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IN VITRO REGENERATION OF *ARISTOLOCHIA TAGALA* CHAMP. - A RARE MEDICINAL PLANT OF CHITTAGONG HILL TRACTS

Animesh Biswas[1?] M A Bari[1], Mohashweta Roy[1] and S K Bhadra [2]

[1]Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh
[2]Department of Botany, University of Chittagong, Chittagong, Bangladesh

Abstract

An efficient regeneration protocol through *in vitro* direct organogenesis was developed for a valuable medicinal plant *Aristolochia tagala* Champ. using nodal segments as explants. Multiple shoot buds were induced directly from nodal explants cultured on MS (Murashige and Skoog 1962) basal medium supplemented with 2.0 mg/l BAP (N6- benzylaminopurine) and 0.5 mg/l NAA (a-naphthalenacetic acid). The average number of shoots induced per culture was found to be six. Excised shoot roots were cultured on half-strength MS medium containing 0.5 mg/l IBA. The rooted plantlets were transferred to natural environment after proper acclimatization.

Key words: *Aristolochia tagala*, rare medicinal plant, direct organogenesis.

Introduction

Aristolochia tagala Champ. is a twining herb of the family Aristolociaceae. In Bangladesh, it grows mainly in forest thickets and hill slops of Chittagong Hill Tracts (CHT). The plant is valuable for its bioactive compound aristolochic acid, which is unique to this genus (Wu *et al*. 2004). Roots and leaves of this plant are widely used in the traditional tribal medicine in CHT to treat fever, dysentery, snakebite, rheumatism and toothache (Biswas 2006). Propagation of *A. tagala* relies only seed but the viability of seed is very low due to the presence of scanty endosperm. For this reason, the natural propagation of this plant species is hampered. Moreover, deforestation, jhum cultivation and over exploitation are causes of depletion of this medicinally important plant species. In these consequences, an alternative mass propagation system like *in vitro* propagation technology can efficiently be applied to save this rare medicinally important plant to meet up the demand as well as for future conservation. Tissue culture techniques are being increasingly exploited for clonal multiplication and in vitro conservation of valuable indigenous germplasm threatened with extinction (Anand and Jeyachandran 2004). So far our knowledge goes; no report has been published on *in vitro* propagation of *A. tagala.* Thus an attempt was taken on *in vitro* propagation of this medicinal plant species.

Materials and Methods

Mature seeds of *Aristolochia tagala* Champ. were collected during field survey in the CHT and sown in earthen pots for raising seedlings. Juvenile twigs from 6-month-old plants were used as source of explants. Surface sterilization of young twigs was done with $HgCl_2$ solution (0.1% w/v) for 6-8 min following 5 washes with sterile distilled water. Nodal segments (0.5 cm) of the twigs were cut and cultured on to 8% (w/v) agar gelled MS medium supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kn) at different concentrations and combinations. Sub culturing was done at 14 - 21-day intervals. Shoot buds were further cultured for elongation in the same medium for two-times at 7-day intervals. Elongated shoot buds were

[?] Corresponding author, E-mail: animeshmedp@yahoo.com

jrooted on half strength MS medium 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 ± 20?C under 16/8h photoperiods. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimatization.

Results and Discussion

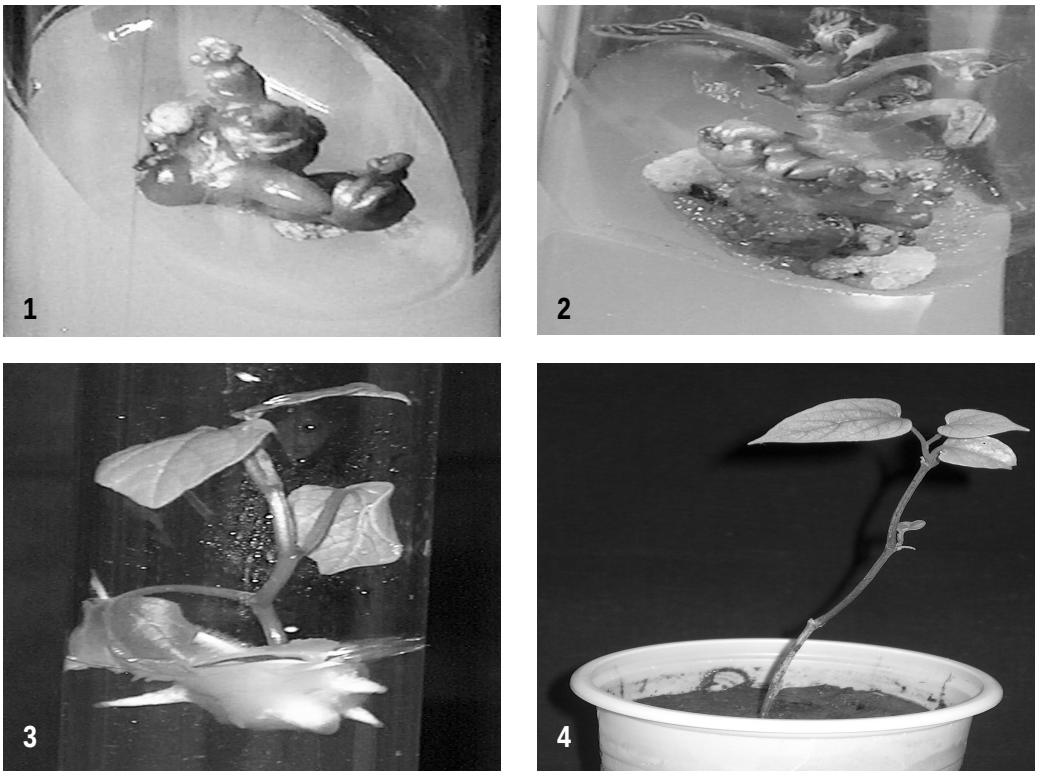
The nodal explants underwent direct organogenesis when cultured on MS medium using various concentrations of BAP (1.0-4.0) and Kn (1.0-4.0) separately or BAP in combination with low concentrations (0.1-1.0 mg /l) of auxins (NAA and IAA). It is observed that BAP was more effective for shoot induction than Kn (Table1).

Table 1. Effect of different concentrations and combinations of growth regulators in MS medium on direct organogenesis from nodal explants of *A. tagala*.

Growth regulators (mg/l)				% of culture induced shoot	*Number of shoot/culture ± SE	*Length (cm) of shoot/culture ± SE
BAP	Kn	NAA	IAA			
PGR free				-	-	-
1				20	0.36 ± 0.13	0.36 ± 0.14
2				31	0.43 ± 0.11	0.46 ± 0.09
3				40	0.66 ± 0.13	0.52 ± 0.14
4				20	0.24 ± 0.08	0.26 ± 0.12
	1			11	0.31 ± 0.24	0.13 ± 0.09
	2			20	0.37 ± 0.13	0.29 ± 0.06
	3			22	0.43 ± 0.13	0.22 ± 0.08
	4			9	0.24 ± 0.09	0.12 ± 0.06
1		0.1		30	1.31 ± 0.24	0.48 ± 0.12
1		0.5		40	1.91 ± 0.20	0.89 ± 0.09
1		1.0		22	0.67 ± 0.16	0.37 ± 0.08
2		0.1		51	2.17 ± 0.21	0.88 ± 0.12
2		0.5		79	3.60 ± 0.09	1.51 ± 0.14
2		1.0		64	2.93 ± 0.08	1.21 ± 0.09
1			0.1	22	0.84 ± 0.16	0.40 ± 0.10
1			0.5	31	0.71 ± 0.14	0.40 ± 0.12
1			1.0	18	0.53 ± 0.08	0.27 ± 0.10
2			0.1	31	0.76 ± 0.09	0.53 ± 0.06
2			0.5	51	1.04 ± 0.10	0.67 ± 0.10
2			1.0	33	0.64 ± 0.10	0.36 ± 0.09

* Values are the mean of three replicates with 15 explants.

Among the different treatments of BAP, 3.0 mg/l BAP supplemented medium gave better response. In this concentration, 40% explants induced to develop shoots. The number of shoot as well as length of shoot per explant was recorded 0.66 ± 0.13 and 0.52 ± 0.14 cm respectively. BAP is considered one of the most useful cytokinins for the multiplication of axillary buds reported by many authors (Joshi and Dhar 2003, Martin 2002 and Sharma *et al*. 1993). In the present investigation, combination of BAP with NAA was found more suitable than BAP alone. Among the BAP - NAA supplemented media the best response was achieved in 2.0 mg/l BAP + 0.5 mg/l NAA after 30-day of culture and 79% explants showed proliferation in this combination (Figs. 1-4). The highest mean number of shoot and shoot length (cm) per culture were 3.60 ± 0.09 and 1.51 ± 0.14 respectively (Table 1). These results are in agreement with the results of Sultana and Handique (2004), Chandramu *et al*. (2003), Sudha *et al*. (2003) and Chen *et al*. (2001). On the other hand, combinations of BAP with IBA and Kn with IBA supplemented media did not give any positive response. In the present study, it was found that the number of shoot per culture was increased with the number of subculture. Rout *et al*. (2000) reported that, a rapid rate of propagation depends on the sub-culturing of proliferating shoots.



Figs. 1-4. Direct shoot regeneration in nodal segments of *Aristolochia tagala*

1. Induction of multiple shoots from nodal segments on MS medium fortified with 2.0 mg/l BAP + 0.5 mg/l NAA after 30 day of incubation. 2. After 42 day of subculture in the same medium. 3. Elongated shoot buds were rooted on half strength MS with 0.5 mg/l IBA. 4. An acclimatized plant implanted in pot.

Rooting experiments were conducted in ½ MS medium supplemented with 0.1-1.0 mg/l either NAA or IBA. Medium containing 0.5 mg/l IBA was proved to be the most effective for rooting of microshoots than that containing any other concentrations of NAA. (Table 2 and Fig. 3).

Table 2. Effect of half-strength MS with different concentrations of auxins on root proliperation in *in vitro* grown shoots of *A. tagala*.

Growth regulators (mg/l)		% of shoot rooted	No. of roots*/shoot ± SE	Average length (cm) of roots* ± SE
Control		-	-	-
NAA	IBA			
0.1		17	0.33 ± 0.11	0.42 ± 0.14
0.5		23	0.63 ± 0.13	0.45 ± 0.11
1.0		40	0.93 ± 0.27	0.72 ± 0.10
	0.1	43	0.96 ± 0.22	0.58 ± 0.13
	0.5	80	1.17 ± 0.12	1.10 ± 0.06
	1.0	51	1.0 ± 0.23	0.93 ± 0.10

* Values are the mean of three replicates with 15 explants.

In this medium the highest 80% percent shoots induced rooting within two weeks of culture and the mean number of root per culture was 1.17 ± 0.12. Martin (2002) and Chandramu *et al*. (2003) reported the effectiveness of IBA in rooting of in vitro induced shoots of medicinal plants. Shoots with strong and stout root system were acclimatized in outside growth chamber for two weeks for hardening and then transferred to plastic pots placed in natural environment containing mixture of soil and manure (1:1). Eighty per cent plants survived and found normal in natural conditions.

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