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IN VITRO PLANT REGENERATION FROM INTERNODE AND LEAF SEGMENTS DERIVED CALLUS IN *Adhatoda vasica* NEES.

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ABSTRACT

The investigation was carried out to observe callus induction and subsequent regeneration potentiality of *Adhatoda vasica* NEES from internode and leaf segments of mature plants. The nature of callus was hard in texture and greenish white in colour in all concentrations of 2,4-D. On the other hand, the nature of callus was loose in texture and creamy in colour in all concentrations of NAA which was achieved from internode and leaf segments on MS medium supplemented with 4.0 mg/l NAA within four weeks of inoculation. The callus produced large number of shoots when cultured on MS medium with 1.0 mg/l BAP +0.1 mg/l NAA within 4 weeks of subculture. *In vitro* raised shoots were rooted on MS medium supplemented with 1.0 mg/l IBA within 4 weeks of subculture. *In vitro* grown plantlets with well root system were successfully established in natural condition through successive phages of acclimatization. The regenerated plants were healthy, uniform and identical to the donor plants and the survival percentage was 80.

Key words: Plant regeneration, internode, leaf segments, callus induction, *Adhatoda vasica*

INTRODUCTION

Herbal medicine is remarkable used by human being. As many as 75 to 95 % of the world rural people rely on herbal medicine for their primary health care. The success of any health care system depends on the availability of suitable drugs on a sustainable basis. Medicinal plants play a key role in the world health care system (Bajaj and Wiliams, 1995). *Adhatoda vasica* Nees, belongs to the medicinal family Acanthaceae, is an evergreen shrub of 1.2-2.4 feet in height with many long opposite branches distributed from the Punjab in the North, and Bengal and Assam in the South-East to the Cylon, Malaya and Singapore in the South. It is one of the most important medicinal plants in this region (Rahman *et al.*, 2004). The plant is valued containing bronchodilator alkaloids mainly vasicine. All parts of the plants are used in herbal medicine and particularly the leaves are credited with insecticidal and parasiticidal properties. The root is useful in strangury, leucorrhoea, bronchitis, asthma, bilious vomiting, sore eyes, fever and gonorrhoea. It is a valuable anticeptic and antiperiodic and anthelmintic. The leaves are considered as a various efficacious remedy for all sorts of coughs and asthma, diarrhoea and dysentery. The leaves are also used for rheumatism. The flowers and fruits are bitter and aromatic antispasmodic. The fresh flowers are used in ophthalmia, lessen strangury and jaundice (Kirtikar and Basu, 1994). Apart from its diverse medicinal and insecticidal uses, the plant is also known for reclaiming degraded soil, artificial ripening of fruits and as a fodder for horses. The stem is used for production of the yellow dye and wood for gun powder, charcoal and beads (Singh *et al.*, 1998). The plant is conventionally propagated by seeds. As the alkaloid content of plant varies with genotype therefore, it is recommended to propagate *A. vasica* plant using vegetative method (Duster, 1985). Chomchalow and Sahavacharin (1981) first attempted regeneration of *A. vasica* through tissue culture. Later Jaiswal *et al.* (1989) reported regeneration of *A. vasica* plantlets *in vitro* by culturing nodal explants on MS medium. However, the limited numbers of plants were produced in both cases. The present investigation was therefore, undertaken to establish protocol for large-scale production of plantlets *in vitro* from the internode and leaf explants of mature plant with a view to clone high alkaloid containing genotypes.

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MATERIALS AND METHODS

Healthy, disease free internode and leaf explants from the mature plant of *Adhatoda vasica* were collected from 2-3 years old plant grown at the Botanical garden, Rajshahi University, Bangladesh. These explants were taken into a conical flask and thoroughly washed under running tap water for 30 minutes to remove loose contaminates attached to explants. Then the explants were washed with distilled water containing 1% savlon (w/v) and 2 drops of Tween 80 for 20 minutes to remove gummy substance. This was followed by successive 3 washing with distilled water to make the material free from savlon. Subsequently, the materials were transferred to running laminar airflow hood. The explants were taken into 3 sterile conical flasks and suspended in concentrations of $HgCl_2$ for 5 minutes to ensure contamination free culture. To remove every trace of the strident, the materials were then washed at least 5 times with sterile distilled water. The explants were cultured on MS (Murashige and Skoog, 1962) media fortified with different concentrations and combinations of plant growth regulators along with 3% sucrose and 0.6% of agar. The pH of the medium was adjusted to 5.7 before autoclaving at 121°C for 20 minutes. The explants were inoculated on MS medium containing different concentrations and combinations of BAP, NAA, 2, 4-D and IBA. All cultures were maintained at 16 hour photoperiod with 3000 lux light intensity at 25 ± 2°C temperature. For each treatment, 20 replicates were used and all experiments were repeated thrice. The well developed plantlets were then rescued very carefully from the tubes and carefully washed out under running tap water. Then the rooted plantlets were transferred to pots containing garden soil, cow dung and sand (1:1:1). The potted plantlets were regularly sprayed with water using a hand spray and covered with polythene sheets to maintain high humidity around juvenile plants. Plantlets were subsequently transferred to larger pots and gradually acclimatized to outdoor condition.

RESULTS AND DISCUSSION

Callus induction

Internode and leaf explants were cultured into MS medium supplemented with different concentrations of 2, 4-D (1.0- 5.0 mg/l) and different concentrations of NAA (1.0- 5.0 mg/l). Results obtained for callus differentiation of the cultured explants are shown in Table 1. The range of cultured producing callus was 20-70% in both explants.

Table 1. Effect of different concentrations of 2,4-D and NAA singly on callus induction from internode and leaf explants of *Adhatoda vasica*

	Growth regulators & Con. (mg/l)	Source of explants									
		Internode					Leaf				
		callus induction (%)	Texture & colour of callus	Fresh weight of callus (gm)	Dry weight of callus (gm)	Degree of Callusing	callus induction (%)	Texture & colour of callus	Fresh weight of callus (gm)	Dry weight of callus (gm)	Degree of callusing
2,4-D	1.0	20	G.W.H	0.800	0.300	+	30	G.W.H	0.780	0.550	+
	2.0	30	G.W.H	0.918	0.428	++	40	G.W.H	0.890	0.518	++
	4.0	50	G.W.H	1.00	0.500	++	60	G.W.H	1.000	0.576	++
	5.0	40	G.W.H	0.840	0.420	++	50	G.W.H	0.900	0.490	++
NAA	1.0	50	C.L	0.458	0.350	+	40	C.L	0.917	0.447	+
	2.0	60	C.L	1.454	0.990	++	60	C.L	1.330	0.743	++
	4.0	70	C.L	2.10	1.00	+++	70	C.L	2.16	0.988	+++
	5.0	50	C.L	1.230	0.750	++	60	C.L	0.928	0.520	++

G= Greenish, W=White, H= Hard, C= Creamy and L = Loose

The highest 70% of callus was recorded when 4.0 mg/l of NAA was used and fresh and dry weight of calli was observed 2.10 gm and 1.00 gm, respectively after 28 days (Fig.1 A&B).

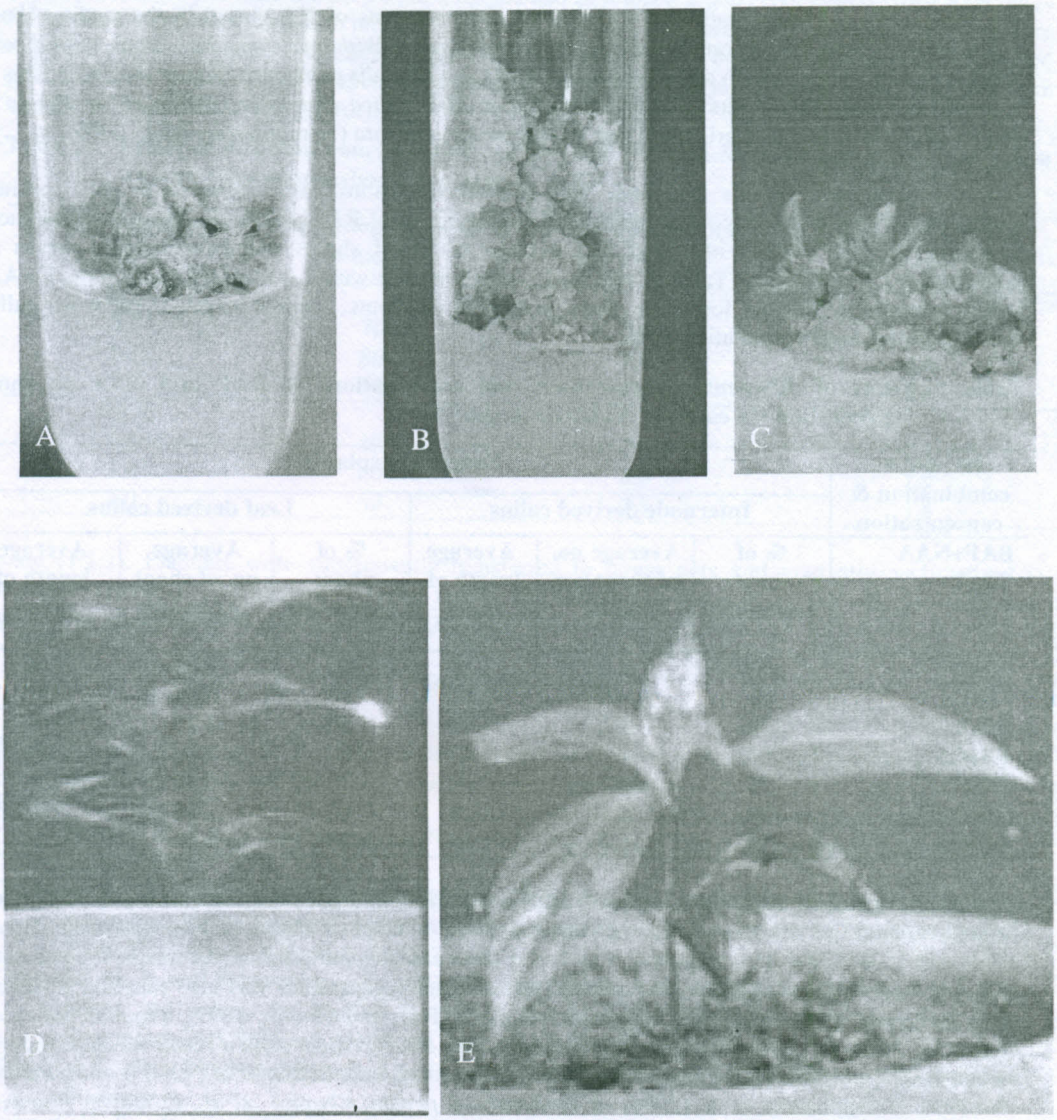

Fig. 1. Plant regeneration from internode and leaf derived callus in *Adhatoda vasica*

A. Callus induction in media with 4.0 gm/l NAA from internode explant
B. Callus induction in media with 4.0 gm/l NAA from leaf explant
C. Shoot regeneration in media with 1.0 mg/l BAP + 0.1 mg/l NAA from leaf derived callus.
D. Induction of roots on *in vitro* raised shoot
E. Regenerated plantlet transferred to the pot

The lowest percentage of callus formation was recorded 20% and fresh weight 0.800gm and dry weight 0.300 gm was obtained at 1.0 mg/l 2,4-D. The nature of callus was hard in texture and greenish white in colour in all concentrations of 2,4-D used in the media. On the other hand, the nature of callus was loose in texture and creamy in colour in all concentrations of NAA used in the media. About similar results were also reported in *Polygonum multiflorum*, *Withania somnifera* (Rani and Grover, 1999), *Centella asiatica* (Nath and Buragohain, 2003) and *Physalis pubescens* (Rao *et al,* 2004). This is in accordance with the results as reported earlier in *Momordica charantia* (Sikdar *et al.* 2003); in *Eclipta alba* (Neeti and Kothari, 2005) and in *Vanasushava pedata* (Karuppusamy *et al.,* 2006)

Shoot regeneration

In this experiment, explants (internode leaf and derived callus) were cultured on MS medium supplemented with different concentrations and combinations of BAP and NAA. Percentages of shoot formation, average number of shoots and average length of shoots per callus after 4 weeks of subculture are shown in the Table 2. MS media supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA, highest percentage of shoot formation, average number of shoots, average length of shoot per callus was higher than other formulation used.

Table 2. Effect of different concentrations and combinations of BAP and NAA on shoot regeneration via callus of *Adhatoda vasica*

Hormonal combination & concentration	Source of explants					
	Internode derived callus			Leaf derived callus		
BAP+NAA (Mg/l)	% of shoot formation	Average no. of shoot per callus	Average length of shoot (cm)	% of shoot formation	Average no. of shoot per callus	Average length of shoot (cm)
0.5+0.1	60	2.0	1.75	70	7.05	2.00
1.0+0.1	80	4.0	2.07	90	10.00	2.10
1.0+0.2	70	3.5	1.83	80	8.25	1.90
1.0+0.5	60	3.0	1.65	80	7.20	1.70
2.0+0.1	50	2.4	1.50	70	5.40	1.50
2.0+0.2	50	2.0	1.28	60	2.10	1.45
2.0+0.5	40	1.5	1.13	50	1.80	1.20

Here percentage of shoot formation of per callus was 80% in internode derived callus and 90% in leaf derived callus (Fig.1 C). It was observed that maximum number of shoots (9.07 in internode derived callus and 10.0 in leaf derived callus) was found from explants cultured in MS media supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA. Such type of plant regeneration was also reported in several medicinal plant species including *Withania somnifera* (Rani and Grover, 1999), *Physalis pubescens* (Rao *et al,* 2004) and *Acmella calva* (Senthilkumar *et al,* 2007). About similar result was also reported earlier by Thiruvengadam and Jayabalan (2000) in *Vitex negundo*, Sikdar *et al.*, (2003) in *Momordica charantia*, Karuppusamy *et al.* (2006) in *Vanasushava pedata*, Chaplot *et al.*, (2006) in *Plumbaga zelanica* and Biswas *et al.*, (2007) in *Abrus precatorius.*

Root induction and acclimatization

For adventitious root induction, *in vitro* grown shoots were excised and transferred to MS medium with different concentrations of auxins (IBA and NAA). Results obtained for root induction are presented in Table 3. The highest 80% of root formation was recorded in 1.0 mg/l IBA after 28 days of culture (Fig.1 D) and followed by 70% in media supplemented with 0.50 mg/l IBA. The lowest 40% of root induction was recorded in media having 0.50 mg/l NAA. The highest average number of roots per shoot was recorded (4.0) in media having 1.0 mg/l IBA. Highest length of roots of 3 cm was recorded in media with 1.0 mg/l IBA and followed by. 2.90 cm in 1.0 mg/l NAA. In most cases, morphology of

roots was fragile, long and thick. Many other workers reported similar results for root induction in various types of medicinal plants, namely Biswas *et al*, (2007) in *Abrus precatorius*, Chaplot *et al*, (2006). When the plantlets were 8-10 cm long and had developed a good root system, they were ready for hardening and transplantation into pots. The caps of the culture vessels containing the plantlets were removed and the plantlets were kept in growth room for 2 days. Then the cultures were transferred gradually from growth room to open room and kept there for 3-4 days. Then the rooted plantlets were transferred to pots containing garden soil, cow dung and

Table 3. Effect of different concentrations of IBA and NAA in MS medium on root induction from in vitro grown shoots explants

Growth regulators (mg/l)	Percentage of root induction	Mean no. of roots per explant	Mean length of the longest root (cm)
IBA			
0.5	70	2.5	2.6
1.0	**80**	**4.0**	**3.0**
2.0	60	3.0	2.8
NAA			
0.5	40	2.0	2.2
1.0	60	3.0	2.9
2.0	50	2.5	1.8

sand (1:1:1). Plantlets were subsequently transferred to larger pots and gradually acclimatized to outdoor condition. The ultimate survival rate of the transferred platelets to soil was 80% and their growth in such condition was satisfactory (Fig.1E).

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