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RESEARCH ARTICLE

IN VITRO REGENERATION OF *RUELLIA TUBEROSA* L. (ACANTHACEAE) THROUGH DIRECT ORGANOGENESIS

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ABSTRACT

Plant tissue culture plays an important role in the production and conservation of rare plant species. An efficient in vitro plant regeneration protocol through direct organogenesis is an important need for the conservation of a valuable medicinal plant. *Ruellia tuberosa* nodal explants were used for in vitro regeneration on Murashige and Skoog (MS) medium containing 3% sucrose and different concentrations and combinations of plant growth regulators. A maximum shoot length of eight centimeter was obtained from nodal explants when cultured on MS medium supplemented with kinetin and BAP at 2.0mg/L concentration. The highest percentage of shoot induction (90%) was obtained in 2.0 mg/L in kinetin with a mean of 8.0±0.89 shoots. The maximum root length (3.63cm) was obtained with 95% and 90% of NAA and IBA respectively at 1 mg/l concentration. Rooted plantlets were transferred to plastic cups and grown in the green house. The survival rate was 90%.

INTRODUCTION

In India over 7500 plant species are being used in traditional medicines (Singh *et al*., 2011). *Ruellia tuberosa.L* is one among them belongs to the family Acanthaceae. It is a perennial weed native to West Indies. It is also found in India, Indonesia and Sri Lanka (Dasanayake and Fosberg, 1998). This plant is used in traditional medicine as antiseptic, depurative, diaphoretic, diuretic, emetic, purgative, for the treatment of bronchitis, constipation, bladder stone, cystitis, fever, leprosy, gonorrhea and other veneral diseases (Alam *et al*., 2009).

This plant is incorporated as a component in herbal drink in Taiwan (Balick *et al*., 2000). Till date there is no report on in vitro regeneration of *R. tuberosa*. In this paper the optimization of culture conditions and plant growth regulators required for shoot induction and rooting plantlets of *R. tuberosa* are reported.

MATERIALS AND METHODS

Plant Selection

The plants were collected from Arakkonam, Vellore District and the species was identified by Dr. G. Jeya Jothi, Assistant professor, Department of Plant Biology and Biotechnology, Loyola College, Chennai. The young plants were collected and used as explants. The axillary buds and the terminal buds were inoculated on MS medium supplemented with different concentrations of BAP and Kinetin.

Surface Sterilization

The young axillary and terminal buds of healthy branches *of R. tuberosa* were collected from disease-free plants. For surface sterilization the collected buds were washed with running tap water for 5 to 10 min and treated with 5% teepol solution for 5 min followed by rinsing with double distilled water. To eliminate the fungal contamination, explants were further treated with 0.1% mercuric chloride for 3 to 5 min followed by 4-5 rinses in sterile double distilled water.

Culture Media And Culture Conditions

Murashige and Skoog (Murashige and Skoog,1962) medium containing 3% sucrose solidified with 1% agar (Tissue culture grade, Hi-media, India) was used. The pH of the medium was adjusted to 5.6 - 5.8 by adding sodium hydroxide and hydrochloric acid (Dode *et al*., 2003) and agar was added before autoclaving at 121°C for 15 minutes under 15 lb pressure. The cultures were incubated for 16 h under photoperiod (2000 lux) provided by cool white fluorescent

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tubes at 25±2°C. MS medium containing different combinations and concentrations of kinetin and BAP (0.5, 1.0, 1.5 and 2.0 mg/L) were used for shooting attributes. Both BAP and kinetin were checked in combinations with 0.1mg/L of IBA and IAA to induce multiple shooting (Anand and Jeyachandran, 2004; Daniel *et al*., 2010).

Shoot Induction

The explants with bud proliferation cultures were transferred to fresh MS media for shoot multiplication. The cultures were maintained by regular subcultures on fresh medium with the same composition. After proper shoot induction, the plantlets were carefully removed from the medium and washed with sterile double distilled water properly to avoid any trace of the roots.

Rooting Of Shoots

The excised shoots with 9 to 10 cm length and with 6-8 compound leaves were transferred to half strength MS medium containing 3% (w/v) sucrose supplemented with different concentrations of NAA and IBA viz., 0.5, 1.0, 1.5 and 2.0 mg /L. The importance of auxin in the root formation during the sub culturing of secondary explants have already been reported (Karuppusamy and Pullaiah, 2007; Abbas *et al*., 2010, Loc and Kiet, 2011). After proper root formation the rooted plantlets were subjected to hardening process. The well developed rooted plantlets were removed from the culture medium.

Hardening

The rooted plantlets were gently extracted from the glass vessels and washed thoroughly with tap water to remove adhered agar and traces of the medium in order to avoid contamination. These plantlets were given a final wash with sterile double distilled water for 5 min. Then the plantlets were transferred to tea cups (8 cm in diameter) containing a mixture of autoclaved red soil, sand and vermicompost in the ratio of 1:1:1 and covered with a plastic bag to maintain the humidity and maintained in the tissue culture laboratory at 22±2°C for 2 to 3 days. The cups were maintained in a polyethylene chamber in the culture room and irrigated every alternative day with a solution of half-strength MS medium. Polyethylene covers were removed gradually and then the plants were transferred to garden soil for further growth in a greenhouse. All the above experiments were repeated thrice and recorded the observations and result. The acclimatized plants were then transferred to normal room temperature for 4 days and finally maintained in green house condition to know the survival rate. A number of plantlets were lost due to damping off and necrosis during acclimatization condition. The invitro propagation is approved out by culturing shoot tips or nodes from field or vegetation grown in greenhouse (Das *et al*., 2005). Because of the genetic constancy, culture of preformed meristems is favored for propagation (Reisinger, *et al*., 1976).

Statistical Analysis

All the above experiments were repeated three times and the data collected were statistically analyzed.

RESULTS AND DISCUSSION

The nodal explants of *R. tuberosa* were cultured on MS medium fortified with different concentrations of kinetin alone and BAP with kinetin for multiple shoot induction and the data are presented in table 1. Nodal buds cultured on MS medium with kinetin showed maximum number of shoots at 2 mg/L concentration within 6 weeks of incubation with an average shoot length of 7cm (Fig d). Increase or decrease in the concentration of kinetin beyond the optimum level showed the development of smaller number of shoot buds. These results were in agreement with earlier findings (Ramasubbu, 2009) in *Pedalium murex* and *Physalis angulata*. An effective shoot formation of 95% by nodal explants was observed on MS medium fortified with the growth regulator (Kinetin) at 2.0 mg/L and BAP at 2.0mg/L concentrations (Table 1).

The results suggested that the cytokinin played an important role on the multiple shoot formation. During subculture, the basal axillary buds underwent initiation. The developed axillary buds enhanced shoot multiplication (Miller *et al*., 1955). Enhanced shoot multiplication in subsequent culture is in accordance with published literature on medicinal plants belonging to Asclepediacean members like *Gymnema sylvestre* (Komalavalli and Rao, 2000). The promotary effect of kinetin shows multiple shoot induction. Of the three cytokinins tested, kinetin and BAP treated explants achieved higher than the other hormone. Both Kinetin and BAP shows multiple shoot induction (fig c, d & e) like *Chlorophytum borivilianum* (Purohit *et al*., 1994) showed multiple shoot formation. After 5 weeks the well developed shoots were transferred to MS medium supplemented with 1.0 mg/L of NAA and 1.0 mg/L of IBA in half strength of MS medium was found to be the most suitable for root induction (Table 2; fig f & g) After hardening, the growth rate of the plantlets were slow initially and increased gradually. New leaves emerged from the hardened plantlets after three weeks. Most of the plantlets survived after hardening. Nearly 90% of the regenerated plantlets survived under green house conditions. Loss of regenerates due to symptoms was observed in *Eucalyptus tereticornis* (Gill *et al*., 1993), *Rauvolfia serpentina* (Illahi, 1993).The pots under natural conditions and survivability in nature was recorded (Jasrai *et al*., 1999) in invitro derived banana plants.

CONCLUSION

The present study supports the *invitro* propagation of medicinal plants. This report provides a simple protocol of micro propagation of *Ruellia tuberosa*. Shoots can be easily derived from nodal cultures on kinetin containing medium and subsequently rooted on NAA containing medium. This protocol could be utilized for conservation and clonal propagation of this traditionally important medicinal plant.

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