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AN EFFICIENT MICROPROPAGATION PROTOCOL FOR *MUCUNA PRURIENS* (L.) DC.

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ABSTRACT

An efficient protocol was developed for rapid micropropagation of *Mucuna pruriens* (L.) DC., an important medicinal plant species of Bangladesh. For the purpose, leaf segments, nodal segments and shoot apices of *in vitro* grown seedlings and cotyledon segments of immature seeds of field grown plants were cultured on agar solidified MS medium supplemented with different combinations of PGRs (Plant Growth Regulators). Only leaf and cotyledon segments produced light green to green friable/ compact callus in some of the media combinations. Combination and concentration of PGRs played important role in induction of such tissue. The induced tissues failed to differentiate on further culture in a wider spectrum of PGR supplemented media. On the other hand nodal segments and shoot apices produced multiple shoot buds when grown on a wide range of PGRs (BAP, NAA, IAA, Kn) supplemented media. Maximum number of multiple shoot buds were produced in medium containing 1.5 mg/l BAP + 1.0 mg/l IAA. The elongated shoot buds on further culture in rooting media produced strong and stout root systems. Half strength MS media fortified with either 1.0 mg/l IAA or 2.0 mg/l IAA was most effective for induction and proliferation of roots. These complete plantlets were successfully acclimatized and established in outside pots through a successive phase of acclimatization.

Key words: *Mucuna pruriens*, micropropagation, acclimatization.

INTRODUCTION

Mucuna pruriens (L.) DC., commonly known as Alkushi or Bilaiachra**,** is an important medicinal plant species belongs to the family Fabaceae. It is grown in the jungle and village thickets of Bangladesh particularly in the hilly areas. The pods of this species are used as active anthelmintic. Hairs of the pods are used as a vermifuge to expel emmengogue; prescribed as a remedy for delirium in fever. Strong infusion of the root mixed with honey is given in cholera. A decoction of the root is a powerful diuretic and cleanser of kidneys. Leaves are applied to ulcers. Like other medicinal plants, the availability of *M. pruriens* in nature is

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decreasing day by day due to excessive, unregulated exploitation and destruction of its natural habitat.

Plant tissue culture technique is one of the best and most successful examples of commercial application of plant propagation. Recently, there has been much progress in this technology for some medicinal plants. Accordingly this technique has been reliably used in propagation of many medicinal plant species (Sivakumar and Krishnamurthy 2003, Selvakumar *et al*. 2001, Wawroch *et al*. 2001, Das and Handique 2002, Lal and Ahuja 1989, Roy *et al*. 1995, Chand *et al*. 1997, Mikulik 1999). With these considerations, the present study was undertaken to develop an efficient and reproducible *in vitro* propagation protocol for *Mucuna pruriens* (L.) DC with the use of different explants.

MATERIALS AND METHODS

Both mature and immature seeds were collected from field grown plants and surface sterilized following Majumder *et al*. (2011). In order to raise *in vitro* seedlings mature seeds were grown on MS media. The seedlings developed were used as a source of aseptic explants. On the other hand the sterilized immature seeds were used for the source of cotyledons. The surface sterilized immature seeds and explants (shoot tip, nodal segments, leaf segments) of *in vitro* grown seedlings were cut into small pieces (0.5 - 1.0 cm) with a sterilized surgical blade and inoculated on MS medium supplemented with different concentrations and combinations of Plant Growth Regulators (PGRs) for induction of tissue formation and differentiation. 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

Cotyledon segments of immature seeds of field grown plants produced green friable and compact callus tissues on some of the used PGR supplemented media. These callus tissues were further grown on MS medium fortified with a wide range of PGR concentrations and combinations but the tissue failed to undergo differentiation on those.

It was then considered important to develop *in vitro* sterile seedlings and use their explants. For this purpose, seeds were aseptically grown on MS basal medium where it failed to germinate (Table 1). Therefore, the seeds were grown on PGRs supplemented media where it germinated and produced a large number of healthy seedlings within 2 - 8 d (Fig. 1A). In some cases more than one shoot system (Fig. 1B) arose from a single seed when grown on MS medium containing different concentrations and combinations of BAP and IAA. The failure of germination of seeds on the basal MS medium indicates the requirement of PGR supplements for *in vitro* germination.

TABLE - 1: RESULTS OF *IN VITRO* GERMINATION OF *M. PRURIENS* 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) required for germination	Callus induction	Type of response
			No.	%			
MS		20	-	-	-	-	-
MS+ 2,4 - D	2.5	8	8	100	3 - 6	+	SSS
MS+ Kn	3.0	10	7	70	2 - 6	-	SSS
MS+BAP+NAA	2.5 + 0.5	6	5	83	4 - 8	-	SSS
MS+Kn+NAA	3.0 + 0.5	10	7	70	3 - 6	-	SSS
MS+BAP+IAA	1.5 + 0.5	12	10	84	2 - 5	-	*MSS or**SSS
	1.5 + 1.0	15	15	100	2 - 6	-	MSS or SSS
	2.0 + 0.5	8	8	100	2 - 8	-	MSS or SSS
MS+Kn+IAA	2.0 + 0.5	6	4	67	3 - 8	+	SSS

"__" = Indicates no response **SSS = Single shoot system,*MSS = Multiple shoot system,

The *in vitro* grown seedlings were used as source of explants *viz*. nodal segments, leaf segments and shoot apices. The explants *were* cultured on a wide varieties of media supplemented with different PGRs. In all these media combinations nodal segments and shoot apices underwent direct organogenesis and produced multiple shoot buds (Fig. 1D). In some cases white friable callus tissue along with multiple shoot buds were also developed from nodal segments (Table 2). The maximum number of multiple shoot buds was produced on MS medium supplemented with 1.5 mg/l BAP + 1.0 mg/l IAA. Such direct organogenesis was also reported in many other medicinal plants including, *Paedaria foetida* (Amin *et al*. 2003), *Rouvolfia tetraphylla* (Faisal *et al*. 2005), *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).

TABLE-2: RESULTS ON THE DEVELOPMENT OF MULTIPLE SHOOT BUDS FROM NODAL SEGMENT AND SHOOT APEX OF *IN VITRO* GROWN SEEDLING OF *M. PRURIENS* WHEN GROWN ON 0.8% (W/V) AGAR SOLIDIFIED MS MEDIUM SUPPLEMENTED WITH DIFFERENT PGRs.

PGRs combinations and concentrations (mg/l)		Explant	% of explant that gave response	Time(d) required for induction	Callus tissue formation	Time(d) required for callus induction	Average* number of multiple shoot buds sprouted from explant (mean ± SE)
BAP + NAA	2.0 + 0.5	SA**	65	22 - 28	-	-	3.30 ± 0.23
		NS***	72	24 - 27	+	20 - 25	3.40 ± 0.24
	2.0 + 1.0	SA	60	20 - 30	-	-	2.75 ± 0.15
		NS	75	21 - 26	+	22 - 26	3.10 ± 0.19
	3.0 + 0.5	SA	62	18 - 25	-	-	3.50 ± 0.26
		NS	68	24 - 29	-	-	3.00 ± 0.17
	3.0 + 1.0	SA	70	20 - 24	-	-	3.10 ± 0.20
		NS	75	20 - 30	-	-	3.85 ± 0.19
Kn + NAA	1.0 + 1.0	SA	64	21 - 27	-	-	4.10 ± 0.21
		NS	60	23 - 30	-	-	3.95 ± 0.18
	3.0 + 0.5	SA	80	22 - 25	-	-	2.75 ± 0.12
		NS	75	24 - 28	-	-	4.15 ± 0.24
	3.0 + 1.0	SA	77	19 - 26	-	-	3.10 ± 0.19
		NS	68	23 - 27	-	-	4.35 ± 0.21
BAP + IAA	1.5 + 0.5	SA	86	18 - 23	-	-	4.35 ± 0.21
		NS	84	20 - 25	+	25 - 30	4.75 ± 0.20
	1.5 + 1.0	SA	93	20 - 24	+	20 - 25	4.85 ± 0.21
		NS	88	24 - 26	+	18 - 25	5.10 ± 0.24
	2.0 + 0.5	SA	85	18 - 24	-	-	3.95 ± 0.19
		NS	96	21 - 28	+	23 - 30	3.70 ± 0.21
Kn + IAA	1.5 + 0.5	SA	65	22 - 30	-		2.90 ± 0.12
		NS	77	24 - 31	+	25 - 30	3.50 ± 0.15
	1.5 + 1.0	SA	60	19 - 28	-	-	2.80 ± 0.13
		NS	75	21 - 27	-	-	3.45 ± 0.19
	2.0 + 0.5	SA	80	20 - 25	-	-	3.10 ± 0.18
		NS	60	25 - 30	+	21 - 28	3.45 ± 0.21

* = values are the mean of five replicates each with 15 explants. SA ** = Shoot Apex, NS *** = Nodal Segment

On the other hand, leaf segments always produced green friable callus tissue (Fig.1C).The callus tissue that developed from leaf segments and nodal segments were grown on MS media fortified with a wide range of PGR concentrations and combinations but failed to undergo differentiation.

Multiple shoot buds which developed directly from nodal segments and shoot apices of *in vitro* grown seedlings underwent rapid elongation on subculture in elongation media. 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 supplement and concentration. The highest response was recorded on MS media supplemented with 1.5 mg/l BAP + 1.0 mg/l IAA (Table - 3). Such enhancement of elongation of *in vitro* induced shoot buds has been noted in many other medicinal plants, such as *Rauvolfia serpentina* (Ahmad *et al.* 2002) and *Vitex negundo* (Rahman *et al*. 2004).

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

PGRs combinations (mg/l)		Average* initial length (cm) of individual shoot bud ±SE	Average* length (cm) of multiple shoot buds after 30d of culture ±SE
BAP+NAA	0.5 + 1.	1.20 ± 0.044	3.53 ± 0.084
	2.0 + 0.5	1.40 ± 0.038	2.95 ± 0.089
	2.0 + 1.0	1.15 ± 0.043	3.10 ± 0.054
	3.0 + 1.0	1.00 ± 0.037	2.80 ± 0.101
BAP+IAA	1.5 + 0.5	1.50 ± 0.038	3.60 ± 0.157
	1.5 + 1.0	1.45 ± 0.040	4.35 ± 0.164
	2.0 + 0.5	1.30 ± 0.042	3.45 ± 0.139
	2.0 + 1.0	1.20 ± 0.045	3.50 ± 0.107
Kn+NAA	1.0 + 0.5	1.00 ± 0.035	2.75 ± 0.137
	1.0 + 1.0	1.25 ± 0.033	2.90 ± 0.094
	3.0+ 0.5	1.30 ± 0.041	3.30 ± 0.105
	3.0 + 1.0	1.45 ± 0.042	3.10 ± 0.048
Kn+IAA	1.5 + 0.5	1.25 ± 0.029	3.90 ± 0.119
	1.5 + 1.0	1.45 ± 0.038	4.20 ± 0.118
	2.0 + 0.5	1.30 ± 0.040	3.85 ± 0.120

* = values are the mean of five replicates each with 15 explants.

Two to three cm long *in vitro* grown shoot buds 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 half strength MS media without auxin. But half strength MS media supplemented with either IAA or IBA induced root formation within 10 - 15d of culture (Fig. 1E) and the data on rooting were recorded after 30d of inoculation (Table 4). Response of shoot to rooting media was very much dependent on the concentration of auxins and strength of MS media. The highest number and length of roots per shoot was noted in half strength MS medium fortified with 2.0 mg/l IAA Such influence of IAA for induction and proliferation of root growth has been reported in other medicinal plants (Chandramu *et al*. 2003, Biswas *et al*. 2007, Jawahar *et al*. 2008).

TABLE - 4: DATA ON THE DEVELOPMENT OF ROOTS IN ELONGATED MULTIPLE SHOOT BUDS OF *M. PRURIENS* WHEN GROWN 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	80.00	1.40 ± 0.117	3.20 ± 0.223
½ MS + 0.5 mg/l IAA	95.00	2.90 ± 0.142	2.10 ± 0.165
½ MS + 1.0 mg/l IAA	100.00	3.20 ± 0.133	3.50 ± 0.263
½ MS + 2.0 mg/l IAA	100.00	3.90 ± 0.187	3.50 ± 0.263
½ MS + 0.5 mg/l IBA	95.00	1.85 ± 0.149	2.20 ± 0.173
½ MS + 1.0 mg/l IBA	75.00	2.10 ± 0.122	1.90 ± 0.155
½ MS + 2.0 mg/l IBA	80.00	2.90 ± 0.213	2.00 ± 0.137
¼ MS	90.00	1.90 ± 0.187	2.30 ± 0.189

* 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 outside pots containing garden soil and compost in the ratio of 1:1 through successive phases of acclimatization. Most of the transferred plants survived (Fig. 1F).

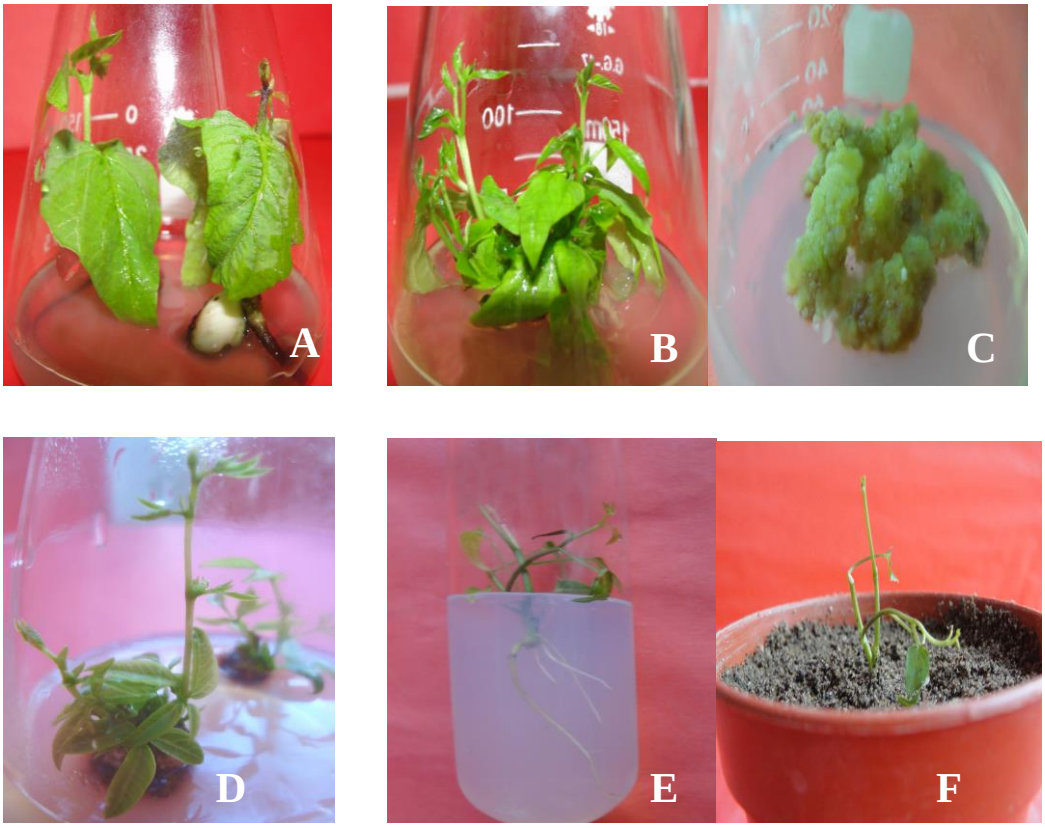

FIGURE-1. DIFFERENT STEPS OF DIRECT SHOOT REGENERATION IN *M. PRURIENS*: A. *IN VITRO* GERMINATION OF SEEDS; B. MULTIPLE SHOOT SYSTEM ARISING FROM SINGLE SEED; C. INDUCTION OF GREEN AND FRIABLE CALLUS TISSUE FROM LEAF SEGMENTS; D. MULTIPLE SHOOT BUDS ARISING FROM NODAL SEGMENTS; E. ELONGATED SOOT BUDS PRODUCED STRONG AND STOUT ROOT SYSTEM; F. *IN VITRO* GROWN SEEDLINGS ESTABLISHED IN OUTSIDE POT.

The overall results revealed that mass micropropagation of *M. pruriens*, an important but rare 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.

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