***In vitro* rhizogenesis and hardening**

In vitro developed shoots having 2–3 pairs of healthy leaves were excised and transferred to rooting medium. The shoots were cultured on MS supplemented with various auxins viz. IBA, IAA and NAA. Well rooted plantlets, derived from nodal explants, were gently washed in sterile water and transferred to plastic cups (10 cm × 8 cm) containing sterilized mixture of sterile soil, sand and coco peat (1:2:1). The plantlets covered in transparent polyethylene bags were kept for 4 weeks in growth chamber at 25 ± 2 ^oC with 16 h photoperiod and 40 mmol m^2 s^1 of irradiation. The plantlets were irrigated with tap water. The irrigation schedule and volume of water was calibrated to keep the pot mixture saturated and prevent flooding. The hardened plants were subsequently transferred to large pots containing normal garden soil and were maintained in an open greenhouse without environmental conditioning for 4 weeks.

***In vitro* flowering**

In order to inducing flowering, *in vitro* developed shoots were transferred on medium containing various combinations and concentrations of plant growth regulators. Each treatment was replicated 12 times and all experiments were repeated at least thrice.

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