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HIGH FREQUENCY *IN VITRO* PROPAGATION OF *ADHATODA VASICA* NEES THROUGH SHOOT TIP AND NODAL EXPLANTS CULTURE

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Abstract

An efficient protocol for *in vitro* propagation of *Adhatoda vasica* Nees was established using shoot tip and nodal explants from field grown mature plant. Proliferation of multiple shoots was achieved on MS medium supplemented with different concentrations and combinations of cytokinins (0.5-4.0 mg/l) and auxins (0.1-1.0 mg/l). Maximum number of shoots per explant (13.0) was obtained on MS medium supplemented with 2.0 mg/l BAP + 0.2 mg/l NAA. Among two types of explants used in this study, nodal explants showed better response in respect of multiple shoot production. The elongated shoots were excised and subcultured for rooting on MS medium supplemented with different concentrations of auxins (IBA and NAA). Highest 80% rooting was achieved; and three to four roots per shoot were recorded in medium with 1.0 mg/l IBA within 4 weeks of culture. The *in vitro* raised plantlets were acclimatized and successfully transferred to natural condition in pot. The regenerated plants were healthy, uniform and identical to the donor plants and the survival percentage was 80%.

Key words: Micropropagation, *Adhatoda vasica*, shoot tip, nodal explant.

Introduction

Herbal medicine is one of the most remarkable uses of plant based bio-diversity. As many as 75 to 95 % of the worlds 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 fruit 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 a yellow dye and the wood for gun powder, charcoal and beads (Singh *et al.* 1998). As the alkaloid content of plant varies with genotype therefore, it is recommended to propagated *A. vasica* plant using vegetative method (Duster 1985).

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The plant is conventionally propagated by seeds and stem cuttings. Chomchalo 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 number of plant produces in both cases. The present investigation was therefore, undertaken to establish protocol for large-scale production of plantlets *in vitro* from the nodal and shoot tip explants of mature plant.

Materials and Methods

Healthy, disease free, shoot tips and nodal 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 using before autoclaving at 121°C for 20 minutes. Shoot tip and nodal segments were aseptically excised from field grown mature plant and inoculated on MS medium containing different concentrations and combinations of BAP, NAA, KIN and IBA. All cultures were maintained at 16 hour photoperiod with 3000 lux light intensity at 25±2°C. For each treatment, 20 replicates were used and all experiments were repeated thrice.

Results and Discussion

Proliferation of multiple shoots was obtained with high frequency from the inoculated shoot tips and nodal segments. These explants were capable of directly developing multiple shoots on MS media containing different concentrations and combinations of cytokinin and auxin. Different concentrations (0.5-4.0 mg/l) of cytokinins (BAP and KIN) in singly were tested to observe their effect on shoot initiation as well as multiplication from shoot tips and nodal segments. Results of this study are presented in Table 1. In this study 90% of explants (both for shoot tip and nodal segments) showed shoot initiation as well as multiplication when explants were cultured in MS medium supplemented with 2.0 mg/l BAP. Maximum number of shoots was recorded 7.0 and 6.7 in shoot tips and nodal segments explants respectively, the highest shoot length of 4.9 cm was obtained on MS fortified medium with 2.0 mg/l BAP attained after 28 days of culture. But when both explants were cultured in MS media supplemented with various concentrations of KIN (0.5-4.0mg/l), single healthy shoots were produced in all media composition. 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) In the present study, higher concentration of cytokinin (3.0 mg/l and above) reduced the shoot number as well as shoot length. A similar response was observed in *Centella asiatica* (Nath and Buragohain 2003) and in *Vanasushava pedata* (Karuppusamy *et al.* 2006).

Table 1. Effect of different concentrations of BAP and KIN singly in MS media on direct proliferation of shoots from shoot tips and nodal segments of *Adhatoda vasica* (Data were collected after 28 days of inoculation).

Hormonal supplement (mg/l)	Sources of explants					
	Shoot tips			Nodal segments		
BAP	Percentage of explants produced shoots	Average no. of shoots per explant	Mean shoot length (cm)	Percentage of explants produced shoots	Average no. of shoots per explant	Mean shoot length (cm)
0.5	50	3.5 ± 0.6	3.0 ± 0.8	70	4.1 ± 1.0	3.2 ± 0.6
1.0	60	4.1 ± 0.8	3.3 ± 1.2	80	5.5 ± 0.8	3.4 ± 0.9
1.5	70	5.8 ± 1.0	4.0 ± 0.3	80	5.8 ± 0.7	4.2 ± 1.2
2.0	90	7.0 ± 0.7	4.3 ± 0.6	90	6.7 ± 1.2	4.9 ± 0.6
3.0	80	5.7 ± 1.3	3.8 ± 1.0	70	6.0 ± 0.9	4.0 ± 1.4
4.0	60	5.1 ± 1.2	3.1 ± 0.9	60	5.2 ± 0.6	3.2 ± 1.1
KIN						
0.5	40	1.0 ± 0.5	1.5 ± 0.2	50	1.0 ± 0.3	2.0 ± 1.3
1.0	50	1.0 ± 0.7	2.0 ± 0.9	60	1.0 ± 0.5	2.2 ± 0.8
1.5	60	1.0 ± 0.6	2.9 ± 0.3	70	1.0 ± 0.2	2.4 ± 1.0
2.0	70	1.0 ± 0.5	3.1 ± 0.4	80	1.0 ± 0.7	3.2 ± 0.6
3.0	60	1.0 ± 0.5	2.7 ± 0.6	70	1.0 ± 0.6	2.8 ± 0.9
4.0	50	1.0 ± 0.2	2.2 ± 0.9	50	1.0 ± 0.8	2.5 ± 0.8

Table 2. Effect of different concentrations and combinations of cytokinin and auxin on shoot multiplication from shoot tips and nodal segments of *Adhatoda vasica* (Data collected after 28 days of cultured).

Growth regulators (mg/l)		Source of explants					
		Shoot tip			Nodal Segment		
BAP	NAA	% of explants produced shoot	Mean no. of shoot per explants	Average shoot length (cm)	% of explants produced shoot	Mean no. of shoot per explants	Average shoot length (cm)
2.0	0.1	70	9.8 ± 0.8	5.0 ± 0.3	80	10.5 ± 0.9	4.7 ± 0.5
2.0	0.2	80	11.0 ± 0.6	5.2 ± 0.8	90	13.0 ± 0.8	5.0 ± 0.9
2.0	0.3	50	9.0 ± 1.1	4.9 ± 0.5	70	9.0 ± 0.2	4.5 ± 0.2
2.0	0.5	40	7.0 ± 0.3	4.7 ± 0.9	60	7.5 ± 0.6	4.4 ± 1.0
2.0	1.0	30	6.0 ± 1.3	4.5 ± 0.7	40	7.0 ± 0.4	4.2 ± 0.7
KIN	NAA						
2.0	0.1	60	1.0 ± 0.6	3.0 ± 0.6	60	1.0 ± 0.4	2.7 ± 0.6
2.0	0.2	70	1.0 ± 0.7	3.2 ± 0.8	70	1.0 ± 0.5	3.0 ± 0.8
2.0	0.3	60	1.0 ± 0.4	2.7 ± 0.7	60	1.0 ± 0.8	2.6 ± 0.7
2.0	0.5	50	1.0 ± 0.8	2.6 ± 0.4	60	1.0 ± 0.6	2.6 ± 0.3
2.0	1.0	40	1.0 ± 0.3	2.4 ± 0.6	50	1.0 ± 0.3	2.3 ± 0.9

To find out the combine effect of cytokinin and auxin on shoot tips and nodal segments were cultured in MS medium supplemented with BAP (2.0mg/l) and different concentrations of (0.1-1.0 mg/l) NAA. Results of the above study are presented in Table 2. It is evident from the table that 80% of explants showed shoot initiation for shoot tips and 90% for nodal segments. Among all five treatments best shoot proliferation was observed in 2.0 mg/l BAP + 0.2 mg/l NAA supplemented MS medium (Fig. 1A) from nodal explant. Maximum number of shoots per explants was 11.0 for shoot tips and 13.0 for nodal segment was obtained in medium with 2.0 mg/l BAP + 0.2 mg/l NAA (Fig. 1B).

Shoot tips and nodal segments were cultured in MS medium supplemented with KIN (2.0 mg/l) and various concentration of NAA (0.1-1.0 mg/l). Results of this experiment are also presented in Table 2. Among all five treatments best response was observed in 2.0 mg/l KIN + 0.2 mg/l NAA supplemented MS medium. Percentage of explants producing shoots was recorded 70% for shoot tips and 80 % for nodal segments, and the numbers of shoots per explants was 1.02 for shoot tips and 1.09 for nodal segments. Similar result was

also reported earlier by various groups; 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* .

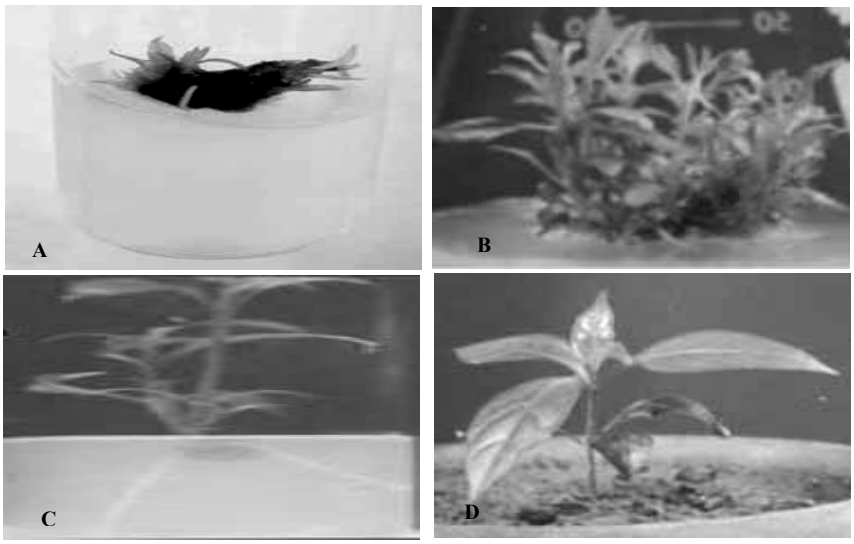

Fig. 1. Plant regeneration from shoot tip and nodal explants in *Adhatoda vasica*; Proliferation of shoots from nodal segments (A), Development of multiple shoots (B), Induction of roots on *in vitro* raised shoot (C). and Regenerated plantlet transferred to the pot (D).

Addition of exogenous auxin to the cytokinin containing medium promoted shoot proliferation and enhanced the growth of culture. One of the advantages of adding auxin at low concentration on the culture media is to nullify the effect of the higher concentration of cytokinin on axillary shoot elongation (Hu and Wang 1983). In the present study, BA (2.0 mg/l) combined with NAA (0.2 mg/l) showed maximum shoot proliferation and highest shoot length potential of *A. vasica.* Multiple shoot formation from shoot tip as well as nodal segments using cytokinin was also reported in many plant species (Bhadra and Hossain 2003). Among cytokinins BA was found to be most effective for inducing adventitious shoot as well as shoot length (Chawla 2000, (Karuppusamy *et al.* 2006).

For adventitious root formation, *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.

Table 3. Effect of different concentrations of IBA and NAA in MS medium on root induction from in vitro grown shoots explants. (Data collected after 28 days of culture and 20 test tubes were inoculated for each concentration).

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 ± 0.7	2.6 ± 0.4
1.0	80	4.0 ± 0.3	3.0 ± 0.7
2.0	60	3.0 ± 0.5	2.8± 0.8
NAA			
0.5	40	2.0 ± 0.5	2.2 ± 0.6
1.0	60	3.0 ± 0.6	2.9 ± 0.3
2.0	50	2.5 ± 0.9	1.8 ± 0.7

The highest 80% of root formation was recorded in 1.0 mg/l IBA after 28 days of culture (Fig. 1C) 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 (3 cm) was recorded in 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. 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 + sand (1:1:1). The potted plantlets were regularly sprayed with water using a hand spray and were covered with polythene sheets to maintain high humidity around juvenile plants. 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. 1D).

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