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In vitro Propagation of *Abrus precatorius* L. - A Rare Medicinal Plant of Chittagong Hill Tracts

Animesh Biswas, Mohashweta Roy, M. A. Bari Miah* and S. K. Bhadra[1]

Institute of Biological Sciences, Rajshahi University, Rajshahi-6205, Bangladesh

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Abstract

An efficient protocol was developed for *in vitro* propagation of *Abrus precatorius* L. through induction of indirect organogenesis in nodal segment derived callus tissue. Yellowish-green nodular callus was induced at the cut surface of the nodal segments cultured on MS fortified with 5.0 mg/l BAP and 0.5 mg/l NAA. The callus differentiated into adventitious shoots when it was subcultured on to MS supplemented with 3.0 mg/l BAP + 0.5 mg/l Kn + 0.5 mg/l NAA. On an average 6.87 ± 0.26 shoots/culture developed. These microshoots were rooted in half-strength MS containing 1.0 mg/l IBA and the rooted plantlets were transferred to soil after proper acclimatization.

Introduction

Abrus precatorius L. commonly known as 'Kunch' in Bengali is a deciduous woody climber of the family Fabaceae. It can be easily recognized by shiny scarlet coloured seeds with a black spot at one end. Since last long this plant species has been in use for its medicinal value (Kirtikar and Basu 1980, Biswas and Ghosh 1973). Different plant parts of this species contain various kinds of alkaloids such as glycerrhizin, precol, abrol, abrasine, abrin A and abrin B which impart its medicinal value (Joshi 2000, Ghani 2003). The herbalists of Chittagong Hill Tracts (CHT) use seeds, leaves and roots of *A. precatorius* to induce abortion, pains and skin diseases. In CHT this medicinally important plant species is facing extinction due to indiscriminate collection, large scale deforestation and *Jhum* cultivation.

In nature the propagation of *A. precatorius* through seeds is difficult because of their hard seed coat - a trait which explains its sparse distribution. It is, therefore important to develop a protocol for *in vitro* propagation to save this medicinally important taxon from further depeletion of its population, at the same time to meet up the demand of the traditional medicine industry.

*Corresponding author. [1]Department of Botany, Chittagong University, Chittagong-4331, Bangladesh.

In vitro propagation has proven as a potential technology for mass scale production of medicinal plant species (Lui and Li 2001, Wawrosch et al. 2001, Martin 2002, 2003, Azad et al. 2005, Faisal et al. 2003, Hassan and Roy 2005). So far our knowledge goes, no report has been published on *in vitro* propagation of *A. precatorius*. The present investigation reports the *in vitro* propagation technique that can be used as a potential tool for large scale production of this medicinal plant.

Materials and Methods

Mature seeds of *Abrus precatorius* L. were collected from a local herbalist of CHT and sown in earthen pots for raising seedlings. Juvenile twigs from one-year-old mature plants were used as source of explants. Juvenile twigs were surface sterilized with $HgCl_2$ solution (0.1% w/v) for four - six min. Thereafter, five washes were done with sterile distilled water. Nodal segments of twigs were cut (0.5 cm) and cultured on 8% (w/v) agar solidified MS supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kn) at different concentrations and combinations. Subculturing was done at an interval of 14 - 20 days. Once the shoot buds developed, they were further cultured for elongation in the same medium. Elongated shoot buds were rooted on half strength MS fortified with different concentrations of auxins (NAA and IBA) alone. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 25 ± 2^{o}C under 16/8 hr photoperiod. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimation.

Results and Discussion

Within seven to 15 days of culture callus formed at the cut surfaces of nodal explants, when grown on MS supplemented with 2, 5 and 8 mg/l BAP and Kn either alone or in combination with 0.1 - 1.0 mg/l NAA, IAA and IBA (Table 1). Maximum (80%) callus formation took place on MS fortified with 5.0 mg/l BAP with 0.5 mg/l NAA after two successive subcultures. In this combination light yellowish green and nodular callus developed (Fig. 1). Callus was also induced in BAP and Kn supplemented medium. However, BAP was found to be more effective than Kn for callus induction (Table 1). According to Preece et al. (1991), callus forms frequently at the basal cut ends of nodal explants on cytokinin enriched medium in species exhibiting strong apical dominance.

The highest number of shoots (6.87 ± 0.26/callus) developed in MS with 2.0 mg/l BAP + 0.5 mg/l Kn + 0.5 mg/l NAA (Table 2, Figs. 2 - 3). There were significant differences in regeneration frequencies, number of shoots/culture and length of shoots/culture. As stated by Martin (2002) the high morphogenic efficiency of node segments derived callus may be due to the presence of some

internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds developed from callus culture elongated. This continued in two subsequent subcultures made up of identical constituents at an interval of 15 days. Shoot regeneration *via* a callus phase was the simplest

Table 1. Effect of different concentrations and combinations of growth regulators on MS for callus induction in *A. precatorius* from nodal explants.

Growth regulators (mg/l)					% explants producing callus*	Days to callus induction*
BAP	Kn	NAA	IAA	IBA		
Growth regulator free medium					-	-
2					20 ± 3.84b	12 ±1.52a
5					51 ± 1.22a	12 ± 1.52a
8					40 ± 1.21ab	12 ± 2.08a
	2				18 ± 2.22b	15 ± 3.21a
	5				31 ± 4.44ab	14 ± 3.03a
	8				20 ± 1.21b	14 ± 3.46a
5		0.1			40 ± 3.84bc	10 ± 1.0bc
5		0.5			82 ± 2.22a	7 ± 1.15c
5		1.0			55 ± 0.92b	8 ± 1.52c
5			0.1		20 ± 3.84d	15 ± 2.51a
5			0.5		44 ± 4.44bc	10 ± 1 bc
5			1.0		27 ± 1.13cd	15 ± 3.5a
5				0.1	31 ± 5.87bcd	14 ± 3.51ab
5				0.5	40 ± 7.69bcd	14 ± 2.0ab
5				1.0	20 ± 1.21cd	15 ± 3.05a

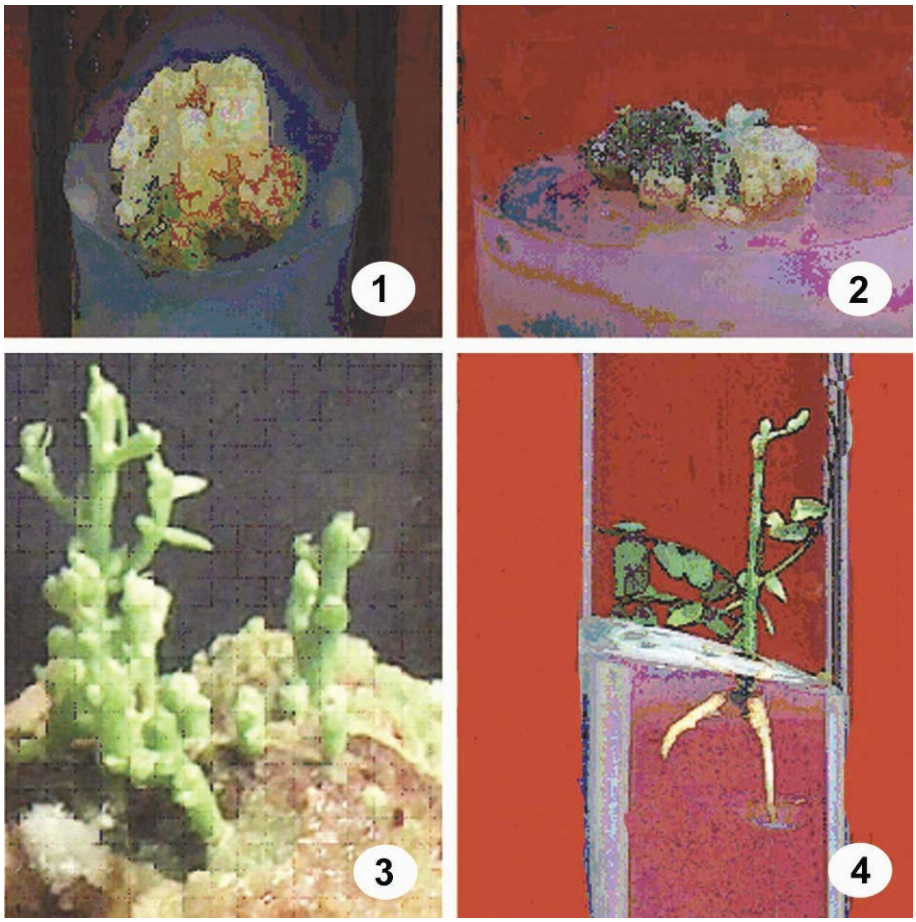
*Mean values within columns followed by the same letter are not significantly different at 5% level.

Table 2. Effect of different concentrations and combinations of growth regulators on MS for the adventitious shoot regeneration from the nodal callus of *A. precatorius*.

Growth regulators (mg/l)					% callus tissue producing shoots	Mean No. of shoots*/ callus	Length (cm) of shoots*
BAP	Kn	NAA	IAA	IBA			
Growth regulator free					-	-	
5.0	0.1				-	-	-
5.0	0.5				-	-	-
5.0	1.0				-	-	-
5.0	0.1	0.1			40c	2.43 ± 0.35c	0.90 ± 0.10c
5.0	0.5	0.5			80a	6.87 ± 0.26a	1.93 ± 0.17a
5.0	1.0	1.0			50b	3.33 ± 0.18b	1.28 ± 0.13b
5.0	0.1		0.1		30e	1.10 ± 0.1d	0.67 ± 0.15d
5.0	0.5		0.5		43d	2.0 ± 0.55c	0.82 ± 0.31cd
5.0	1.0		1.0		27d	0.8 ± 0.01de	0.18 ± 0.07cd
5.0	0.1			0.1	20e	0.43 ± 0.14e	0.34 ± 0.05e
5.0	0.5			0.5	30d	1.15 ± 0.10d	0.65 ± 0.16d
5.0	1.0			1.0	-	-	-

*Mean values within columns followed by the same letter are not significantly different at 5% level.

way to induce somaclonal variation and thus pave the way for improvement of the species (Thorpe et al. 1991). Such indirect organogenesis was reported in many medicinal plant species including *Asparagus cooperi* (Ghosh and Sen 1989), *Plumbago zeylanica* (Das and Rout 2002), *Holostema ada-kodien* (Martin 2002), *Rotula aquatica* (Martin 2003), *Gloriosa superba* (Sivakumar et al. 2003), *Phellodendron amurense* (Azad et al. 2005).



Figs. 1 - 4 : Indirect shoot regeneration of *A. precatorius*. 1. Light yellowish green nodular callus. 2. Initiation of shoot buds from callus 15 days after transfer. 3. Shoot buds elongated in the same medium. 4. Elongated shoot buds were rooted.

When shoot buds started elongation and leaves developed in the nodal zone, quick abscission of leaves took place. It remained a problem for keeping the shoot buds healthy. Similar results were previously reported in other medicinal plants species (Patnaik and Debata 1996, Saxena et al. 1997). Martin (2002) considered that necrosis and abscission of leaves and shoots were due to the accumulation of ethylene, they used $AgNO_3$ or $CoCl_2$ for resolving this problem.

But in the present study abscission could not be resisted by the use of either $AgNO_3$ or $CoCl_2$.

Table 3. Effect of half-strength MS with different concentrations of auxins on root proliferation in *in vitro* grown shoots from callus cultures of *A. precatorius*.

Growth regulators (mg/l)		% of shoots producing roots	No. of roots*/shoot	Average length (cm) of roots*
Control		-	-	-
NAA	IBA			
0.1		-	-	-
0.5		27cd	0.98 ± 0.25cd	0.45 ± 0.11f
1.0		40cd	1.43 ± 0.27bc	0.72 ± 0.10de
1.5		-	-	-
	0.1	-	-	-
	0.5	50bc	1.17 ± 0.12cd	1.13 ± 0.06bc
	1.0	70b	1.93 ± 0.23b	1.33 ± 0.10b
	1.5	47bc	1.67 ± 0.35bc	0.91 ± 0.10cd

*Mean values within columns followed by the same letter are not significantly different at 5% level.

Rooting experiments were conducted in MS supplemented with 0.1-1.0 mg/l either NAA or IBA. Medium containing 1.0 mg/l IBA proved to be the most effective for rooting of microshoots than that of any concentration of NAA (Table 3, Fig. 4). In this medium the highest per cent (70) and number (3.23 ± 0.27) of root formed at the cut end of microshoots within two weeks of culture. The effectiveness of IBA in rooting has been reported in many medicinal plants (Martin 2002, Chandramu et al. 2003). Shoots with strong and stout root system were acclimatized outside growth chamber for one week and then transferred to earthen pots placed in natural environment containing mixture of soil and manure (1 : 1). Seventy five per cent plants survived in nature.

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