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In vitro Shoot Proliferation and Plant Regeneration of *Phlogacanthus thyrsiflorus* Nees. a Rare Medicinal Shrub of Bangladesh

A.K. M. Sayeed Hassan*, Nadira Begum, Rebeka Sultana and Rahima Khatun

Biological Research Division, BCSIR Laboratories, Dhaka-1205, Bangladesh

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Abstract

An efficient protocol was developed for shoot proliferation and plant regeneration of *Phlogacanthus thyrsiflorus* Nees. (Acanthaceae) - a rare medicinal shrub of Bangladesh, through *in vitro* culture using shoot tip and nodal explants. Best shoot induction was observed on MS with 1.0 mg/l BAP + 0.5 mg/l NAA, in which 84.2% of nodal explants responded to produce maximum number (12.4 ± 0.66) of shoots per culture. *In vitro* raised shoots rooted on half-strength MS with 0.5 mg/l IBA + 0.5 mg/l NAA. For acclimation and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 85%.

Introduction

Medicinal plants are important source of traditional and synthetic medicines containing different types of organic compounds with therapeutic properties. Approximately 80% of people in developing countries still rely on traditional medicines for their primary health care. This usually involves the use of plant extracts (Vieira and Skorupa 1993). Many medicinal plant species are disappearing at an alarming rate, as a result of rapid agricultural and urban development, deforestation and indiscriminate collection. The tissue culture technique is very efficient in rapid mass propagation and conservation of these rare and endangered medicinal plants (Fay 1992, Sagare et al. 2000, Lakshmi and Mythili 2003).

*Author for correspondence. <aksayeedsc@gmail.com>.

Phlogacanthus thyrsiflorus Nees. commonly known as 'Ram Basak' belongs to - Acanthaceae, is a rare medicinal shrub of Bangladesh. A stoutish shrub, which looks similar to *Adhatoda zeylanica*, with large, elliptic leaves narrowly tubular, orange flowers in terminal thyrses and elongated capsules, often cultivated as an ornamental plant for its handsome, laurel-like foliage and long spikes of flowers (Anon. 1969). Various parts of this plant are quite popularly used in indigenous medicines in the treatment of disease of the respiratory system. Burnt leaves and fruits are taken as a specific for fevers. Decoction and infusion of the leaves are used as expectorants in coughs, bronchitis and asthma and as mild bronchial antiseptics (Ghani 2003). Fruit and leaf ash mixed in equal amount is taken orally with water to treat fever (Jaiswal 2010).

This plant species has become rare in Bangladesh and needs to be propagated rapidly to meet up the medicinal demand and also for conservation purposes. In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni 1998, Prakash et al. 1999). Commercial exploitation and elimination of natural habits consequent to urbanization has led to gradual extinction of several medicinal plants. It is important, therefore to develop an efficient micropropagation technique for *Phlogacanthus thyrsiflorus* Nees. for rapidly disseminate superior clones. So far no report has been published on *in vitro* propagation of *Phlogacanthus thyrsiflorus*. The present study was therefore undertaken to develop a protocol for shoot proliferation and plant regeneration of this important medicinal shrub through *in vitro* culture.

Materials and Methods

Explant of *Phlogacanthus thyrsiflorus* Nees. was collected from Curzon Hall Campus of Dhaka University. Shoot tip and nodal explants with a single axillary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface sterilized with 0.1% (w/v) mercuric chloride for 5 min, followed by five times rinse with sterile distilled water under laminar air flow cabinet. The surface sterilized explants were sized to 1.0 - 1.5 cm length containing a single node with an axillary bud or a shoot tip with an apical bud. Explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for shoot regeneration.

MS with different concentrations of hormone was used for shoot proliferation and shoot regeneration and half strength MS was used for *in vitro* root induction. All media were supplemented with 30 g/l sucrose, 7 g/l agar

(Difco) and dispensed into 15 × 150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 1.9 kg/cm^2 pressure at 121°C for 20 min. The cultures were incubated for a 16 hrs photoperiod at 24 ± 2°C under 1200 lux/m^2 fluorescent light.

Shoot proliferation from shoot tip and nodal explants was obtained in two separate sets of experiments. In the first experiment 0.5 - 2.0 mg/l BAP and 0.5 - 2.0 mg/l Kn were incorporated into MS to select the best cytokinin for the response of shoot induction. In the second set, combination of BAP (0.5 - 2.0 mg/l) with NAA (0.1 - 0.5 mg/l) and BAP (0.5 - 2.0 mg/l) with IAA (0.1 - 0.5 mg/l) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded after every week of inoculation.

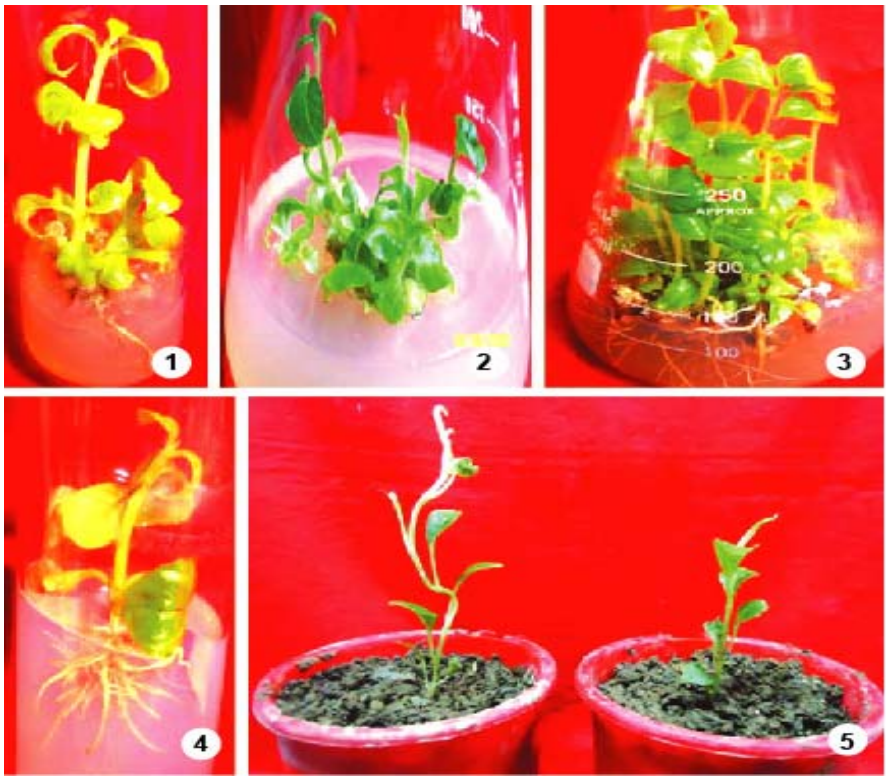
For *in vitro* rooting, individual shoots (3 - 5 cm long) were excised from the proliferated shoot cultures and implanted onto half strength MS media with different concentrations and combinations of NAA, IBA and IAA.

The rooted plantlets were taken out from the culture tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with soil and compost (1 : 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2°C for a 12 hrs photoperiod under 1500 lux/m^2 sunlight for acclimation. Established plantlets were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tip and nodal explants of *Phlogacanthus thyrsiflorus* Nees. were cultured on MS with various concentrations of BAP alone and with NAA or IAA for multiple shoot regeneration. The explants were found to be swollen and they produced 2 - 3 shoots within three weeks after inoculation (Fig. 1) on MS containing BAP alone but the number of shoots increased up to 9 when the explants were cultured in MS with 1.0 mg/l BAP + 0.5 mg/l NAA (Fig. 2). Both the explants responded in the same medium but highest number of micro-shoots were induced from nodal explants (Table 1). BAP alone or BAP with IAA were not suitable than BAP with NAA for shoot induction (Table 1) and combinations of Kn with NAA or IAA were also not suitable for shoot induction. Newly initiated shoots were separated and subcultured repeatedly in fresh MS with 1.0 mg/l BAP + 0.5 mg/l NAA, where the number of shoots increased up to 12.4 ± 0.66 per culture (Table 1, Fig. 3). In different medicinal plant, it was also observed that multiple shoots were found by using different concentrations of cytokinin with auxins by other researchers (Faisal et al. 2003, Gawde and Paratkar 2004, Baskaran and Jayabalan 2005, Hassan and Roy 2005, Gopalakrishnan et al. 2009, Hassan and Khatun 2010, Ugraiah et al. 2010).

Eighty two per cent regenerated shoots rooted (Fig. 4) when cultured individually on root induction medium consisted of half-strength MS with 0.5 mg/l IBA + 0.5 mg/l NAA (Table 2). Use of auxins singly or in combination for rooting was also reported (Sahoo and Chand 1998, Ajithkumar and Seeni 1998, Rai 2002, Sivakumar and Krishnamurthy 2000, Bhadra et al. 2009, Hassan and Sikdar 2010).



Figs 1 - 5: *In vitro* regeneration of *Phlogacanthus thyrsiflorus* Nees. from nodal explants. 1. Induction of shoots in three weeks of culture on MS +1.0 mg/l BAP + 0.5 mg/l NAA from nodal explants. 2. Development and multiplication of shoots on MS + 1.0 mg/l BAP + 0.5 mg/l NAA from nodal explants after six weeks of culture. 3. Development and multiplication of shoots on MS + 1.0 mg/l BAP + 0.5 mg/l NAA from nodal explants after nine weeks of culture. 4. Rooting of *in vitro* regenerated shoots cultured on half-strength MS + 0.5 mg/l IBA+ 0.5 mg/l NAA in third weeks. 5. Acclimated regenerated plants of two months old.

After four weeks the rooted shoots were transferred to pots. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 85 per cent of the transplanted plantlets of *Phlogacanthus thyrsiflorus*. survived if the plantlets in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and

Table 1. Effect of growth regulators in MS on morphogenic response of *Phlogacanthus thyrsiflorus* Nees. Webster shoot tips and nodal explants.

Growth regulators (mg/l)	Shoot tips		Nodal explants	
	% of explants forming shoots	Mean No. of shoot/explant	% of explants forming shoots	Mean No. of shoot/explant
BAP				
0.5	61.2 ± 2.58	04.0 ± 0.59	64.4 ± 2.10	07.4 ± 0.91
1.0	63.4 ± 1.57	05.6 ± 0.45	71.4 ± 2.38	08.6 ± 0.72
1.5	57.6 ± 2.16	04.4 ± 0.72	67.6 ± 2.16	07.4 ± 1.18
2.0	34.8 ± 2.58	03.8 ± 0.76	33.6 ± 1.84	06.4 ± 0.82
BAP + NAA				
0.5 + 0.1	42.6 ± 0.87	05.2 ± 0.59	68.6 ± 1.70	09.6 ± 1.14
1.0 + 0.2	61.4 ± 2.87	06.6 ± 0.77	73.6 ± 0.51	10.6 ± 0.91
1.0 + 0.5	**72.4 ± 2.89**	**08.4 ± 0.76**	**84.2 ± 2.80**	**12.4 ± 0.66**
1.5 + 0.5	48.8 ± 1.77	06.0 ± 0.63	61.2 ± 2.47	09.8 ± 0.95
2.0 + 0.5	28.2 ± 1.66	04.4 ± 0.45	52.2 ± 0.66	08.8 ± 0.95
BAP + IAA				
0.5 + 0.1	22.2 ± 1.96	04.0 ± 0.39	56.8 ± 2.14	07.2 ± 0.76
1.0 + 0.2	26.6 ± 1.66	04.2 ± 0.65	67.6 ± 2.10	08.2 ± 0.51
1.5 + 0.5	21.0 ± 1.14	03.4 ± 0.45	52.6 ± 1.63	07.4 ± 0.91
2.0 + 0.5	16.2 ± 0.86	02.4 ± 0.45	48.4 ± 0.93	06.4 ± 0.66

Results are mean ± SE of three experiments with 15 replications.

Table 2. Effect of auxin(s) on root induction in regenerated shoots of *Phlogacanthus thyrsiflorus* Nees. on half-strength MS.

Growth regulators (mg/l)			% of shoots roducing roots (± SE)	No. of roots/ shoot (± SE)
IBA	NAA	IAA		
0.5			80.4 ± 0.64	9.8 ± 0.59
0.75			67.2 ± 1.53	8.8 ± 0.65
1.0			63.2 ± 1.46	7.2 ± 0.76
	0.5		71.0 ± 0.10	8.6 ± 0.72
	0.75		57.8 ± 1.85	7.2 ± 0.76
	1.0		54.2 ± 1.53	6.0 ± 0.63
0.5	**0.5**		**82.0 ± 0.71**	**11.8 ± 0.59**
1.0	1.0		59.4 ± 1.08	9.2 ± 0.71
0.5		0.5	65.2 ± 1.16	8.8 ± 0.65
1.0		1.0	61.4 ± 0.75	7.6 ± 0.96
0.5	0.5	0.5	62.6 ± 0.93	7.6 ± 0.72
1.0	1.0	1.0	56.2 ± 1.34	6.2 ± 0.65

Data were recorded after 4 weeks of culture. Results are mean ± SE of 15 replications.

reared for three weeks. The plantlets were reared under semi-controlled temperature (30 ± 2ºC) and light (1500 lux) in a chamber with 80 per cent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and healthier (Fig. 5).

After three weeks, plantlets were transferred to an open place and gradually acclimated to outdoor conditions, where 85 per cent plants survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation for sustainable use.

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