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High frequency plant regeneration through adventitious multiple shoot organogenesis in epicotyl explants of sensitive plant (*Mimosa pudica Linn*)

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Abstract
In vitro rapid propagation was deliberated from shoot tip, nodal and epicotyl explants of *Mimosa pudica L*. The explants were cultured on (MS medium supplemented with B5 vitamins (Murashige and Skoog 1962). In various concentrations of Cytokinins and Auxins ranging from 0.1mg to 3.0mg combinations of BAP, KIN, was good response from shoot tip, nodal, and epicotyl explant. Root induction of explant 2.0 mg IBA and IAA 2.5 mg. Highest number of callus induced in the concentrations of 2.5mg BAP 2mg NAA and 2-4D 0.5mg. Multiple shoot was noticed in 2.5mg BAP and KIN 1.5mg NAA 3.mg and 2mg IBA and shoot elongation in GA3 1.0 mg for good response after transplantation of gardening. The present study enables the large scale production of *Mimosa pudica L*. using *in vitro* conditions and disease free plants.

Keywords: *In vitro culture*, MS medium, *Mimosa pudica*, growth hormone

Introduction
Mimosa pudica L. is a creeping annual or perennial herb often grown for its curiosity value, as the compound leaves fold inward and droop when touched and open within minutes. (Ghani 2003) [10] It belongs to the family Mimosaceae Mimosa pudica L. is native to Brazil, but is now pan tropical and subtropical weed. It is otherwise known as humple plant, shame plant, touch me not, sensitive plant, sleeping grass, (tropical biological association) and prayer plant etc,. (Arthi and Murugan 2011) [1].

Scientific classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnolipsida
Order: Fabales
Family: Fabaceae
Subfamily: Mimosoideae
Genus: Mimosa
Species: pudica L.

Synonyms
Sanskrit: Samanga Varakranta, Namaskari,
Assamese: Lajubilata, Adamalati,
Bengali: Lajaka, Lajjavanti,
English: Touch me not,
Gujrati: Risamani, Lajavanti, Lajamani,
Hindi: Chhumui, Lajauni,
Kannada: Muttidasenui, Machikegida, Lajjavati,
Malayalam: Thottavati,
Marathi: Lajalu
Oriya: Lajakuri
Pujabi: Lajan
Tamil: Tottavadi, Tottalchurungi
Telugu: Mudugudamara
Urdu: Chhuimui

Plant movement
Movement in which the day nictinastic bullet leaves a whole duck and droop down until sunrise, seismonastic bullet movement and when were holding the leaves daughters ashamed shake, heat the leaves or stimulated by chemical or if a daughter of shame have a shortage of water the plant would shy daughter will cause the leaves to duck at the same time and for a whole leaf the leaves will droop down. Raven *et al*., 1991.
The name Mimosa pudica L ‘’Mimic’’ means to " allude and ‘’pudica’’ means "bashful" results the name Mimosa is one of the largest genera which distribute more than 500 species tropical and subtropical rain forest. M.pudica is an indoor plant having fascinating behavior (Gibson, 1966) [11]. The whole plant are widely used in several preparations of medicinally in ayurvedic and folk medicine. phytochemical studies revealed the presence of secondary metabolites in toxic alkaloids such as mimosine, orientin, isoorientin, D-pinitol, norepinepherine, (Karthikeyan etal 2009) [5]. It contains active constituents an toxic alkaloids such as mimosine, mucilage, tannins, non protein, amino acids (mimosine), flavonoids, C-glycosides, steroids, terpenoids, fattyacids, saponins and coumarin.(Minoru veda etal 2003) [3]. Roots of Mimosa contain, ash, calcium crystals, and alkaloids mimosine were also determined. Oudhia *et al* 2006.
Ayurvedic medicine has whole plant is used medicinally and important sources that cure and therapeutic agent the root is bitter, acrid, cooling vulnerary, alexipharmic and treatment of leprosy, dysentery, vaginal and uterine complaints, inflammations, burning sensation, asthma, leukoderma and fatigue and blood disease etc. NPGS/GRIN. The unani system

of medicine root is resolvent alternative treatment, blood impurities, bilious fevers, piles, jaundice and leprosy etc. It is also used diarrhoea, amoebic, dysentery, bleeding piles (Chauhan *et al* 2009) [12] gynaecological disorder all part of the plants used as Indian system of medicine and traditional healthcare system. It is mainly used for bronchitis, bleeding, wound healing activity, amnesia, mental stress. Vaidyaratanm, P S 2001. According to different researches done so for properties present in plant antioxidant, antimalarial, wound healing, antimicrobial, antihepatotoxic, anti-inflammatory, anticonvulsant, antidiarrheal, antihelminthic and antivenom, antifertility Mukesh Chandra *et al*. 2010. Traditionally the plant is used treatment of hydrocele, dysentery, antihelminthic, anti-inflammatory, and substantial neutralisation of snake protein, such as hyalouronidase, and protease (Girish etal., 2004) [6]

Materials and Methods

Plant collection

Seeds of Mimosa pudica L. were collected from the various place in Mannargudi, Thiruvarur (dt) Tamil nadu, India. The freshly seeds collected during the month September 2016.

Surface Sterilization of seeds. The seeds were washed thoroughly under running tap water for 30 minutes followed by treatment in liquid detergent solution (Teepol-5% v/v) and 0.1%(w/v) Bavistin (Bayer, India) for 10min and rinsed with double distilled water. They were then surface sterilization with an aqueous solution of 0.1% (w/v) mercuric chloride (HgCl2) for 5 min and rinsed with double sterile distilled water under the laminar air flow chamber to remove all tracing of sterilizing agent. The disinfected seeds were inoculated in 150mm x 25mm borosil tubes 10 matured seeds for each tube containing sugar -agar medium of 3% w/v sucrose and 0.8% (w/v) agar in distilled water. Epicotyls from 1-week and 2-week-old axenically grown seedling were excised and used for in vitro shoot induction and multiplication.

Table 1: Composition of MS medium and B5 Vitamins

Ingredients	Stock solution of Weight in g/vol	Volume of stock Solution taken for one Litre medium	Mg / litre of Medium
Macronutients			
Mgso4 7H2O	3.700		370
Cacl2 2H2O	4.400	50ml	440
KNO3	19.000		1900
NH4 NO3	1.700		1650
KH2 PO4			170
Micronutrients			
MnSO4 7H2O	2.230		22.3
Zn SO4 7H2O	0.860	2.5ml	8.6
H3 BO3	0.620		6.2
Minor nutrients			
Na2MoO4 7H2O	0.125		0.25
CuSO4 5H2O	0.012	1.0ml	0.025
CaCl2	0.012		0.025
KI	0.0833	2.5ml	0.83
Iron			
Na2EDTA	1.8625	5.0ml	37.25
FeSO4	1.3925		27.85
Vitamins			
Thaimin HCl	10mg		0.1
Pyridoxin HCl	50mg		0.5
Nicotinic acid	50mg		0.5
Glycine	200mg	1.0ml	2.0
Meso-inositol			100mg
Sucrose			30g
Agar			8g

Culture medium

Nutritional support must be essential for optimal growth of a tissue by *in vitro*. The nutritional supplementation in the medium vary with the species. Selection an preparation of particular media is one of the steps in the *in vitro* studies. The nutrient media consist of inorganic nutrients, carbon source and organic supplements. In addition, vitamin and growth regulators are also added to this media. In the present study MS basal (Murashige and skoog 1962) [4] medium with B5 vitamin and different combination of growth regulators are used. For shoot induction MS salts (Murashige and Skoog 1962) [4] supplemented with sucrose (3%, w/v) and BAP at a range of concentrations (0.5 to 4.0 mg) was used, either alone or in combination with IAA, KN 0.5 to 3.0 mg. primary shoots regenerated through in vitro culture were multiplied by successive culture of nodal explants in MS containing the optimum concentration of BAP 3.0 mg and IAA 2.0 mg. For in vitro rooting IBA 1.0 -2.0 mg and NAA 2.0 -3.0 mg was incorporated in the agar gelled half-salt -strength MS basal medium (½ MS). The P^H of media was adjusted to 5.8 prior to gelling with 0.8% (w/v) agar and autoclaving at 121'C and 104 kpa for 15 min. Depending on the requirement, media were dispensed into either 150mm x 25mm borosil tubes 15ml/tube and 250 ml Erlenmeyer flasks. For shoot regeneration, treatment consisted of 7 replicates (1 explant per culture tube) and for rooting, 15 replicates per treatment. Each

experiment was repeated three times. All cultures were incubated in a culture room maintained at 55-60% relative humidity, 27'c under a 16 h photoperiod. Rooted plantlets (3-4- week old) from separate rooting treatments (IBA and NAA) were taken out from the culture vessels washed thoroughly in running tap water to remove any remains of the nutrient -agar medium and planted in polypots containing autoclaved vermiculture saturated with Hoagland's solution. The plantlets were maintained inside a mist chamber set 28 +2.c, 85-90% relative humidity and 16h photoperiod. The acclimatized plants were then transferred to the polybags containing sand, soil and farmyard manure (1:1:1) and maintained in a green house under intermittened misting and daylight conditions for 2 months.

Results

Explants of field grown Mimosa pudica L. (seedling epicotyl and shoot tip explants) was washed by 0.1% percent mercuric chloride ($HgCl_2$) 4 min and 70% ethanol for 2 min) after sterilization explant were inoculated MS basal medium. To observed the growth of explants various hormone NAA, BAP, IBA, IAA, 2-4D and GA3 (Table. 2) are used at different concentrations (0.5 to 4.0 mg/l) are used. BAP is more essential for shoots initiation and regeneration. (Munshi.,*et al.*, 2009)

Table 2: Effect of BAP, NAA, IAA and constant concentration of (2-4 D, 0.5 mg) in callus induction on MS media from shoot tip explants after 2 weeks of culture

Growth regulators (mg/l)			Culture showing response (%)	Basal callus
2-4,D	NAA	IAA		
0.5	0.5	-	52	+
0.5	1.0	-	60	+
0.5	1.5	-	70	++
0.5	2.0	-	75	++
0.5	2.5	-	**65**	+
0.5	0.5	0.5	40	+
0.5	1.0	1.0	60	+
0.5	1.5	1.5	80	++
0.5	2.0	2.0	90	+++
0.5	2.5	2.5	50	+
0.5	0.5	0.5	70	+
0.5	1.0	1.0	88	+++
0.5	1.5	1.5	80	++
0.5	2.0	2.0	70	++
0.5	2.5	2.5	60	+

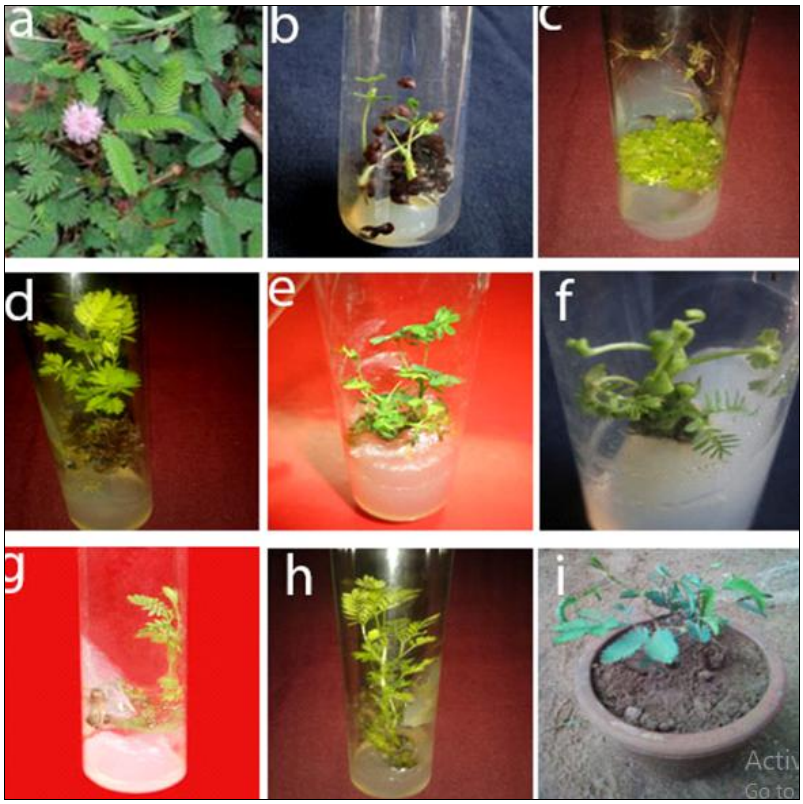

Fig 1: a) Habit. b) Seed germination on MS – B5 medium with in 2 weeks. c) Callus induction on MS -B5 medium with NAA,3.0mg/l and 2,4D 0.5mg/l after 2 weeks. d) Shoot induction in 4 weeks of culture on MS +3.0mg/l BAP, 2.0mg/l NAA and 1.0mg/l KIN from epicotyl explant. e & f) Multiple shoots in 8 weeks of culture on MS +3.0mg/l BAP,2.5mg/NAA from shoot tip explants. g) Rooting of *in vitro* regenerated shoots cultured on half strength MS + 2.0mg/l IBA and IAA 1.0mg/l in third weeks. h) Shoot elongation cultured on MS+0.5mg GA3 in 8 weeks. i) Acclimatized regenerated one months old plants

Table 3: Effect of various concentration of BAP, NAA, IAA in shoot regeneration from on MS medium after 4 weeks of culture.

Growth regulators (mg/l)			Culture showing response (%)	Mean shoot / explant	Mean shoot length (cm)
BAP	NAA	IAA			
0.5	-	0.5	40	2	1.5
1.0	-	1.0	60	3	3.8
1.5	-	1.5	60	3	4.0
2.0	-	2.0	85	6	5.8
2.5	-	2.5	50	2	2.1
-	0.5	0.5	50	1	2.5
-	1.0	1.0	70	3	3.5
-	1.5	1.5	60	2	2.0
-	2.0	2.0	55	2	2.0
-	2.5	2.5	60	1	2.2
0.5	0.5	0.5	55	2	2.3
1.0	1.0	1.0	60	2	3.9
1.5	1.5	1.5	50	4	3.2
2.0	2.0	2.0	50	3	2.6
2.5	2.5	2.5	40	1	1.1

Table 4: Effect of various concentrations of BAP, KIN, IBA in shoot multiplication on MS medium after 8 weeks of culture.

Growth regulators (mg/l)			Culture showing response (%)	Mean on roots	Mean root length (cm)
BAP	KIN	IBA			
0.5	-	-	40	12.6	3.5
1.0	-	-	60	13.6	5.1
1.5	-	-	60	14.5	4.5
2.0	-	-	80	17.6	5.0
2.5	-	-	85	23.5	6.1
-	0.5	-	75	16.3	5.9
-	1.0	-	60	13.3	4.7
-	1.5	-	50	13.3	3.0
-	2.0	-	50	10.5	3.2
-	2.5	-	40	11.5	2.0
-	-	0.5	40	13.4	1.6
-	-	1.0	60	13.7	1.7
-	-	1.5	80	14.5	5.5
-	-	2.0	60	11.5	3.0
-	-	2.5	50	13.6	3.9

Table 5: Effect of various concentrations of BAP, IAA, IBA in roots formation on MS medium after 3 weeks of culture

Growth regulators (mg/l)			Culture showing response (%)	Mean shoot / explant	Mean shoot length (cm)
BAP	KIN	IBA			
0.5	0.5	-	60	2	2.2
1.0	1.0	-	70	3	4.5
1.5	1.5	-	67	3	4.0
2.0	2.0	-	65	4	3.0
2.5	2.5	-	70	4	4.7
0.5	0.5	-	50	2	1.5
1.0	1.0	-	60	3	2.2
1.5	1.5	-	75	4	3.0
2.0	2.0	-	70	4	4.0
2.5	2.5	-	90	3	6.1
0.5	0.5	0.5	60	2	2.0
1.0	1.0	1.0	65	2	3.2
1.5	1.5	1.5	71	3	3.8
2.0	2.0	2.0	75	4	4.0
2.5	2.5	2.5	70	14	2.2

The callus regeneration along with the cut ends of the various explants followed by callus growth was observed (Fig -1.a.). The response of explants to different concentrations and combination of growth regulators used in the present study was shown (Table -2). The epicotyl derived two weeks old seedling the observations indicate that 2, 4-D (0.5 mg/l) in combination with NAA (3.0 mg/l) and IAA (2.0 mg/l) are produced maximum percentage of callus(Fig. 1.b)(95 %) was the most effective in producing callus in term of percentage of response per epicotyl explant (Table -2)

Shoot elongation

A highest frequency of shoots (Table -3) from epicotyl and shoot tip explants was observed in MS media containing 2.5 mg BAP showed 95% response. It was found most suitable and multiplication rates continuously increasing. Duration of shoot formation on Mimosa pudica L. regeneration from shoot tip and epicotyl explants 15 – 25 days Fig.1 d. The shoots are aggregated. The mean of shoot length 6.69. 0.25 was measured. Among the different concentration (0.5 to 4.0 mg/l) BAP, NAA. IBA, are used. 3.0 mg/l BAP was given 80 % growth of mean shoot length was 7 cm multiple shoots were observed Fig-8. The maximum shoot regeneration percentage (80 %) maximum number of regenerated shoots (15.7 + 0.78) and an average shoot length (5.96 + 0.33 cm) was obtained. The cultures were incubated at 25 + 2 C under 16/8 h light / dark photoperiod (Fig-1.i.)

Rooting and Hardenig

The shoots was transferred to rooting medium well developed roots from explants were excised and transferred to rooting medium (MS) containing IBA, NAA, and IAA. Basal medium did not support the induction of roots. Hence various concentrations of IBA and IAA were used in the range of 0.5 to 4.0 mg /l. Root induction occurred in 7 to 28 days of culturing with highest root induction (90 %) on MS medium containing 2.0 mg/l (IBA) with more than no of roots (20.15 + 4.5 cm) and average length of roots (18 + 4.5 cm) (Table 4)

Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand, 1998; Ajithkumar and Seeni, 1998; Sivakumar and Krishnamurthy, 2000 Hassan and Ray, 2004; Baksha *et al*.,2007; Hassan, 2008) [16, 14, 15, 19, 18]. The well rooted plantlets were transferred to paper cups containing sterile sand soil and vermiculture (1:1:1 v/v/v) and covered with polythene bags to make sure high relative humidity (85%). The plantlets were maintained under controlled environmental conditions for two weeks and were wet with water once in two days during this period to stay away from desiccation. The plantlets were subsequently transferred to earthen pots containing composition mixture as mentioned above and grown in greenhouse.

Discussion

In present study the highest frequency of shoots from leaf explants was observed in MS media containing 2.5 mg BAP +2.5 mg KIN this combination showed 82 % response. Root explants (E. axillare) were cultured on MS medium supplemented with (KIN, BAP,) used alone or in combinations (2.22 m in combination with 4.64 m of KIN induced the maximum number of adventitious shoot buds (24.60 + 0.54) shoots per explants with the shoot length of 1.54 + 0.36). Explants from in vitro shoots in BAP supplemented medium were found with more response than those of wild plants. Saritha and Naidu (2007) [21] The type of callus is determined by the explants used and organ of the plant, the hormones and their concentrations, the chemical constituents of the culture medium. The combinations of external growth regulators (Cytokinins with Auxins) aer essential requirement to stimulate shoots formation from callus. The difference in response are depends on regeneration media could be due to the kind of endogenous hormones in cells which control many circumstances expressed by cells.

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