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In Vitro Mass Propagation of *Mimosa pudica* L., Using Shoot Tip and Nodal Explants

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Abstract

An efficient protocol was established for *in vitro* mass propagation of a valuable medicinal shrubby plant, *Mimosa pudica* Linn., from shoot tip and nodal explants. Optimum *in vitro* shoot induction was observed from nodal explants on MS basal medium supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA, in which 88.2% of the explants produced 9 shoots per culture within 3-4 weeks. Repeated subcultures in the same medium, resulted rapid shoot multiplication with 20.4 ± 1.20 shoots per culture within 12 weeks. The healthy *in vitro* raised shoots rooted on half strength MS medium with 0.5 mg/l IBA. For acclimatization and transplantation, the plantlets in the rooting culture tubes were kept in normal room temperature for 7 days before transplanting in pots where plantlets were reared for three weeks. The survival rate of regenerated plantlets was 80%.

Key words : *Mimosa pudica*, Medicinal plant, Shoot proliferation, *In vitro* mass propagation, Acclimatization

Introduction

Mimosa pudica L. commonly known as 'Lajjabati' belonging to the family - Mimosaceae, is a stout strangling prostrate shrubby plant with compound leaves, sensitive to touch, spinous stipules and globose pinkish flower heads. It grows in almost all parts of Bangladesh (Ghani, 2003). It originated in South America and naturalized almost throughout the tropical and subtropical parts of India (Fosberg, 1980). The plant is regarded as diuretic, astringent and antispasmodic. Leaves and roots are used in the treatment of piles and fistula. Paste of leaves is applied to hydrocele. Cotton impregnated with juice of leaves is used for dressing sinus. The plant is also useful in the treatment of sore gums and is used as a blood purifier. It is also used for treating convulsions of children (Ghani, 2003). A decoction of the root of the plant is considered useful in gravel, other urinary complaints (Anonymous, 1988), all bilious disease, fevers jaundice, leprosy, scabs and pox (George, 1891).The seeds yield 17% of a greenish yellow, fatty oil that resembles soybean oil and may find similar uses (Kirtkar and Basu, 1915).

In recent years, there has been an increased interest in *in vitro* culture techniques which offer a viable tool for mass multiplication and germplasm conservation of rare, endangered and threatened medicinal plants (Ajithkumar and Seeni, 1998; Prakash *et al*., 1999). Commercial exploitation and elimination of natural habit at consequent to urbanization has led to gradual extinction of several medicinal plants. Micropropagation is an effective approach to conserve such germplasm. Further, genetic improvement is another approach to augment drug-yielding capacity of the plant (Tejavathi and Shailaja, 1999). *In vitro* propagation has proven as a potential technology for mass scale production of medicinal plant species (Azad *et al*., 2005; Faisal *et al*., 2003; Hassan and Roy, 2005; Lui and Li, 2001; Martin, 2002 and 2003; and Wawrosch *et al*., 2001). Therefore it is important to develop an efficient micropropagation technique for *Mimosa pudica* Linn. to rapidly disseminate superior clones (Munshi *et al.*, 2001). There have been a few reports to date on micropropagation of *Mimosa pudica* Linn. using shoot tips and nodal explants. The present study was therefore undertaken to develop a protocol for mass clonal propagation of this important medicinal shrub through *in vitro* culture.

Materials and Methods

The experiment was conducted at the Biological Research Division of the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. Healthy and profusely growing vine of *Mimosa pudica* Linn. was collected from the

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BCSIR campus, Dhaka and used as source of explants. Shoot tips and stem nodes with a single axillary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with 0.1% (w/v) mercuric chloride for 8 min, followed by five rinses with sterile distilled water in front of a laminar air flow cabinet. The surface-sterilized explants were sized to 1-1.5 cm length containing a single node with an axillary bud or a shoot tip with an apical bud. The explants were placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were further used as an explants for adventitious shoot regeneration.

MS (Murashige and Skoog, 1962) basal medium was used for shoot proliferation and adventitious shoot regeneration and half strength MS was used for in vitro rooting. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15x150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 121^0 C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2^0 C under a fluorescent light.

Shoot proliferation from shoot tips and nodal explants was obtained in two separate sets of experiments. In the first experiment 0-2.0 mg/l BAP and 0-2.0 mg/l Kn were incorporated into MS medium to select the best cytokinin for the response of shoot induction. In the second set, combination of BAP (0-2.0mg/l) with NAA (0.1-0.5 mg/l) and BAP (0-2.0mg/l) with IAA (0.1-0.5 mg/l) were assessed for shoot multiplication. Number of new shoot proliferation of each culture was recorded after every week of inoculation.

For *in vitro* rooting, individual healthy shoots (3-5 cm) were excised from the proliferated shoot cultures and implanted onto half strength MS with different concentrations and combinations of NAA, IBA and IAA.

The rooted plantlets were taken out from the culture tubes, washed to remove agar gel adhered to the roots and transplanted to plastic pots with soil and compost (1: 1) for hardening. The plantlets were kept in a polychamber at 80% relative humidity, 32 ± 2^0 C under a 12 h photoperiod for acclimatization. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

Results and Discussion

Shoot tips and nodal explants of *Mimosa pudica* Linn. were cultured on MS media supplemented with various concentration of BAP alone and BAP with NAA or IAA for shoot regeneration. The explants were found to be swollen and they produced four to five shoots within three-four weeks after inoculation (Fig. 1a) on MS medium containing BAP

Table I. Effect of growth regulators in MS medium on morphogenic response of *Mimosa pudica* Linn. shoot tips and nodal segments

Growth regulators(mg/l)			Shoot tips		Nodal segments	
BAP	NAA	IAA	% of explants forming shoots	Mean No. of Shoot/explant	% of explants forming shoots	Mean No. of Shoot/explant
Control			-	-	-	-
0.5			22.2±1.96	8.6± 1.07	32.2±0.66	8.8± 1.06
1.0			28.2±1.66	9.2±0.86	33.6±1.84	10.2±1.42
1.5			34.8±2.58	10.4± 1.12	43.6±0.51	12.6± 0.35
2.0			21.0±1.14	9.2±0.86	41.2±2.47	10.6±0.77
0.5	0.1		57.6±2.16	9.4± 0.92	68.6±1.70	13.4± 1.88
1.0	0.2		63.4±1.57	10.0± 1.14	71.4±2.38	16.2± 1.46
1.5	0.5		72.4±2.89	12.2± 1.15	88.2±2.80	20.4± 1.20
2.0	0.5		48.8±1.77	10.4± 1.12	56.8±2.14	14.8± 0.99
0.5		0.1	16.2±0.86	9.2± 0.86	18.4±0.93	11.2± 0.76
1.0		0.2	42.6±0.87	9.4± 0.92	47.6±2.10	13.2± 0.59
1.5		0.5	61.4±2.87	11.0± 1.70	67.6±2.16	15.4± 0.45
2.0		0.5	26.6±1.66	10.4± 0.72	32.6±1.63	10.4± 1.12

Results are mean ± SE of three experiments with 15 replications

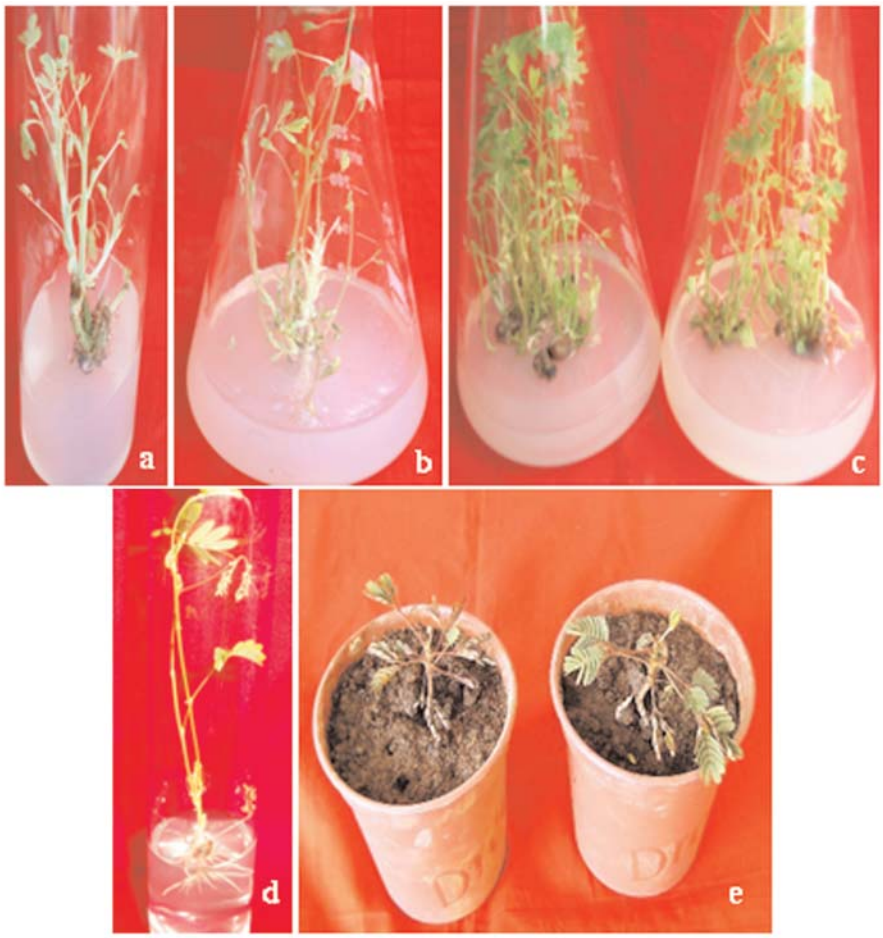

Fig. 1. *In vitro* regeneration of *Mimosa pudica* L. from shoot tips and nodal explants

(a) Induction of shoots in four weeks of culture on MS + 1.5 mg/l BAP + 0.5 mg/l NAA from nodal explants.

(b) Development and multiplication of shoots on MS + 1.5 mg/l BAP + 0.5 mg/l NAA from nodal explants after eight weeks of culture.

(c) Development and multiplication of shoots on MS + 1.5 mg/l BAP + 0.5 mg/l NAA from nodal explants after twelve weeks of culture.

(d) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 0.5 mg/l IBA in third weeks.

(e) Acclimatized regenerated two months old plants.

alone but the number of shoots increased up to 9 when the explants were cultured in MS with 1.5 mg/l BAP + 0.5 mg/l NAA (Fig 1b). Both the explants responded in the same medium but highest numbers of micro shoots were observed to be induced from nodal explants. Combinations of BAP with IAA were not found preferable to BAP with IAA for shoot induction (Table I) and combination of Kn alone and Kn with NAA were also not found suitable for shoot induction (Data not shown). Newly initiated shoots were separated and cultured repeatedly in fresh MS with 1.5 mg/l BAP + 0.5 mg/l NAA, where the number of shoots increased up to 20.4±1.20 per culture within 12 weeks ((Table I, Fig. 1c). Munshi *et al*., (2001) reported that maximum nodal segments and shoot tips obtained from *Mimosa pudica* L. were used for shoot multiplication via direct or indirect organogenesis. Maximum callus formation was obtained on MS medium in presence of 0.5 mg/l 2,4-D and 1.0 mg/l NAA when shoot tips were taken as explants. The maximum shoot regeneration from these calli was observed on MS medium containing 2.0 mg/l BAP and 0.2 mg/l NAA and highest frequency (92%) of direct multiple shoot formation was

obtained from nodal segment on MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l 2ip. It was also observed that multiple shoots were found by using different concentration of cytokinin with auxins in different medicinal plant, (Gawde and Paratkar, 2004; Baskaran and Jayabalan, 2005; Sinha *et al.*, 2005; Husain and Anis, 2006; Han *et al.*, 2007; Afroz *et al.*, 2008; Hassan *et al.* 2009).

Ninety five percent regenerated shoots rooted (Fig. 1d) when cultured individually on root induction medium consisting of half-strength MS medium with 1.0 mg/l IBA (Table II). Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand, 1998; Ajithkumar and Seeni, 1998; Rai, 2002; Sivakumar and Krishnamurthy, 2000; Hassan and Roy, 2004; Baksha *et al.*, 2007; Hassan, 2008).

The rooted shoots were transferred to pots after four weeks. None of the plantlets survived when directly transferred from rooting medium to the pot under natural conditions. About 80 percent of the transplanted plants of *Mimosa pudica* L. survived when the plants in the rooting culture tubes were kept in normal room temperature for seven days before transplantation in pots and reared for three weeks. The plantlets were reared under semi-controlled temperature (30 ± 2^0 C) and light (2000 lux) in a chamber with 80 percent humidity. During this period of acclimation shoots elongated, leaves expanded and turned deep green and became healthier (Fig. 1e).

After three weeks, the plants were transferred to an open place and gradually acclimated to outdoor conditions, where 80 percent plants survived. The technique described here appears to be readily adaptable for large scale clonal propagation and plantation for sustainable use in the industry.

Table II. Effect of auxin(s) on root induction in regenerated shoots of *Mimosa pudica* L. on half strength MS medium

Growth regulators (mg/l)			% of shoots peoducint roots (±SE)	No. of roots/ shoot (±SE)
BAP	NAA	IAA		
0.5			85.2±0.86	13.4±0.24
0.75			67.2±1.53	11.8±0.37
1.0			63.2±1.46	09.2±0.37
	0.5		71.0±0.10	09.6±0.51
	0.75		57.8±1.85	09.2±0.37
	1.0		54.2±1.53	08.0±0.71
0.5	0.5		82.0±0.71	11.8±0.37
1.0	1.0		59.4±1.08	08.2±0.37
0.5		0.5	65.2±1.16	09.8±0.58
1.0		1.0	61.4±0.75	08.6±0.51
0.5	0.5	0.5	62.6±0.93	09.6±0.51
1.0	1.0	1.0	54.4±1.63	09.2±0.73

Data were recorded after four weeks of culture.
Results are mean ± SE of 15 replications

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