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Effect of Different Concentrations of Plant Growth Regulators for Micropropagation of *Eugenia singampattiana* Beddome Endangered Tree Species

P. Pavendan and C. Sebastian Rajasekaran

Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, South India

Corresponding Author: P. Pavendan, Department of Botany, Bishop Heber College, Tiruchirappalli, Tamil Nadu, South India

ABSTRACT

In vitro techniques have found increasing use in the conservation of threatened plants in recent years and this trend is likely to continue as more species face risk of extinction. Effects of auxins and cytokinins (1 6-benzylaminopurine (BAP) and Thidiazuron (TDZ) on shoot induction and Indole-3-butyric acid on root induction were assessed at various combinations/concentrations. Growth hormones at different combinations brought out remarkable variations in shoots and root induction. 21 shoots per explant were obtained on Murashige and Skoog (MS) medium containing benzylaminopurine and Thidiazuron (1 mg L^{-1}) within 4 weeks. The elongated shoots were separated individually and transferred to rooting medium. The highest percentage of rooting was observed with Indole-3-butyric acid (0.5 mg L^{-1}). Rooted plantlets were transferred to plastics pots filled with mixture of autoclaved garden soil, sand, vermiculate (2:1:1) and irrigated with MS basal salt once in three days. More than 95% of the rooted plants were transferred to the field after hardening for 15 days. The combined effect of BAP and TDZ on shoot regeneration was found to be highest in the concentration of (1.0 mg L^{-1}) and recorded 93% of explants produced shoots and maximum number of shoots per explant was recorded 21.7 with 5.8 cm shoot length. The effect of IBA on root induction was found to be highest in the concentration of IBA (0.5 mg L^{-1}) MS media. 98% of shoots produced roots and maximum number of roots per shoot was recorded 11.6 with root length varying between 6.3 cm and it took 17 days for emergence of roots. Future conservation biotechnology research and its applications must be aimed at conserving highly threatened, mainly endemic, plants from conservation hotspots.

Key words: Korandi, singampatti hills, critically endangered, thidiazuron, plant growth regulator

INTRODUCTION

Medicinal plants have been the subject of man's curiosity since time immemorial. Almost every civilization has a history of medicinal plant use (Singh *et al.*, 2009). The tissue culture methodology is envisaged as a means for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale revegetation and for genetic manipulation studies (Sundaram *et al.*, 2011). Plant cell cultures were introduced as an important tool for studying and producing plant secondary metabolites in the mid 1960s (Venkatramalingam and Ebbie, 2011). Improved tissue culture technologies would help in producing the active compounds *in vitro* with better productivities without cutting down the natural resources (Siwach *et al.*, 2011).

Combinations of *in vitro* propagation techniques and cryopreservation may help in conservation of biodiversity of locally used medicinal plants (Singh *et al.*, 2009).

Eugenia singampattiana Beddome is a species of in the Myrtaceae family, locally known as 'Korandi' by anni tribes in Tirunelveli district, Tamil Nadu is one of the endemic and threatened tree species of the southern Western Ghats in Peninsular India with medicinal value. Earlier records related to its natural distribution are few and found only from Singampatti and Papanasam hills at Kalakad Mundandurai Tiger Reserve Forest (KMTR-17th Tiger Project of India) in Tirunelveli district, Tamil Nadu, in the hills at an altitudinal range of 300-900 m. The plant leaves and roots are used for a variety of purposes in traditional Indian medicine (Pavendan *et al.*, 2011). For example, the plant parts were pharmacologically proven to possess hypoglycemic, antibacterial, anti-HIV activity and anti-diarrhea effects (Bhuiyan *et al.*, 1996; Kusumoto *et al.*, 1995). Earlier Slowing *et al.* (1994) reported the anti-inflammatory activity of leaf and barks. The photochemical parameters of *Eugenia singampattiana* have similar medicinal properties as *Syzygium cumini* (Sarcar *et al.*, 2006).

During literature review no report on micro-propagation of *Eugenia singampattiana* has been found. Hence, the current study was undertaken: to explore the possibility of developing an efficient culture medium for multiplication of *Eugenia singampattiana*. There are many reports showing that the application of Thidiazuron (TDZ) results in a better shoot regeneration capacity in comparison with other cytokinins (Babaoglu and Yorgancilar, 2000; Zhang *et al.*, 2001). The plant species exhibiting increased morphogenesis in the sole presence of TDZ has continued to increase over the years, facilitating the improvement of tissue culture technology (Lone *et al.*, 2011). In this study, an efficient procedure for *in vitro* multiplication of *Eugenia singampattiana* which may facilitate conservation efforts of this threatened medicinal plant is being reported.

MATERIALS AND METHODS

The plants were collected from evergreen forests of Singampatti and Papanasam Reserved Forests in (Singampatti Hills) Tirunelveli district, Tamil Nadu, during the month of September 2009 to March 2010. The nodal segments (0.5-1 cm long) from field grown plants were used as explants. The explants were surface sterilized with 70% (v/v) ethyl alcohol for 1-5 min followed by 0.1% HgCl₂ for three min. The explants were washed four times with sterile distilled water to remove traces of HgCl₂ (Satyavani *et al.*, 2011). The single node (0.5-1 cm long) was dissected out of the cuttings and blotted on sterile filter paper.

Which were thoroughly washed in running tap water for 10 min and then washed with an aqueous solution of 5% Teepol for 3 min followed by rinsing with distilled water and 1% Bavistin fungicide for 3 min and washed with sterile water three times. The explants were disinfected with 0.1 N HgCl₂ Solution for three minutes, then thoroughly rinsed with sterile water 3-5 times. The single node (0.5-1 cm long) was dissected out of the cuttings and blotted on sterile filter paper.

Murashige and Skoog (1962) medium with 3% (w/v) sucrose were used in the study. The plant regulators tested for multiple shoots included various concentrations of BAP and TDZ and for rooting IBA. The pH of the medium was adjusted to 5.7 with 1 N NaOH or 1 N HCl before addition of 0.8% of (w/v) agar. In all the experiments, the chemicals used were of analytical grade (Mimedia, Qualigens, Fischer and Sigma). About 15 mL of was distributed to each culture tube (150×25 mm), autoclaved at 120°C at 115 kPa for 15 min. All the culture tubes were incubated in

a growth chamber at temperature of 24°C and 16 h photoperiod provided with a light intensity of 2000 Lux using white fluorescent lamps (Otroshy *et al.*, 2011). Subsequent sub culturing was done at three weeks interval.

The nodal explants were cultured on MS medium along with different concentrations of plant growth substances, including BAP and TDZ (0.3, 0.5, 1.0, 1.5 and 2.0 mg L⁻¹). The elongated shoots were excised and transferred to MS medium supplemented with different concentrations of IBA (0.1, 0.3, 0.5 and 1.0 mg L⁻¹) depending upon the objective of the experiment.

Well developed rooted plantlets were removed from the culture medium, thoroughly washed under the running tap water. The plantlets were transferred to plastics containing plastics pots containing a mixture of autoclaved garden soil, sand and vermiculate (2:1:1). They were irrigated with MS basal salt once in three days. All the potted plantlets were covered with polythene bag for maintaining high humidity. The related humidity was reduced gradually and after 15 days the plantlets were transferred to the field condition.

Statistical analysis: Ten replicates for each treatment were tested for shooting medium and 10 replicates for each treatment were tested for rooting medium. The design of experiments was completely randomized and the obtained data were statistically analyzed using Standers Error (SE) according to the method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Explants of *Eugenia singampattiana* were inoculated in MS medium supplemented with different concentration of Cytokinins and there effect on shoot regeneration was summarized. The combined effect of BAP and TDZ on shoot regeneration was found to be highest in the concentration of (1.0 mg L⁻¹) (Table 1) and recorded 93% of explants produced shoots and maximum number of shoots per explant was recorded 21.7 with shoot length 5.8 cm. Figure 1a and b shows *in vitro* regeneration of *Eugenia singampattiana* through nodalexplants include in MS medium containing BAP and TDZ (mg L⁻¹). Sub-culturing regenerated maximum rate of shoot multiplication on a medium with above this concentration BAP and TDZ (1.0 mg L⁻¹). It was found that above this concentration in the medium enhances the shoot proliferation (Fig. 1c), thus acted as a potent growth regulator for shoot organogenesis in *Eugenia singampattiana*, also reported earlier in other plants viz., *C. maritime* Linn. (Banerjee *et al.*, 2004), *Hypericum perforatum* (Murch *et al.*, 2000), *Rosa damascene* (Kumar *et al.*, 2001) and *Artemisia judaica* L. (Liu *et al.*, 2003).

Different combinations of auxin were used for root induction in *Eugenia singampattiana*. The effect of IBA on root induction was found to be highest in the concentration of IBA (0.5 mg L⁻¹) (Table 2) MS media. 98% of shoots produced roots and maximum number of roots per shoot was

Table 1: Effect of different concentrations of BAP and TDZ for multiple shoot induction from nodal explants in *Eugenia singampattiana*

Plant growth regulators BAP and TDZ (mg L ⁻¹)	Percentage of explants response	No. of shoots per explants (Mean±SD)	Length of shoots in cm (Mean±SD)
0.3	26	1.7±0.9	1.5±0.4
0.5	63	11.0±0.8	11.0±0.2
1.0	93	21.7±0.4	5.8±0.8
1.5	60	9.3±1.2	2.3±0.5
2.0	53	8.7±1.2	2.7±0.5

The data were recorded after four weeks of culture from 10 replicates

Table 2: Effect of different concentrations of IBA on rooting of micro shoots *Eugenia singampattiana*

IBA concentration	Percentage of shoots rooted	No. of roots/shoots (Mean±SD)	Average length of roots in cm (Mean±SD)
0.1	27	3.7±0.5	3.5±0.4
0.3	48	4.3±0.9	3.7±0.5
0.5	98	11.6±1.4	6.3±0.5
1.0	73	0.7±0.8	4.8±0.6

The data were recorded after four weeks of culture from 10 replicates

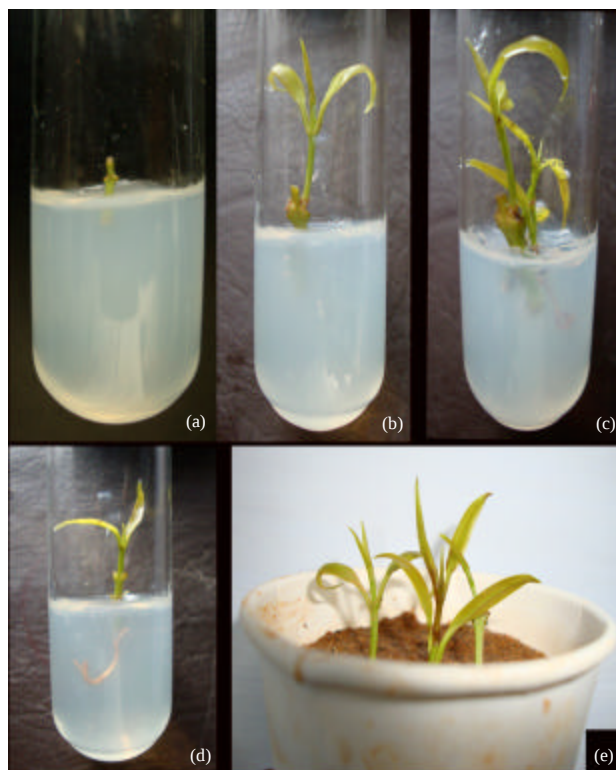


Fig. 1(a-e): *In vitro* regeneration of *Eugenia singampattiana* through nodal explants (a) Nodal explant induced MS medium containing BAP and TDZ (mg L^{-1}), (b, c) shoots formation, multiple shoot elongation, (d) Rooting of elongated shoot on MS medium containing IBA (mg L^{-1}) and (e) Hardened plants

recorded 11.6 with root length varying between 6.3 cm and it took 17 days for emergence of roots (Fig. 1d). The above results were in concordance with the findings of the Lone *et al.* (2011) in *Barleria prionitis* and Radha *et al.* (2011) in *Rubia cordifolia*. In addition, Litz and Jaiswal (1990) found IBA to be a superior auxin compared with IAA for the *in vitro* rooting of apple shoots.

During hardening, the plantlets were irrigated with one fourth strength of MS basal medium for one week. This helped the plantlets to recover the shock resulting from a change of environment. Earlier, Lone *et al.* (2011) reported maintenance of plantlets in half strength of MS medium, prior to their transfer to the soil. Humidity was maintained by covering it with rigid plastic cover and frequently spraying of water. Similar process of maintaining humidity was practiced for hardening of Caper (Musallam *et al.*, 2010). The hardened plants were transferred to the pots after

45 days of good growth (Fig. 1e). Almost 95% of plantlets survived in pots. All hardened plants were successfully established in field, where the plants appeared morphologically uniform with normal leaf form, shape and growth pattern. Thus the protocol Snedecor and Cochran (1967) described here could be of considerable commercial importance for large scale propagation of *Eugenia singampattiana* an important and threatened medicinal plant.

CONCLUSION

In vitro propagation can become an important alternative to conventional propagation for wide range of plant species. Conclusively, a fruitful protocol was set up for *Eugenia singampattiana* through multiple shoot induction. This protocol can be exploited for commercial propagation and conservation of potential endangered medicinal plant resources.

We observed that following combined effect of BAP and TDZ on shoot regeneration. The highest shoot regeneration was found in the concentration of 1.0 mg L⁻¹ and recorded 93% of explants produced shoots and maximum number of shoots per explant was recorded 21.7 with 5.8 cm shoot length. The effect of IBA on root induction was found to be highest in the concentration of IBA 0.5 mg L⁻¹ MS media. The 98% of shoots produced roots and maximum number of roots per shoot was recorded 11.6 with root length varying between 6.3 cm and it took 17 days for emergence of roots.

This present study provide some information on the nature of the growth and culture conditions for the *in vitro* regeneration of the *Eugenia singampattiana* plant which will be used further biotechnological research on *Eugenia singampattiana* medicinal plant.

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