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In vitro propagation of *Andrographis paniculata* Nees.-A threatened medicinal plant of Bangladesh

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Abstract

An efficient protocol was developed for *in vitro* regeneration of plantlets from shoot tip and nodal segment explants of *Andrographis paniculata* Nees. Nodal segment explants produced the highest number of shoots (18±1.24) when they were cultured on MS supplemented with 11.10 µM/l BAP. Addition of 10% coconut water and 2.0 g/l activated charcoal to the above mentioned medium increased the number of shoots (30) per culture. Shoot tip explant also showed better performance in the same medium. Addition of 100 mg/l urea and 2.0 g/l activated charcoal to the medium showed proper shoot elongation. The isolated shoots rooted well (90%) on half-strength MS fortified with 9.80 µM/l IBA, where average number of roots per shoot was 28-30. The plantlets were acclimatized successfully in poly bags containing a mixture of soil, sand and compost in 2:1:1 ratio. Finally acclimatized plantlets were transferred to experimental field.

Key words: *In vitro* propagation, multiple shoots, *Andrographis paniculata* Nees.

INTRODUCTION

Andrographis paniculata Nees. is an erect annual herbaceous plant in the family-Acanthaceae. It is extremely bitter in taste in all parts of the plant body. *Andrographis paniculata* commonly known as Kalmegh in Bengali. It is distributed in tropical Asian countries, often in isolated patches. It can be found in a variety of habitats, such as plains, hill slopes, wastelands, farms, dry or wet lands, sea shores and even road sides. The herb is available in Bangladesh, India, Pakistan, Malaysia, Sri Lanka and China. It has been used as medicinal herb in several traditional systems of medicine all over the world. It is extensively used in Ayurveda and Unani medicines as home remedy for various diseases (Sivarajan & Balachandran, 1994). The therapeutic value of Kalmegh is due to its mechanism of action by enzyme induction. It is an important cold property herb, used in fevers and to dispel toxins from the body. It is used to treat gastrointestinal tract and upper respiratory infections, herpes, sore throat, hepatitis and a variety of other chronic and infectious diseases (Chopra *et al*., 1956; Purkayastha *et al*., 2008). It exhibits antibacterial, antimalarial, antidiarrhoeal, cardiovascular activities and protection of liver and gallbladder. The herb and its isolates like andrographolide, neoandrographolide, dehydroandrographolide etc. are reported to possess anti-inflammatory, hepatoprotective, astringent, anodyne, tonic and anti-pyretic properties and helps in arresting dysentery, cholera, diabetes, influenza, bronchitis and gonorrhoea (Prajapati *et al*., 2003; Niranjan *et al*., 2010). Mass scale collection of this plant from natural habitats is leading to a depletion of plant resources.

Conventional vegetative propagation of this important medicinal plant is very difficult and too slow to meet the commercial quantities required. Variability among the seed-derived progenies and scanty and delayed rooting of seedlings curb its propagation via seeds (Martin, 2004; Prathanturarug *et al*., 1996).

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 & Roy, 2005). 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

The study was conducted in the Plant Biotechnology and Genetic Engineering Laboratory, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka. Plant materials, namely shoot tips and nodal segments were collected from the garden of Plant Biotechnology and Genetic Engineering division. They were washed thoroughly with running tap water for 45 min and then with house hold detergent (Trix) to remove the traces of dust particles. Shoot tips and nodal segments were then surface sterilized with aqueous solution of 0.1% $HgCl_2$ for 6 min under aseptic conditions in laminar air flow cabinet and rinsed 4-5 times with autoclaved distilled water to wash away the traces of $HgCl_2$ completely. After surface sterilization, shoot tips and nodal segments were divided into small pieces (approx. 1.0-1.5 cm). These were used as explants and cultured onto culture medium. MS (Murashige & Skoog, 1962) containing 3% sucrose was used for all shoot regeneration studies. But half-strength MS was used for *in vitro* root formation. Shoot tip and nodal segment explants were cultured on MS supplemented with different cocentrations and combinations of cytokinins (BAP & Kin) and auxin (NAA) for shoot regeneration. Media supplements such as coconut water (CW) (5-20%) collected from local market and urea (Sigma) (50-200 mg/l) with constant cocentration of activated charcoal (AC) (Sigma) (2.0 g/l) were added to the medium for the determination of their effects on shoot multiplication and elongation respectively. Well developed shoots were excised from the elongation medium and cultured individually in rooting media containing different concentrations and combinations of IBA, IAA and NAA. The pH of the media was adjusted to 5.8 before adding agar. All media were gelled with 0.7% Difco Bacto agar and autoclaved for 20 min at 121ºC under 1.1kg/cm^2 pressure. The cultures were regularly sub-cultured at three weeks intervals on fresh medium. Cultures were maintained at 25±2ºC with 16h photoperiods. For hardening, the test tubes containing rooted shoots were kept at room temperature and light for 5 days. Thereafter, the well rooted plantlets were taken out from the test tubes and gently washed them to free from medium. After washing they were transferred to poly bags containing a mixture of soil, sand and compost in 2:1:1 ratio. They were finally transferred to experimental field for study of their mortality rate and growth in natural conditions. .

RESULTS AND DISCUSSION

In vitro technique provides viable alternative method of mass production of healthy plants with uniform characteristics. Both shoot tip and nodal segment explants cultured on MS supplemented with different concentrations of BAP, Kin and NAA alone or in various combinations for multiple shoot regeneration. Between two types of explants, the best shoot induction rate was observed in nodal segment explant and it was 88% at the concentration of 11.10 µM/l BAP in MS medium (Table 1). In this combination, the regenerated shoots per explant was 18±1.24 (Table 1, Fig. 1). In the same medium, multiple shoots induction from shoot tip explant was 15±1.22 (Table 1). Purkayastha *et al.* (2008) reported that MS medium supplemented with 10µM/l BAP was optimum to regenerate multiple shoots in *Andrographis paniculata* from nodal explant. It is concluded that BAP combination is more effective on shoot proliferation from nodal explant of *Andrographis paniculata.* Addition of 10% CW and 2.0 g/l activated charcoal to the medium increased the number of shoots (30 in case of nodal explant) per culture (Fig. 2). Different concentrations of urea (50-200 mg/l) and constant concentration of activated charcoal (2.0 g/l) were added to the medium to determination of their effects on shoot elongation. Addition of 100mg/l urea and 2.0 g/l AC to the medium increased the length of shoots (Fig. 3). Roy (2008) reported that addition of 10% CW in the medium increased the number of shoots in *Boerhaavia diffusa* L. culture. Rahman *et al*. (1999) also observed similar effect of CW (10%) on *in vitro* shoot multiplication of *Emblica officinalis.* Ahmed *et al*. (2001) reported that urea was fruitful media supplement for *in vitro* shoot elongation of *Holarrhena antidysenterica* L.

Regenerated shoots need to root formation for their healthy growth. So, well developed and elongated shoots were excised and implanted in the rooting medium containing half-strength MS with different concentrations and combinations of IBA, IAA and NAA. The best result was obtained in half-strength MS supplemented with 9.80 µM IBA (Table 2). In this combination, it was observed that 90% shoots rooted well within three weeks of culture and each microcutting produced 28-30 roots (Fig. 4). Purkayastha *et al*. (2008) reported that IBA was more effective for *in vitro* rooting of *Andrographis paniculata*. They observed that MS supplemented with 2.5µM/l IBA was suitable combination for best rooting. After sufficient development of roots, plantlets were transferred to poly bags containing a mixture of soil, sand and compost in 2:1:1 ratio (Fig. 5). The plantlets were kept in a shade and misted twice a day. After two weeks of hardening, plantlets were transferred to experimental field. Forty eight plantlets out of sixty were survived (i.e. 80%) in natural conditions (Table 3).

In conclusion, I report an efficient and easy to handle protocol for rapid micropropagation of *Andrographis paniculata*. The application of this protocol can help minimize the pressure on wild population and contribute to the conservation of this important medicinal plant.

Table 1. Effects of different concentrations and combinations of cytokinins (BAP, Kin) and auxin (NAA) on shoot proliferation of *Andrographis paniculata* Nees. from shoot tip and nodal segment explants

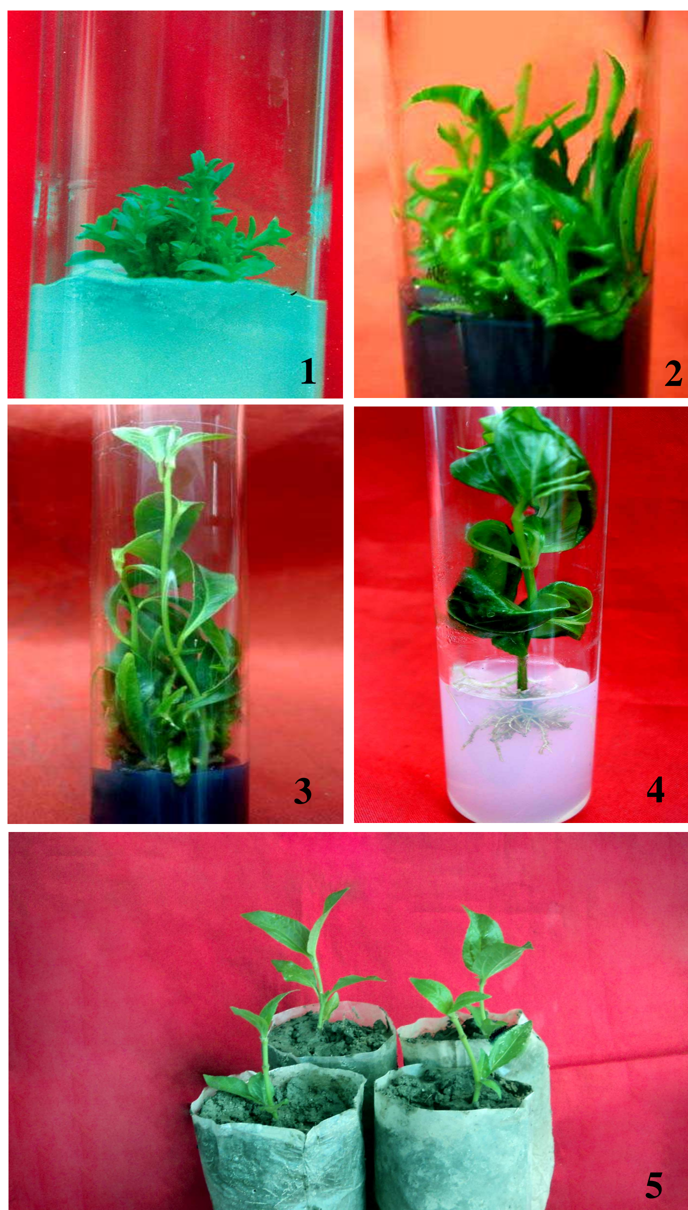
Growth regulators (µM/l)	Explants			
	Shoot tip		Nodal segment	
	% of explants produced shoots	Average No. of shoots/ culture (± SE)	% of explants produced shoots	Average No. of shoots/ culture (± SE)
BAP				
2.22	-	-	-	-
4.44	30	4±0.22	30	5±0.42
6.66	48	6±0.30	40	6±0.34
8.88	60	10±1.12	64	12±1.20
11.10	**80**	**15±1.22**	**88**	**18±1.24**
13.32	50	10±0.90	52	10±0.84
15.54	40	5±0.32	40	6±0.82
Kin				
2.32	-	-	-	-
4.64	-	-	-	-
6.96	-	-	-	-
9.28	10	5±0.33	20	6±0.24
11.60	30	8±0.48	40	10±0.82
13.92	-	-	10	5±0.32
16.24	-	-	-	-
BAP+NAA				
2.22+1.34	-	-	-	-
4.44+1.34	-	-	-	-
6.66+1.34	20	5±0.22	30	5±0.62
8.88+1.34	30	6±0.42	38	8±0.48
11.10+1.34	20	4±0.12	20	4±0.22
13.32+1.34	-	-	-	-
6.66+2.68	-	-	-	-
8.88+2.68	10	4±0.42	28	5±0.12
11.10+2.68	-	-	-	-
13.32+2.68	-	-	-	-
Kin+NAA				
4.64+2.68	-	-	-	-
6.96+2.68	10	3±0.24	30	5±0.24
9.28+2.68	30	5±0.33	34	5±0.42
11.60+2.68	38	8±0.44	40	6±0.75
13.92+2.68	20	4±0.22	20	3±0.22

Table 2. Effects of different concentrations of IBA, IAA and NAA singly or in combination in half-strength MS on root induction from regenerated shoot cuttings of *Andrographis paniculata* Nees

Auxins (µM/l)	% of shoots rooted	Days required for rooting	No. of roots per shoot
IBA			
2.45	30	20-22	10-12
4.90	50	20-22	15-17
7.35	70	18-20	18-20
9.80	**90**	**15-17**	**28-30**
12.25	50	18-20	12-14
14.70	40	20-22	10-12
IAA			
2.85	-	-	-
5.70	-	-	-
8.55	-	-	-
11.40	20	20-22	12-14
14.25	10	20-22	10-12
17.10	-	-	-
NAA			
2.86	-	-	-
5.36	-	-	-
8.04	20	18-20	10-12
10.72	10	20-22	08-10
13.40	-	-	-
16.08	-	-	-
IBA+IAA			
4.90+2.85	20	18-20	12-14
7.35+2.85	30	18-20	14-16
9.80+2.85	60	16-18	18-20
12.25+2.85	40	18-20	12-14
IBA+IAA+NAA			
4.90+2.85+2.68	20	20-22	10-12
7.33+5.70+2.68	40	20-22	12-14
9.80+8.55+5.36	50	18-20	16-18
12.25+11.40+8.04	30	20-22	10-12

Table 3. Survival rate of *in vitro* grown plantlets (*Andrographis paniculata* Nees) in experimental field

Transferred steps	No. of transferred plantlets in experimental field	No. of survived plantlets	Average survived plantlets (%)
Step-1	20	16	
Step-2	20	14	
Step-3	20	18	80%
Total	60	48	



Figs. 1-5. ***In vitro*** **regeneration of** ***Andrographis paniculata Nees.*** **1. Multiple shoot formation from nodal explant on MS with 11.10 µM/l BAP. 2. Positive effect of coconut water (10%) and activated charcoal (2.0g/l) on increase the number of shoots. 3. Elongated shoots on MS supplemented with 11.10 µM/l BAP, 100 mg/l urea and 2.0 g/l AC. 4.** ***In vitro*** **root induction on half-strength MS supplemented with 9.80 µM/l IBA. 5. Regenerated plantlets in poly bags containing soil, sand and compost (2:1:1)**

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