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MASS SCALE MICROPROPAGATION OF *ANDROGRAPHIS PANICULATA* (BURM. F.) WALL EX NEES

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ABSTRACT

A reliable and efficient protocol was developed for *in vitro* propagation of *Andrographis paniculata* (Burm. f.) Wall ex Nees with the use of nodal and shoot tip explants. Nodal segments and shoot apex of *in vitro* grown seedlings were cultured on agar solidified MS medium supplemented with different concentrations and combinations of plant growth regulators (PGRs). Both the explants underwent direct organogenesis and produced multiple shoot buds. In case of shoot apex, maximum number of multiple shoot buds were formed when cultured on MS medium containing 1.5 mg/l Kn + 1.0 mg/l IAA. But nodal segments produced highest number of shoot buds on MS medium fortified with 1.5 mg/l BAP + 1.0 mg/l IAA. Multiple shoot buds underwent rapid elongation on elongation media and maximum elongation took place on MS medium fortified with 2.0 mg/l Kn + 1.5 mg/l IAA. Elongated shoot buds, on further culture in rooting media, produced strong and stout root systems. Half strength MS medium fortified with 2.0 mg/l IAA was most effective for induction and proliferation of roots. The healthy complete plantlets were successfully acclimatized and established in outside pots through successive phases of acclimatization.

Key words: *Andrographis paniculata*, micropropagation, acclimatization.

INTRODUCTION

Andrographis paniculata (Burm. f.) Wall ex Nees., commonly known as Kalomegh, belongs to the family Acanthaceae and grow in different areas of our subcontinent. Medicinally this species is very valuable as it contains alkaloids andrographin, panicolin, kalmaghin, neoandrographolide, which is used in anti-inflammation, anthelmintic, antimalarial, antipyretic, antiallergic, anticholastatic and other complaints (Sivaranjan and Balachandran 1994). Because of its over and unregulated exploitation by common people and traditional communities this species become endangered. It is therefore important to take immediate steps for large scale propagation of this medicinal plant species to save it as well as to meet the demand of traditional ayurvedic industries.

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Conventional vegetative propagation of this important plant is very difficult and too slow to meet the commercial quantity required. In recent years plant tissue culture techniques gained greater momentum on commercial application in the field of plant propagation. A number of medicinally important plant species have been successfully propagated by *in vitro* techniques (Lal and Ahuja 1989, Roy *et al*. 1995, Chand *et al.* 1997, Mikulik 1999). The *in vitro* propagated medicinal plants furnish a ready source of uniform, sterile and compatible plant constituents (Wakhlu and Bajwa 1986, Miura *et al*. 1987). No comprehensive *in vitro* studies have been reported on *A. paniculata* in Bangladesh. In this perspective, it is necessary to establish protocol for *in vitro* clonal propagation of important and rare indigenous medicinal plants of Bangladesh. Therefore, this research study was undertaken to develop an efficient protocol for micropropagation of *Andrographis paniculata* (Burm. f.) Wall ex Nees.

MATERIALS AND METHODS

The mature seeds of field grown plants were collected and made aseptic through proper procedure. These seeds were aseptically grown on MS (Murashige and Skoog 1962) medium with or without PGRs supplements. The germinated seedlings were used as a source of explants. In case of multiple shoot bud formation, elongated shoot buds at a height of 2 - 3 cm were separated individually and cultured on rooting medium containing half strength MS medium fortified with different concentrations and combinations of auxins (IAA, IBA). In all the cases the media were solidified with 0.8% (w/v) agar (Sigma) and pH was adjusted to 5.8 before autoclaving for 30 minutes at 121^0C under a pressure of 1.1 kg/cm^2. All culture vessels containing inoculated explants were incubated in a culture room at 25 ± 2^0C under a regular cycle of 14 hours of light and 10 hours of dark. After complete regeneration the plantlets were transferred to pots containing a mixture of soil and compost (1:1) following successive phases of acclimatization. For acclimatization, the mouth of the culture vessels were kept open for one day in the culture room and they were then kept outside the culture room for 6 hours of the following day. Later on, those were kept outside the culture room for 12 hours. Finally, the seedlings were taken out of the culture vessels and rinsed with running tap water for complete removal of medium attached to the roots. Statistical analysis was done for computation of standard error (SE) for different sets of data.

RESULTS AND DISCUSSION

Seeds were aseptically grown on MS basal medium to induce juvenile seedlings. Seeds germinated at a low frequency and took long time to sprout. Then seeds were grown on PGRs supplemented media. In all these media seeds germinate very fast and developed healthy seedlings within 15 – 35 d (Fig. 1A). The highest germination rate was recorded when seeds were grown on MS medium fortified with 3.0 mg/l Kn + 1.0 mg/l NAA (Table 1).

TABLE - 1: RESULTS OF *IN VITRO* GERMINATION OF *A. PANICULATA* SEEDS ON 0.8% (W/V) AGAR SOLIDIFIED MS MEDIUM SUPPLEMENTED WITH OR WITHOUT PGR COMBINATION.

Medium with or without PGRs combinations (mg/l)		Total number of seeds used	Number of seeds germinated		Time (d) requirred for germination
			No	%	
MS		60	15	25.00	35 - 45
MS + BAP + NAA	2.0 + 0.5	50	38	76.00	25 - 32
	2.0 + 1.0	50	35	70.00	20 - 28
MS + Kn + IAA	1.5 + 0.5	60	45	75.00	20 - 25
	2.0 + 0.5	70	49	70.00	22 - 30
MS + Kn + NAA	3.0 + 1.0	60	51	85.00	20 - 30
MS + BAP + IAA	1.5 + 0.5	90	54	60.00	20 - 27
	1.5 + 1.0	90	47	52.22	18 - 25
	2.0 + 0.5	75	50	66.67	15 - 20

In order to induce organogenesis and embryogenesis nodal segments and shoot apices of *in vitro* grown seedling were cultured on a wide variety of media supplemented with different PGRs. In all these media both the explants underwent direct organogenesis and produced multiple shoot buds. The maximum number of shoot apices produced multiple shoot buds on MS medium containing 1.5 mg/l Kn + 0.5 mg/l IAA (Table 2). In case of nodal segments best response was obtained on MS medium supplemented with 1.5 mg/l BAP + 1.0 mg/l IAA (Fig. 1B & Table 2). Such direct organogenesis was reported by (Martin 2004) in *A. paniculata* and in many other medicinal plants including *Curculigo orchioides*

(Wala and Jasrai 2003), *Paedaria foetida* (Amin *et al*. 2003), *Withania somnifera* (Supe *et al*. 2006), *Eclipta alba* (Hassan *et al*. 2008), *Boerhaavia diffusa* (Roy 2008, Biswas *et al*. 2009), *Ficus religiosa* (Hasan *et al*. 2009). *Rauvolfia serpentina* (Singh *et al.* 2009), *Plumbago indica* (Bhadra *et al*. 2004), *Wedilia chinensis* (Sultana and Handique 2004).

TABLE - 2: RESULTS ON THE DEVELOPMENT OF MULTIPLE SHOOT BUDS FROM NODAL SEGMENT AND SHOOT APEX OF *A. PANICULATA* ON 0.8% (W/V) AGAR SOLIDIFIED MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGRs.

PGRs combinations and concentrations (mg/l)		Explants	% of explants gave response	Time (d) required for induction	Average* number of multiple shoot buds sprouted from explants (mean±SE)
2, 4-D	2.0	SA**	45	25 - 35	2.25 ± 0.153
		NS***	40	20-30	1.75 ± 0.182
	3.0	SA	35	22 – 30	1.90 ± 0.163
		NS	40	25-32	1.78 ± 0.127
BAP+NAA	3.0+0.5	SA	65	25 – 35	1.95 ± 0.165
		NS	70	20-28	1.65 ± 0.142
	2.0+0.5	SA	65	21 – 30	2.50 ± 0.215
		NS	58	23-30	1.85 ± 0.183
	2.0+1.0	SA	78	24 – 31	2.75 + 0.182
		NS	65	25-35	2.30 ± 0.150
Kn+NAA	2.0+0.5	SA	95	20 – 30	3.10 ± 0.175
		NS	87	22-28	2.88 ± 0.238
	2.0+1.0	SA	90	18 – 27	2.90 ± 0.240
		NS	85	20-25	3.30 ± 0.252
BAP+IAA	1.5+0.5	SA	85	18 – 25	3.45 ± 0.256
		NS	80	20-25	3.20 ± 0.215
	1.5+1.0	SA	90	22 – 30	3.75 ± 0.228
		NS	78	25-35	3.55 ± 0.283
	2.0+1.0	SA	84	20 – 28	2.95 ± 0.248
		NS	75	20-30	3.15 ± 0.212
Kn+IAA	1.5+0.5	SA	100	22 – 28	3.85 ± 0.192
		NS	100	25-34	3.10 ± 0.175
	2.0+0.5	SA	95	25 – 35	3.30 ± 0.250
		NS	88	22-35	3.15 ± 0.221
	2.0+1.0	SA	100	22 – 27	3.70 ± 0.195
		NS	90	20-32	3.45 ± 0.256

* Values are the mean of five replication each with 15 explants. ** Shoot Apex *** Nodal Segment

Multiple shoot buds which developed directly from shoot apex and nodal segment of *in vitro* grown seedlings underwent rapid elongation on subculture in elongation media (Fig. 1C). The elongation media was prepared based on MS basal medium supplemented with different concentration and combinations of PGRs. The efficiency of the media for the promotion of elongation was assessed in terms of enhancement of elongation of shoot buds and it depended on the PGR supplements and concentrations. The highest rate of elongation was recorded on MS media supplemented with 2.0 mg/l Kn + 1.5 mg/l IAA (Table 3). Such enhancement of elongation of *in vitro* induced shoot buds has also been noted in many other medicinal plants, such as *Rauvolfia serpentina* (Ahmad *et al.* 2002), *Vitex negundo* (Rahman *et al*. 2004), *Catharanthus roseus* (Islam *et al*. 2001), *Plumbago indica* (Bhadra *et al*. 2004).

TABLE - 3: DATA ON THE ELONGATION OF MULTIPLE SHOOT BUDS OF *A. PANICULATA* THAT DEVELOPED THROUGH DIRECT ORGANOGENESIS ON 0.8% (W/V) AGAR SOLIDIFIED MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGRs.

PGR supplements (mg/l)		Average* initial length (cm) of individual shoot bud (mean±SE)	Average* length (cm) of multiple shoot buds after 30d of culture (mean±SE)
BAP + NAA	2.0 + 0.5	1.30 ± 0.048	2.75 ± 0.078
	2.0 + 1.0	1.20 ± 0.035	2.60 ± 0.087
	3.0 + 0.5	1.45 ± 0.047	2.75 ± 0.084
BAP + IAA	1.5 + 0.5	1.20 ± 0.034	3.10 ± 0.187
	1.5 + 1.0	1.35 ± 0.045	2.85 ± 0.089
	2.0 + 0.5	1.10 ± 0.045	2.70 ± 0.070
Kn + IAA	1.5 + 0.5	1.50 ± 0.055	2.80 ± 0.093
	2.0 + 0.5	1.40 ± 0.042	2.65 ± 0.084
	2.0 + 1.5	1.35 ± 0.038	3.35 ± 0.150
Kn + NAA	2.0 + 0.5	1.00 ± 0.038	3.00 ± 0.095
	2.0 + 1.5	1.30 ± 0.045	3.20 ± 0.103

* Values are the mean of five replication each with 15 explants.

Elongated shoot buds which attained a height of 2 - 3 cm were separated and individually transferred to rooting media. Full and half strengths MS medium fortified with different concentrations of auxins (IAA, IBA) was used for induction of roots. The shoot buds failed to produce any root in MS media without any auxins being provided, but MS media supplemented with either IAA or IBA produced root tissue within 12 - 20d of culturing and the data on rooting were recorded after 30d of inoculation. Response of shoot to rooting media was very much dependent on the concentrations of auxins and strength of MS media. The highest number of roots per shoot and mean length of roots was noted in half strength MS medium fortified with 2.0 mg/l IAA (Table 4). Such influence of IAA for induction and proliferation of root growth has been reported in other medicinal plants including *Vitex negundo* (Chandramu *et al*. 2003, Jawahar *et al.* 2007), *Abrus precatorius* Biswas *et al*. 2007), *Eclipta alba* (Hassan *et al*. 2008), *Withania somnifera* (Supe *et al*. 2006).

TABLE 4: RESULTS OF THE DEVELOPMENT OF ROOTS ON ELONGATED MULTIPLE SHOOT BUDS OF *A. PANICULATA* ON 0.8% (W/V) AGAR SOLIDIFIED ROOTING MEDIA.

Rooting media	Percentage of shoots producing root	Average* length (cm) of roots after 30d of culture ±SE	Average* number of roots/shoot ±SE
Full MS+0.5 mg/l IAA	70.00	1.30 ± 0.160	2.80 ± 0.255
½ MS + 0.5 mg/l IAA	85.00	2.20 ± 0.130	1.90 ± 0.135
½ MS + 1.0 mg/l IAA	90.00	3.10 ± 0.125	2.50 ± 0.263
½ MS + 2.0 mg/l IAA	100.00	3.50 ± 0.187	3.20 ± 0.250
½ MS + 0.5 mg/l IBA	90.00	1.75 ± 0.129	2.00 ± 0.145
½ MS + 1.0 mg/l IBA	70.00	1.85 ± 0.143	1.90 ± 0.155
½ MS + 2.0 mg/l IBA	85.00	2.70 ± 0.125	1.80 ± 0.137

* Values are the mean of five replicates each with 10 explants.

The *in vitro* grown complete plantlets were finally transferred from the culture room to keep outside pots containing garden soil and compost of ratio of 1:1 through successive phases of acclimatization (Fig. 1D). The survival rate of the transferred plants was 60%.

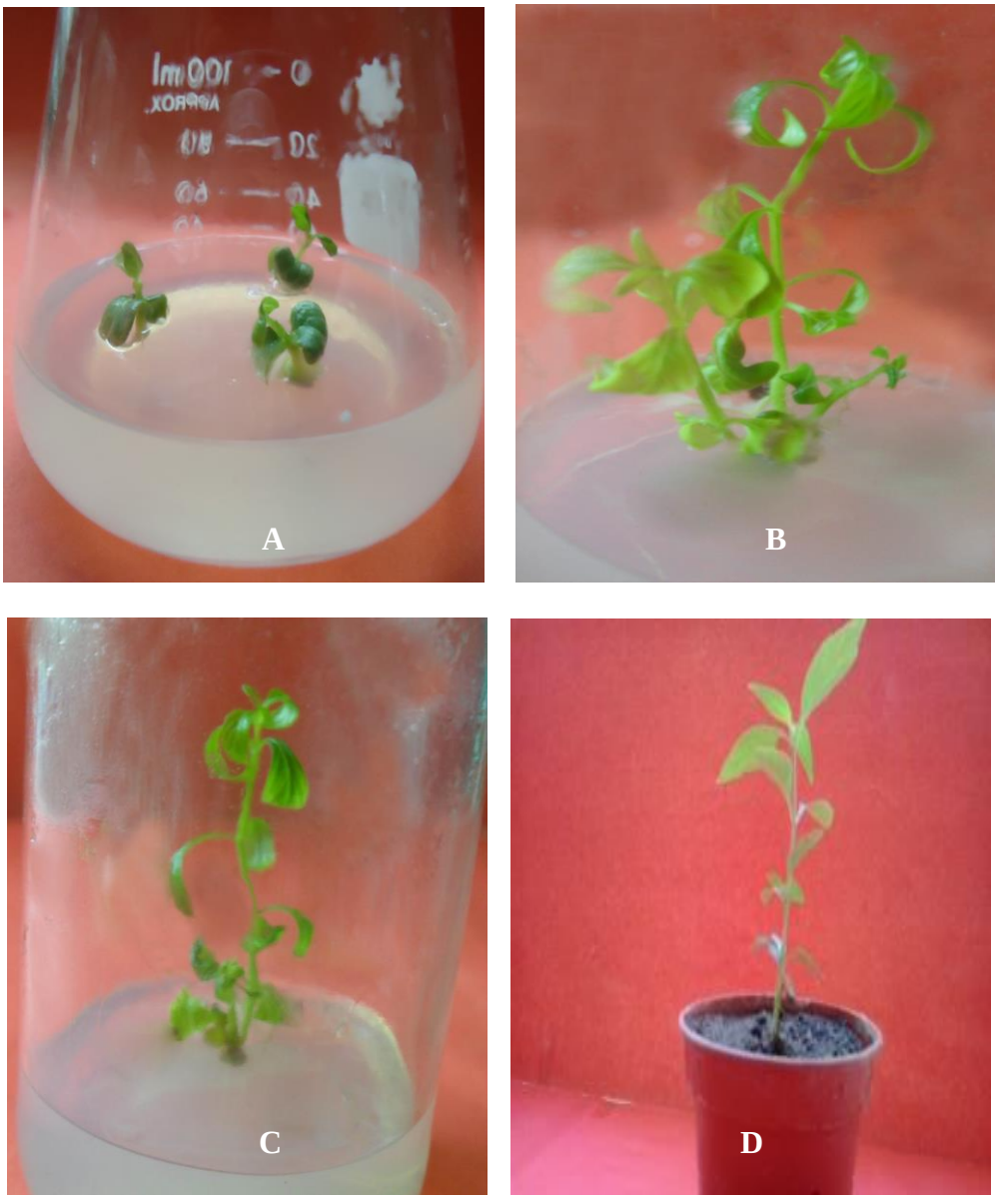

FIGURE-1. DIFFERENT STEPS OF DIRECT SHOOT REGENERATION IN *A. PANICULATA*: A. *IN VITRO* GERMINATION OF SEEDS; B. MULTIPLE SHOOT BUDS ARISING FROM NODAL SEGMENTS; C. SHOOT BUDS IN ELONGATION MEDIA; D. *IN VITRO* GROWN SEEDLINGS ESTABLISHED OUTSIDE POT.

The overall results revealed that mass micropropagation of *A. paniculata*, an important medicinal plant species of Bangladesh, was possible with the use of this culture protocol. It was further proved that induction of tissue growth and its differentiation was dependent on PGR supplements of the culture medium. Though the percentage of survival of the transferred plantlets to the outside environment was not very high, this technique could still be adopted profitably by our herbal industries

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