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Standardization of protocol for *in vitro* propagation of an endangered medicinal plant *Rauwolfia serpentina* Benth.

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The present investigation was carried out with a view to standardize an *in vitro* culture technique for mass propagation of an endangered medicinal plant *Rauwolfia serpentina* Benth. Shoot tip was used as explant for initial culture. The explants were cultured on MS (Murashige and Skoog, 1962) medium supplemented with different concentration and combination of NAA and BA for primary shoot proliferation. The best shoot proliferation was observed in MS medium containing 0.1 mg L^{-1} NAA and 2.5 mgL^{-1} BA, where 92% of plants showed proliferation. For rooting, half strength MS medium supplemented with 0.4 mgL^{-1} NAA and 0.1 mgL^{-1} IBA showed maximum root formation (91%). After acclimatization and transplantation, 90% of the *in vitro* derived plants survived in *ex vivo* condition.

Key words: *Rauwolfia serpentina*, *in vitro* culture, naphthalene acetic acid, benzyl adenine, indole buteric acid, MS medium.

INTRODUCTION

Sarpagandha (*Rauwolfia serpentina*) belongs to family Apocynaceae. In Sanskrit sarpagandha means one which smells like serpent. The plant is found in almost all parts of India up to an altitude of about 1000 m. The plant is indigenous to India, Bangladesh and other regions of Asia and found to grow in the wild in many parts of the country (Ghani, 1998). It is found in West Bengal, Orissa, Bihar, Chhattisgarh, Madhya Pradesh, Andhra Pradesh, Tamil Nadu and Kerala states of India.

The roots yield 0.8 to 1.3% total alkaloids, consisting of ajmalicine, ajmaline, serpentine, serpentinine and yohimbine. The roots of sarpagandha have great medicinal value. The chemical reserpine is an alkaloid first isolated from roots of *R. serpentina* and is used to treat hypertension (Vida, 1953). The roots are bitter, acrid, laxative, thermogenic, diuretic and possess sedative properties. It is highly reputed for hypertension and is useful in stangury, fever, wounds, insomnia, epilepsy and dyspepsia (Prakash, 2001). The decoction of root is used to increase uterine contractions. The juice of the leaf is used as a remedy for the removal of opacities of the cornea. Root is also considered as anthelmintic and antidote to snake venom. Due to indiscriminate and unsustainable harvesting, Rauwolfia has attained the status of “red” listed plant in India (Ravikumar and Ved, 2000).

IUCN has also kept this plant under endangered status. In view of the increasing demand there is a need to develop approaches for efficient propagation. The major constraint in conventional propagation through seed is low germination of seeds ranging from 20 to 25%. Whereas vegetative propagation through stem and root

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Table 1. Effect of different concentrations of NAA and BA on *in vitro* shoot formation of *Rauwolfia serpentine.*

Treatment (mg L^{-1})	Average No. of shoots/culture	Average No. of useful shoots	Average length of shoot (cm)	Shooting percentage
0.5 NAA + 0.5 BA	2	1.8	2.1	57
1.0 NAA + 0.5 BA	1.9	1.6	2.3	40
2.0 NAA + 0.5 BA	1.8	1.4	2.2	45
2.5 NAA + 0.5 BA	1.7	1.1	2.4	50
0.1 NAA + 0.5 BA	2.5	1.9	2.9	53
0.1 NAA + 1.0 BA	3	2.8	3.3	63
0.1 NAA + 2.0 BA	4.3	4	3.2	75
0.1 NAA + 2.5 BA	5.8	5.6	6.8	92

cuttings leads to destructive harvesting. In view of this there is an urgent need to develop *in vitro* methods for the micro propagation and conservation of this valuable endangered medicinal plant. At present application of plant tissue culture offers valuable ways to overcome all the problems that is found in natural propagation. *In vitro* techniques offer a powerful tool for mass multiplication. This technique may rapidly increase the number of propagules for cultivation. In earlier studies regeneration through callusing in leaf and direct regeneration using apical and axillary nodes has been achieved (Bhatt et al., 2008; Prabhatsingh et al., 2009). The present study was undertaken to develop more efficient protocol for *in vitro* propagation system using shoot tip as an explant.

MATERIALS AND METHODS

The experiment was carried out at Tissue culture laboratory of Herbal Garden, Rajendranagar, Hyderabad, India. Shoot tips of field grown *R. serpentina* were used as explants. The collected explants were kept for half an hour in Bavistin 0.1% solution and then cut in to small pieces and cleaned thoroughly under running tap water. This is followed by mercuric chloride treatment (0.1%) for 10 min and washed with sterile water for 4 times and then placed on sterile filter paper to remove moisture. The surface sterilized shoot tips were cultured on Murashige and Skoog (MS) medium supplemented with inositol (100 mg l^{-1}), thiamine-HCL (0.5 mg l^{-1}), pyridoxine-HCL (1 mg l^{-1}), nicotinic acid (0.5 mg l^{-1}), sucrose (30 g l^{-1}) and different concentration and combination of NAA (Naphthalene acetic acid) and BA (Benzyl adenine). The pH was adjusted to 5.7 and solidified by 8 gm l^{-1} agar and dispensed into culture bottles. The medium was sterilized by autoclaving. After inoculation all the cultures were grown under photoperiod of 16/8 h light and dark conditions under 3000 lux light intensity at a temperature of 25±3°C. Each treatment consisted of three replications and for each replication 10 explants were used. Data on percentage of explants showing shoot proliferation, number of shoots per culture and average length of longest shoot were recorded after 4 weeks of culture initiation and the results were shown in Table 1.

In rooting experiments proliferated shoots of approximately 2 to 4 cm length were collected aseptically from culture vessels and transferred to freshly prepared half strength MS medium with different concentration and combination of NAA and IBA (Indole buteric acid). Rooted plantlets were removed from the culture bottles and agar was carefully washed away. The plantlets were then transplanted into small plastic pots containing coco peat, garden soil and sand (2:1:1). For hardening they were kept in poly house for two weeks. The acclimatized plantlets were finally established in the field (Figures 1 to 3).

RESULTS AND DISCUSSION

The explants cultured on MS medium supplemented with different concentrations and combination of NAA and BA showed varied response for shoot formation. The percentage of shoot formation and length of shoot was minimum at lower concentrations of BA. Among the different concentrations of NAA in combination with BA, the highest percent of shoot formation (92%) was induced with 0.1 mgL^{-1} NAA and 2.5 mgL^{-1} BA. No. of shoots per culture were low in shoot tips cultured on higher concentration of NAA in combination with lower concentration of BA. The results indicated that higher concentration of cytokinin (BA) have a positive effect on shoot multiplication. Multiple shoot formation was recorded with higher concentration of BA. Highest number of shoots (5.6) per culture and longest shoot length (6.8 cm) was found in 0.1 mgL^{-1} NAA and 2.5 mgL^{-1} BA. From the results it is evident that higher concentration of cytokinin in combination with auxin could induce multiple shoot elongation. Multiple shoot formation from nodal segments was reported by Salma et al. (2008), Bhatt et al. (2008), Verma et al., (2002), and Selvakumar et al. (2001). Among the various combination and concentration of growth regulators tested for root induction, the percentage of rooting is low at lower concentration of NAA. Root induction was found to be higher in half strength MS medium supplemented with 0.4 mg l^{-1} NAA and 0.1 mg l^{-1} IBA resulted in 91% root induction (Table 2) and took minimum number of days (27 to 32 days) to root formation. Root induction was lowest in shoots cultured on 0.1 mgL^{-1} NAA and 0.1 mgL^{-1} IBA (45%). Average length of longest root (6.5 cm) and no. of roots per culture (16) were maximum in medium containing 0.4 mgL^{-1} NAA and 0.1 mgL^{-1} IBA.

Table 2. Effect of different concentrations of NAA and IBA on *in vitro* root formation of *Rauwolfia serpentine.*

Treatments (mg l^{-1})	Percent rooting (%)	Days to root formation	Average length of root (cm)	No. of roots per culture
NAA 0.1+ 0.1 IBA	45	35 to 40 days	2.6	6.3
NAA 0.1+ 0.2 IBA	55	35 to 40 days	2.5	6.8
NAA 0.2+ 0.1 IBA	55	30 to 35 days	5	5.5
NAA 0.2+ 0.2 IBA	55	30 to 35 days	3.5	6.3
NAA 0.3+ 0.1 IBA	75	30 to 35 days	5.5	6
NAA 0.3+ 0.2 IBA	70	30 to 35 days	5.7	11
NAA 0.4+ 0.1 IBA	91	27 to 32 days	6.5	16
NAA 0.4+ 0.2 IBA	78	27 to 32 days	6	10

Figure 1. Multiple shoot formation from 0.1 NAA + BA 2.5 mg L^{-1}.

Figure 2. Rooting response from NAA 0.4+ IBA 0.1 mg L^{-1}.

Figure 3. Acclimatized plantlets.

Increase in rooting percentage as well as no. of roots per culture was observed with increase in concentration of NAA. The effectiveness of half strength MS medium supplemented with auxins on root induction was reported by Ahmed et al. (2002) and Huda et al. (2003). The *in vitro* rooted micro shoots survived and grew well in the coco peat in the first stage of acclimatization in poly house. These established plants exhibited 90 to 95% survival under field conditions.

Conclusions

A suitable protocol was established for *in vitro* mass multiplication of *R. serpentina*. Form the present study it was found that in *R. serpentina* shoot formation was best in MS medium supplemented with 0.1 mgL^{-1} NAA and 2.5 mgL^{-1} BA. Rooting was highest in half strength MS medium supplemented with 0.4 mgL^{-1} NAA and 0.1 mgL^{-1} IBA.

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