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In vitro Regeneration and Conservation of Rare Medicinal Plant *Dregea volubilis* Benth.

[1]N. Yogananth, [2]R. Bhakyaraj, [3]A. Chanthuru and [4]S. Palanivel
[1]Management Trustee of Ragavendra Medical Institute of Electropathy, Erode, Tamil Nadu, India
[2]Indian Institute of Crop Processing Technology, Thanjavur, Tamil Nadu, India
[3]J.J. College of Arts and Science, Pudukkottai, Tamil Nadu, India
[4]Government Arts College, Karur, Tamil Nadu, India

Corresponding Author: N. Yogananth, Management Trustee of Ragavendra Medical Institute of Electropathy, Erode, Tamil Nadu, India

ABSTRACT

A micropropagation protocol for the important medicinal plant species *Dregea volubilis* was developed using nodal explants by culturing on Murashige-Skoog (MS) media fortified with different concentration of KIN and BAP. The highest number of multiple shoots (9.36±0.67^e) was noticed at the KIN level of 3.0 mg L^{-1} followed by 8.26±0.62^e shoots in KIN 4.0 mg L^{-1}. The *in vitro* raised micro shoots produced highest percentage of rooting (78%) in MS media augmented with 1.5 mg L^{-1} IBA. The hardened plantlets were acclimatized in the green house conditions and then reintroduced in the Herbal garden attached to the Centre in which 75% of the plants survived.

Key words: *Dregea volubilis*, micropropagation, nodal culture, plant growth regulators

INTRODUCTION

Dregea volubilis Benth. (Family-Asclepiadaceae) is a large climber with green flowers in drooping umbels, with smooth bark and ash-coloured, leaves rounded at the base. It is found in India and South East Asia. The root is applied to snake bites and given to women to cure headache after child birth. Leaf and stem extracts have been reported to possess pharmacological activities, including anti-inflammatory activity, anti-diabetic and anti-cancer (Sahu *et al*., 2002; Biju *et al*., 2007). It is also used for treating rheumatic pain, cough, fever and severe cold (Muthu *et al*., 2006; Rajadurai *et al*., 2009).

In nature, this plant propagates *via* seeds but poor seed germination is one of the limitations of natural propagation and vegetative stem cuttings, is also rather too difficult. Also, the normal propagation method of *Dregea volubilis* requires a lot of time (about 6-8 months) to grow a developed plantlet from seed. Therefore, it is necessary to device a method for the development of a potentially large scale multiplication protocol for commercial production of this endangered species. *In vitro* propagation methods offers powerful tool for germplasm maintenance and multiplication.

MATERIALS AND METHODS

The healthy mother plants of *D. volubilis* were collected from J.J. College Medicinal Plant Garden, Pudukkottai, Tamilnadu, India. The nodal explants were washed thoroughly under running tap water and then treated with a few drops of Tween-80 and 1% Savlon for 10 min with constant shaking. This followed by successive three washing with distilled water to make the

material free from savlon. Again the explants were washed with 70% ethyl alcohol for few seconds and washed with distilled water for 3-4 times. After that, the explants were transferred to laminar air flow chamber and disinfected with 0.1% $HgCl_2$ for 2 min and washed with sterile distilled water for 5-7 times. Then, the explants were placed in sterile Petri plates before inoculation. The sterilized explants were injured all over the surface and used for shoot induction.

Then the nodal explants were placed on MS medium supplemented with sucrose 3% (w/v). Various concentrations of Benzyl amino purine (BAP) (1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}), Kinetin (1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1}) and Indole-3-Butyric Acid (IBA) (1.0, 1.5, 2.0, 2.5 and 3.0 mg L^{-1}) were used for shoot and root induction. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving and then adding 0.8% agar for preparing a solid medium. About 15 mL of the medium was dispensed in each culture tube and plugged with non-absorbent cotton plugs prior to autoclaving at 121°C for 15 min. All the cultures were maintained at 12 h photoperiod with 2000 lux light intensity at 25±2°C.

The rooted micropropagules were thoroughly washed to remove the traces of agar and planted in polycups containing a mixture of soil, sand and farmyard mixture in the ratio of 1:1:1 and covered with perforated plastic bags. Once in two days half MS liquid medium without sucrose was added and kept in the culture room for 2 weeks after which they were transferred to the green house for acclimatization. The percentage of survival was recorded one month after transfer. All the experiments were repeated three times with ten replicates per treatment, observations were made regularly and the details were carefully recorded.

RESULTS AND DISCUSSION

Multiple shoot induction in nodal explants was observed in MS medium with KIN (1.0-5.0 mg L^{-1}) and BAP (1.0-5.0 mg L^{-1}). Initially two or three shoot buds per explant emerged 20 days after inoculation and gradually the number of shoot buds per explant increased up to 9.36±0.67^e on MS medium fortified with 3.0 mg L^{-1} KIN alone. The Shooting response to both cytokinins treatments is shown in Table 1. The maximum percentage of response and shoot length was recorded in 3.0 mg L^{-1} of KIN (74.93±0.77^e and 7.52±0.56^e, respectively). Further increase in the concentration of KIN and BAP did not show any improvement. Of the two types of Cytokinins, KIN was found to be comparatively more effective than the other Cytokinin of BAP. Similarly, Kinetin induced multiple adventitious shoots as was reported in another *Asclepiadaceous*

Table 1: Effect of different concentrations of KIN and BAP on multiple shoot induction in *D. volubilis* from nodal explants after four weeks

Hormones (mg L^{-1})	% of response	Mean No. of shoots/explant	Mean shoots/length (cm)
KIN			
1.0	59.76±0.67^a	4.23±0.33^a	4.52±1.02^a
2.0	62.83±0.39^b	6.30±0.72^b	6.48±0.45^b
3.0	74.93±0.77^e	9.36±0.67^e	7.52±0.56^e
4.0	71.13±0.13^d	7.60±0.19^d	5.65±0.76^c
5.0	69.33±0.78^c	6.96±0.67^c	6.42±0.42^d
BAP			
1.0	49.33±0.60^a	3.40±1.00^a	3.43±0.36^a
2.0	53.70±1.01^b	5.50±0.94^b	3.42±0.67^c
3.0	62.06±0.67^c	6.70±0.88^c	5.92±0.88^c
4.0	68.66±0.67^e	8.26±0.62^e	6.46±0.65^d
5.0	65.36±0.76^d	7.53 ±0.36^d	4.82±0.82^b

Each experiment was performed with 10 replicates and was repeated thrice. Values are expressed in Mean±SEM, Level of Significance = 0.5%

Table 2: Effect of different concentrations of IBA and NAA on *in vitro* rooting in *D. volubilis* after six weeks. (20 replicates per treatment)

Hormones (mg L^{-1})	% of response	Mean No. of roots/explant	Mean roots/length (cm)
IBA			
1.0	70.26±1.46^{b}	3.14±0.24^{b}	5.117±0.521^{d}
1.5	78.70±0.92^{e}	5.79±0.32^{e}	6.031±0.502^{e}
2.0	77.34±0.69^{d}	4.09±0.20^{c}	4.116±0.259^{c}
2.5	75.33±1.67^{c}	2.13±0.23^{a}	2.324±0.248^{a}
3.0	67.16±0.67^{a}	4.98±0.17^{d}	3.241±0.288^{b}
NAA			
1.0	64.03±1.24^{c}	2.53±0.28^{d}	2.79±0.17^{d}
1.5	66.56±1.67^{d}	2.15±0.56^{c}	3.21±0.37^{e}
2.0	67.73±1.28^{d}	1.82±0.34^{b}	2.55±0.68^{c}
2.5	61.86±1.64^{b}	2.83±0.15^{e}	2.02±0.37^{b}
3.0	59.93±1.63^{a}	1.26±0.35^{a}	2.02±0.37^{b}

Each experiment was performed with 10 replicates and was repeated thrice. Values are expressed in Mean±SEM, Level of Significance = 0.5%

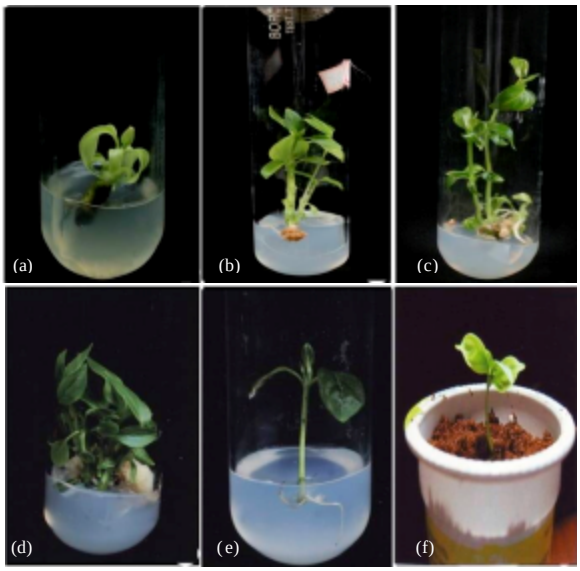

Fig. 1(a-f): Effect of KIN on plant regeneration from nodal explants of *Wattakaka volubilis*. (a) Shoot bud development, (b and c) Multiple shoot in earlier and stage, (d) Well developed multiple shoots, (e) Rooted plantlet and (f) Plantlet transferred in plastic cup

members *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Tylophora indica* (Faisal and Anis, 2003) and *Hemidesmus indicus* (Siddique *et al*., 2003).

Well developed shoots were excised from culture tubes and cultured on half strength MS containing different concentrations of IBA (1.0-3.0 mg L^{-1}) and NAA (1.0-3.0 mg L^{-1}). The percentage of root frequency, number of roots per shoot and length of roots were recorded after 4-5 weeks of culture. The rooting response to both auxin treatments is shown in Table 2. Of the two types of auxins, IBA was found to be comparatively more effective than the other auxin of NAA. IBA (1.50 mg L^{-1}) was found to be the best concentration of auxin for proper rooting where 78.70+0.92^{e}% of the shoots rooted within 6 weeks of culture. The highest average number of roots was (5.79+0.32^{e}) with root length (6.031+0.502^{e} cm) (Table 2, Fig. 1a-f). Similarly, IBA has

been used successfully to obtain the highest rooting frequency of *Decalepis hamiltonii* (Obul Reddy *et al*., 2001), *Dregea volubilis* (Vinothkumar *et al*., 2011) and *Andrographis paniculata* (Basu and Yogananth, 2011). At higher concentrations of auxins profuse callus was produced at the basal end of microshoots which inhibited the growth and elongation of roots. The present investigation has resulted in a protocol which could be used for mass propagation of *Dregea volubilis* to meet the increasing demand of the pharmaceutical industry as well as for conservation of this important medicinal plant.

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