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In vitro shoot multiplication of the biodiesel plant *Jatropha curcas* L. through direct organogenesis

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Abstract

An efficient protocol was established for *in vitro* shoot multiplication of the biodiesel plant, *Jatropha curcas* L. (Euphorbiaceae) through direct organogenesis using shoot tip and nodal explants. Best shoot induction was observed on MS basal medium supplemented with 1.5 mg/l BAP + 0.5 mg/l NAA, in which 86.2% of nodal explants responded to produce maximum number (7.2 ± 0.68) of shoots per culture. *In vitro* raised shoots rooted on half strength MS medium with 1.0 mg/l IAA. The survival rate of regenerated plantlets was 85%.

Keywords:: *Jatropha curcas;* Biodiesel plant; Multiplication; Organogenesis; Acclimatization

Introduction

Jatropha curcas L. commonly known as 'Jamal Gota' belongs to Euphorbiaceae, an evergreen shrub or small tree with long-petioled ovate-cordate lobed leaves, small flowers in axillary cymes and angular fruits, grows wild in waste places throughout the Bangladesh (Ghani, 2003). Seeds and seed oil are used as purgative, more drastic than those of *Ricinus communis*; they are used against warts and cancers; seed oil is applied topically in rheumatism, herpes and pruritis; sap of the plant is used to cure toothache and as a styptic; pounded leaves and juice are applied to wounds and ulcers and also used in the treatment of scabies, eczema and ringworm; roots are used as emetic and purgative (Ghani, 2003).

Jatropha curcas a drought tolerant perennial plant species has received global attention due to its seed which contains 40-50% semi drying oil; it is used as an efficient substitute for diesel fuel (Takeda, 1982; Banerji *et al.* 1985; Martin and Mayeux, 1985; Muhlbauer *et al.* 1998). Realizing the importance of renewable energy sources, countries like Cape Verde, Madagascar, Nicaragua, Brazil, Mali etc. are using the seeds of *Jatropha curcas* for large scale production of biodiesel (Heller, 1996). The center of origin of this species is known to be South America (Brazil); later is spread all tropical regions (Dehgan and Webster, 1979).

The oil yield from kernels is estimated between 46 and 58% derived from semi-drying oil (iodine value 93 - 100%) containing mainly oleic (37 - 63%), linoleic (19 - 40%) and palmitic (12 - 17%) acids as constituents of fatty acids. Medicinally the oil is used as a purgative and emetic and against coetaneous disease (Godbole *et al.*,1966). It is also used for burning and spinning in the manufacture of hard soaps and candles, paint and lubricants (Roy, 1990).

The seeds of Jatropha curcas are of great commercial interest in the recent decade for fuel; to exploit the oil at a maximum extent, it is necessary to propagate and select the best genotype for oil contents and quality; the genetic diversity in the natural population appears narrow since no remarkable morphological differences have been observed (Sujatha and Mukta, 1996). In order to meet the demand of fuel in the near future, the development of appropriate technology for the rapid regeneration at a large scale of this species is essential. The conventional method of propagation through seeds will not solve the problem; under this situation *in vitro* regeneration of this species through tissue culture offers a powerful method to overcome the problem (Kalimuthu *et al*., 2007). There have been a few reports on the establishment of micropropagation protocol for *Jatropha podagrica* (Spera *et al.*, 1997), *Jatropha integarrima* L. (Sujatha and Pabakaran, 2003; Sujatha and Dhingra, 1993) and *Jatropha curcas* L.

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(Kalimuthu *et al*. 2007). Protocols developed for micropropagation of *Jatropha* species by Sujatha and Mukta (1996), Sujatha and Pabakaran (2003), Rojore and Batra (2005), Sujatha *et al.* (2005) were not promising because the multiplication rate was low. *In vitro* propagation has been proved as a potential technology for mass scale production of medicinal plant species (Hassan and Roy, 2005; Lui and Li, 2001; Martin, 2002; 2003). It is important, therefore to develop an efficient micropropagation technique for the biodiesel plant, *Jatropha curcas* L. for rapidly disseminate superior clones. Kalimuthu *et al*. (2007) reported that the biodiesel plant, *Jatropha curcas* L. was micropropagated using nodal explants on MS supplemented with 1.5 mg/l BAP, 0.5 mg/l Kn and 0.1 mg/l IAA and somatic embryos were induced directly from green cotyledon explants on MS fortified with 2.0 mg/l BAP. The present study was therefore, undertaken to develop a protocol for *in vitro* shoot multiplication of the biodiesel plant, *Jatropha curcas* L. through direct organogenesis using shoot tip and nodal explants.

Materials and Methods

Jatropha curcas L. at Medicinal Plants Garden of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, was used as a source of explants. Shoot tip and nodal explants with a single axillary bud were used for this purpose. The explants were washed thoroughly under running tap water, pre-soaked in Tricks 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 5 min, followed by five times rinse with sterile distilled water under laminar air flow cabinet. The surface-sterilized explants were cut into 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 root induction. All media were supplemented with 30 g/l sucrose, 7 g/l agar (Difco) and dispensed into 15×150 mm culture tubes and 250 ml conical flasks. The pH of the media was adjusted to 5.8 before autoclaving at 1.9 kg/cm^2 pressure at 121°C for 20 min. The cultures were incubated for a 16 h photoperiod at 24 ± 2°C under 1200 lux/m2 fluorescent light.

Shoot proliferation from shoot tip and nodal explants was obtained in two separate sets of experiments. In the first experiment 0.1-2.0 mg/L BAP were incorporated into MS media to select the best cytokinin for the response of shoot

Table I. Effect of BAP, NAA and IAA in MS on morphogenic response of *Jatropha curcas* shoot tips and nodal explants

Growth regulators (mg/l)			Shoot tips		Nodal explants	
BAP	NAA	IAA	% of explants forming shoots	Mean No. of shoot/explant	% of explants forming shoots	Mean No. of shoot/explant
0.1			43.4±0.87 h	1.6±0.77 d	58.6±1.70 i	2.4±0.91 de
0.3			52.6±1.66f g	2.2±0.63 cdef	64.4±0.51 g	2.6±0.66 d
0.5			53.4±1.57 f	2.6±0.76 cd	67.2±2.47 ef	2.6±0.72 d
1.0			57.6±2.16 ef	2.6±0.45 cd	71.4±2.38 d	3.4±1.18 cde
1.5			64.8±2.58 c	3.4±0.72 bc	77.6±2.16 b	3.4±0.82 cde
2.0			61.4±2.87 de	2.8±0.76 c	63.6±1.84 gh	4.6±1.14 bc
0.5	0.1		52.6±0.87 fg	2.2±0.59 cdef	68.6±1.70 e	3.6±0.91 cd
1.0	0.2		68.2±1.66 b	3.6±0.77 b	73.6±0.51 c	4.4±0.66 bcd
1.5	**0.5**		**72.4±2.89 a**	**4.8± 0.92 a**	**86.2±1.80 a**	**7.2± 0.68 a**
2.0	0.5		62.2±1.96 d	3.0±0.63 bcd	71.2±2.47 de	4.8±0.95 b
0.5		0.1	48.8±1.77 g	2.4±0.45 cde	52.2±0.66 j	2.2±0.76 def
1.0		0.2	56.6±1.66 efg	3.0±0.39 bcd	66.8±2.14 f	3.2±0.51 cdef
1.5		0.5	61.0±1.14 ef	3.6±0.65 b	77.6±2.10 b	4.4±0.91 bcd
2.0		0.5	58.4±1.96 e	2.2±0.45 cdef	62.6±1.63 h	3.8±0.76 c

Mean values ± SE followed by the same letter within a column are not significantly different at $p < 0.05$ by DMRT.

induction. In the second set, combination of BAP (0.5-2.0 mg/L) with NAA (0.1-0.5 mg/L) and BAP (0.5-2.0 mg/L) with IAA (0.1-0.5 mg/L) were assessed for shoot multiplication. Number of new proliferated shoot of in each culture was recorded after every week of inoculation. For *in vitro* rooting, individual shoots (3-5 cm) were excised from the proliferated shoots and implanted onto half strength MS with different concentrations and combinations of NAA, IBA and IAA.

The rooted plants were 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°C temperatures for a 12 h photoperiod under 1500 lux/m^2 sun light for acclimation. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

All experiments were repeated three times. Each treatment had 15 replicates. The morphologenetic response of explants for microshoot induction was evaluated in eight weeks of culture. For microshoots proliferation and plantlet formation, results were evaluated in eight weeks of culture. Morphogenetic response was expressed as percentage of explants with microshoots in relation to the number of surviving explants. For acclimatization 50 or 25 plantlets were taken for each treatment. Data were statistically analyzed and in

Fig. 1. *In vitro* regeneration of *Jatropha curcas* L. from nodal explants.
(a) Induction of shoots from nodal explants after three weeks. (b) Development and multiplication of shoots from nodal explants after six weeks. (c) Development and multiplication of shoots from nodal explants after nine weeks. (d) Rooting of *in vitro* regenerated shoots cultured in three weeks. (e) Acclimatized regenerated plants of one year old.

some parameters means were compared using DMRT (Duncan, 1955).

Results and discussion

Shoot tip and nodal explants of *Jatropha curcas* L. were cultured on MS media supplemented with various concentration of BAP alone and with NAA or IAA for multiple shoot regeneration. The explants were found to be swollen and they produced two to three shoots within three weeks after inoculation (Fig.1a) on MS media containing BAP alone but the number of shoots increased up to 7.2 ± 0.68 when the explants were cultured in MS with 1.5 mg/l BAP + 0.5 mg/l NAA (Table I, Fig.1b). Both the explants responded in the same medium but highest numbers of micro shoots were observed from nodal explants (Fig.1c). Combinations of BAP alone or BAP with IAA were not suitable than BAP with NAA for shoot induction (Table I). Kalimuthu *et al.* (2007) reported that the biodiesel plant, *Jatropha curcas* was micropropagated using nodal explants on MS supplemented with 1.5 mg/l BAP, 0.5 mg/l Kn and 0.1 mg/l IAA and somatic embryos were induced directly from green cotyledon explants on MS fortified with 2.0 mg/l BAP. In *Jatropha curcas* and other medicinal plants, it was also observed that multiple shoots were found by using different concentration of cytokinin with auxins by others (Faisal *et al.*, 2003; Mallikadevi *et al*., 2008; Sahoo and Debata, 1998; Spera *et al.* 1997; Sujatha and Mukta, 1996; Sujatha *et al.* 2005). 84.4% regenerated shoots rooted (Fig.1d) when cultured individually on root induction medium consisted of half-strength MS medium with 1.0 mg/l IAA (Table II). Use of auxins singly or in combination for rooting was also reported by others (Baskaran and Jayabalan, 2005; Bhadra *et al.*, 2009; Hassan and Khatun, 2010; Sivakumar and Krishnamurthy, 2000).

Table II. Effect of IBA, NAA and IAA on root induction in regenerated shoots of *Jatropha curcas* on half strength MS.

Growth regulators (mg/l)			% of shoots producing roots (±SE)	No. of roots/ shoot (±SE)
BAP	NAA	IAA		
0.5			47.2±1.53 i	1.2±0.65 d
0.75			53.2±1.46 hi	1.8±0.65 cd
1.0			61.0±0.10 fgh	2.2±0.76 bcd
1.5			57.8±1.85 g	1.6±0.72 cde
	0.5		54.2±1.53 h	1.2±0.76 d
	0.75		62.0±0.71 f	2.0±0.63 c
	1.0		69.4±1.08 c	2.8±0.59 b
	1.5		65.2±1.16 e	2.2±0.71 bcd
		0.5	61.4±0.75 fg	1.8±0.65 cd
		0.75	72.6±0.93 b	2.6±0.96 bc
		1.0	**84.4±1.16 a**	**3.6±0.72 a**
		1.5	68.2±0.71 d	2.2±0.63 bcd

About 85 per cent of the transplanted plants of *Jatropha curcas* survived if 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 ± 2ºC) and light (1500 lux) in a chamber with 80 per cent humidity. During this period of acclimation shoots elongated, leaves expanded and turned to deep green and healthier (Fig.1e).

Following the transfer of plants to an open place and gradually acclimatized to outdoor conditions, where 85 per cent plants were survived. The technique described here appears to be readily adaptable for large scale plant regeneration and plantation of *Jatropha curcas.*

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