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Short Communication

Efficient *In vitro* Plant Regeneration Protocol from Leaf Explant of *Jatropha curcas* L – A Promising Biofuel Plant

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An efficient *in vitro* plant regeneration from leaf-disc culture of *Jatropha curcas* L has been established. Adventitious shoot buds along with callus were induced from leaves of 2-year-old *J. curcas* plants cultured on Murashige and Skoog's (MS) medium supplemented with TDZ (2 µM) BAP (2 µM) and IBA (1 µM), wherein 83.3% leaf explants responded. The multiplication of shoots was achieved from the adventitious shoot buds after transferring them to shoot induction medium. The highest number of shoots (9.7/explant) was achieved after 8 weeks of culture on MS medium containing 3 µM of BAP. The well-developed shoots were rooted on MS medium supplemented with IBA (1.5 µM) with the rooting frequency of 53.3%. Addition of phloroglucinol (200 µM) to the medium enhanced the frequency of rooting to 76.7%. Regenerated plantlets were successfully transferred to field after initial acclimatization.

***Key words*:** Adventitious shoot buds, *Jatropha curcas*, growth regulators, plant regeneration.

Jatropha curcas L (physic nut), a multipurpose tree of Latin American origin, belongs to the family Euphorbiaceae, and is widespread throughout the tropical regions of the World (1). It has been found to be a highly promising species, yielding oil seed as a source of energy in the form of bio-diesel. The short gestation period, easy adaptation to different kinds of marginal and semi-marginal lands, makes this plant species attractive for cultivation (1, 2). Besides its importance as an energy crop, *J. curcas* has many medicinal uses, for instance in treatment of piles, scabies, eczema and ring worm (3). The cultivation of *J. curcas* assumes utmost importance to meet the large-scale demand and ensure continuous supply of the elite material (4). Tissue culture techniques offer rapid and continuous supply of the planting material. Although there are reports of plantlet regeneration in *J. curcas* by some workers, the number of shoots initiated in the explants is relatively low (3, 5, 6). Also, the previous works done on the *in vitro* propagation of *J. curcas* suggest that the incorporation of two or more growth regulators in the culture medium results in its further proliferation and multiplication (4). In the present study, we report an efficient and reproducible method for large-scale propagation of *J. curcas* using leaf explants.

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Abbreviations: BAP, 6-benzylaminopurine; IBA, indole-3-butyric acid; MS, Murashige and Skoog; SE, Standard error; TDZ, thidiazuron.

Leaves were excised from 2-years-old *J. curcas* plant; very young leaves (starting from one to three from nodal tips) were selected for culture. The leaves were thoroughly washed with tap water, then dipped in 1% Bavistin (BASF, India) for 5 min and washed 2-3 times with autoclaved distilled water. Then they were surface sterilized with 0.1% $HgCl_2$ (w/v) solution for 3 min and again washed 4-5 times with sterile distilled water. Subsequently, the leaves were cut into small pieces (5-7 mm) and placed with abaxial side on the MS medium (Fig. 1a) containing salts and vitamins, 30g l^{-1} sucrose and 0.8% agar. For initiation, MS medium supplemented with different growth regulators *viz.*, TDZ, BAP and IBA (0 - 4 µM) both singly and in combination, was used. Induced mass of adventitious shoot buds with callus was removed gently and the mass of shoot buds was cut into two or three pieces, which were subsequently cultured on multiplication medium containing MS medium with BAP (3, 6 and 9 µM).

For initiation of roots, regenerated shoots (1.5-2.0 cm in size) were cultured in MS medium containing IBA (0-3 µM) either singly or with phloroglucinol (100, 200 µM). The pH of the medium was adjusted to 5.8 before autoclaving. The medium was autoclaved at 105 kPa for 15 min at 121°C. All cultures were incubated at 25 ± 2°C under a 14-h photoperiod using cool-white fluorescent lights (60.2 µmol m^{-2} sec^{-1}).

Table 1. Morphogenetic response of *J. curcas* from leaf disc on MS medium supplemented with BAP, TDZ and IBA and effect of BAP on multiple shoot formation

Plant-growth regulator conc* (μM)			Per cent response	Multiplication Medium** (Average number of shoots/leaf disc)		
TDZ	BAP	IBA		BAP (3μM)	BAP (6μM)	BAP (9μM)
0	0	0	-	-	-	-
1	1	0.5	70.0±5.8^{ab}	5.3±0.19^{c}	4.1±0.30^{b}	2.9±0.18^{ab}
2	2	0.5	73.3±3.3^{ab}	8.7±0.41^{b}	5.7±0.57^{a}	3.4±0.28^{a}
2	2	1	83.3±3.3^{a}	9.7±0.53^{a}	6.8±0.44^{a}	3.6±0.35^{a}
4	4	1	73.3±3.3^{ab}	4.3±0.15^{cde}	2.9±0.24^{bcd}	2.1±0.24^{bcd}
2	2	2	70.0±5.8^{ab}	4.8±0.40^{cd}	3.0±0.20^{bcd}	2.5±0.32^{abc}
2	0	1	53.7±3.3^{bc}	3.3±0.31^{ef}	2.6±0.25^{bcd}	1.5±0.23^{cde}
4	0	2	56.3±3.3^{bc}	4.0±0.31^{de}	2.6±0.23^{cd}	1.4±0.17^{de}
0	2	2	40.0±5.7^{c}	1.9±0.11^{f}	1.6±0.10^{d}	1.0±0.18^{de}
0	4	2	40.0±5.7^{c}	2.1±0.17^{f}	1.8±0.15^{d}	0.8±0.08^{e}

*Initiation medium; data recorded after 5 weeks of culture
**Multiplication medium; data recorded after 8 weeks of culture.
Each treatment was replicated three times and each replicate consisted of 10 explants. Values represent the means ± SE.
Means having different letters as superscripts are significantly different from each other
(α = 0.05) according to Tukey's test

Plantlets with well-developed roots were taken out from the flasks and washed with sterile water to remove any traces of agar from the roots. These were dipped in 0.1% (w/v) fungicide solution (Bavistin) for 3-4 min and transferred to the potting mixture containing sand and garden soil in the ratio of 1:1. Ten replicates per treatment were taken and the experiment was repeated thrice. Data were analysed by analysis of variance (ANOVA) to detect significant differences between means, and the means were compared using Tukey's LSD test at 5% probability level.

Leaf-discs placed on the initiation medium started swelling within 5 weeks of culture and a mass of adventitious shoot-buds with callus originated from the leaf-discs (Fig. 1b, c). The explants cultured in basal MS medium failed to give any response and turned yellow subsequently. A maximum of 83.3% of explants formed adventitious shoot buds in medium supplemented with 2 μM TDZ, 2 μM BAP and 1 μM IBA which gave rise to an average of 9.7 shoot buds per explant after 8 weeks of culture on multiplication medium (Table 1). The presence of TDZ, BAP and IBA in the initiation medium significantly enhanced the formation of shoots bud at lower concentrations. However, higher concentration of cytokinins in the medium had an inhibitory effect on shoot bud initiation, reducing the number of shoots buds to 4.3 per explant. Such findings are similar to earlier reports on the effect of cytokinins on organogenesis in plants (7-9). The addition of BAP, TDZ and IBA in combination in the medium had pronounced effect on induction of adventitious shoot bud formation (4).

The initiated mass of adventitious shoot buds on transfer to multiplication medium containing BAP (3-9 μM) gave rise to multiple shoots. An average of 9.7 shoots per explant developed after 8 weeks of culture in the medium containing 3 μM of BAP (Fig. 1d; Table 1). In our study, BAP at a concentration higher than 3 μM had a negative effect on shoot bud regeneration (Table 1). However, the use of BAP for elongation and multiplication has been greatly emphasised by other workers (10-12).

Shoots measuring 1.5-2.0 cm were separated individually and transferred for rooting on MS medium supplemented with IBA and phloroglucinol. After 4 weeks of culture, 53.3% shoots formed roots without any intermediary callus phase, in medium containing 1.5 μM IBA (Fig. 1e). Addition of 200 μM of phloroglucinol along with IBA (1.5 μM) increased the frequency of rooting to 76.7% (Table 2). This enhancement of rooting could be due to the degradation of phloroglucinol to phlorizin which has been reported to improve root formation in woody species (13, 14). The plantlets with well-developed roots were transferred to the potting mixture containing sand and garden soil (1:1 ratio) in pots. Primary hardening took place in 6-8 weeks under high-humidity (80-100%) created

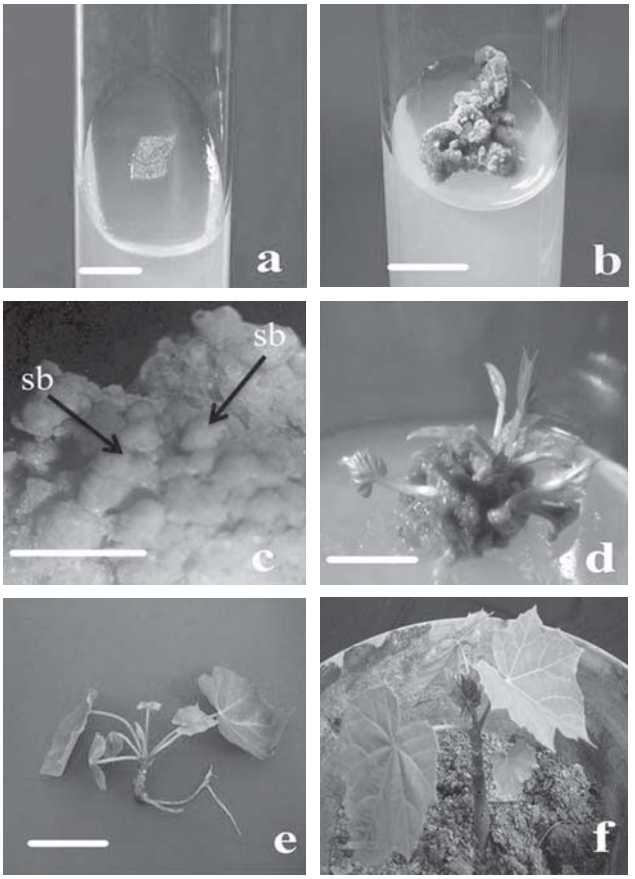

Fig. 1. *In vitro* plant regeneration of *Jatropha curcas* L from leaf explants. **(a)** Leaf explants from the donor plant (bar = 10 mm), **(b & c)** Adventitious shoot buds with callus in MS medium with 2µM (TDZ), 2µM (BAP) and 1µM (IBA) after 5 weeks of culture (sb, shoot bud) (bar = 10 mm), **(d)** Multiple shoot formation in MS medium with 3µM (BAP) after 8 weeks of culture (bar = 10 mm), **(e)** Initiation of roots in *in vitro* shoots in MS medium with 1.5 µM (IBA) + 200 µM (phloroglucinol) after 4 weeks of culture (bar = 10 mm), and **(f)** Hardened plant.

Table 2. Initiation of roots in *in vitro* regenerated shoots of *J. curcas* on MS medium supplemented with IBA and phloroglucinol

IBA (µM)	Phloroglucinol (µM)	Rooting (%)	Base callusing (%)
0	-	0.00	0.00
0.5	-	22.3±3.3	0.00
1	-	43.3±6.7	0.00
1.5	-	53.3±3.3	0.00
2	-	0.00	46.3±6.7
0	100	0.00	0.00
1.5	100	56.7±3.3	0.00
1.5	200	76.7±3.3	0.00

Data recorded after 4 weeks of culture.
Each treatment was replicated three times and each replicate consisted of 10 explants. Values represent the means ± SE.

by covering pots with polythene bags. About 70% of plants survived after 8 weeks of hardening. The well-acclimatized plants were finally transferred to the experimental garden with the percentage of survival of 70% (Fig. 1 f). The present protocol of multiplication of *J. curcas* from leaf explants will be more useful for production of large number of regenerated plants for mass propagation and establishment of this promising bio-fuel plant.

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