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ORIGINAL PAPER

Shoot regeneration from cotyledonary leaf explants of *Jatropha curcas*: a biodiesel plant

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Abstract A simple, high frequency, and reproducible method for plant regeneration through direct organogenesis from cotyledonary leaf explants of *Jatropha curcas* was developed using Murashige and Skoog (MS) medium supplemented with different concentrations of thidiazuron (TDZ) or 6-benzyl aminopurine (BAP). Medium containing TDZ has greater influence on regeneration as compared to BAP. The induced shoot buds were transferred to MS medium containing 10 μM kinetin (Kn), 4.5 μM BAP, and 5.5 μM α-naphthaleneacetic acid (NAA) for shoot proliferation. The proliferated shoots could be elongated on MS medium supplemented with different concentrations and combinations of BAP, indole-3-acetic acid (IAA), NAA, and indole-3-butyric acid (IBA). MS medium with 2.25 μM BAP and 8.5 μM IAA was found to be the best combination for shoot elongation. However, significant differences in plant regeneration and shoot elongation were observed among the genotypes studied. Rooting was achieved when the basal cut end of elongated shoots were dipped in half strength MS liquid medium containing different concentrations and combinations of IBA, IAA, and NAA for 4 days, followed by transfer to growth regulators free half strength MS medium supplemented 0.25 mg l^{-1} activated charcoal. Elongated shoot treated with 15 μM IBA, 5.7 μM IAA, and 11 μM NAA resulted in highest percent rooting. The rooted plants could be established in soil with more than 90% survival rate. The method developed may be useful in improvement of *J. curcas* through genetic modification.

Keywords Cotyledonary leaf · Genotype · *Jatropha curcas* · Regeneration

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Introduction

Energy is an important input for development. It aims at human welfare covering household, agriculture, transport, and industrial complexes. Like other natural resources, energy sources are also renewable as well as non-renewable. Non-renewable hydrocarbons are being used as major energy sources the world over. Besides, the threat of depleted reserves, excessive use of non-renewable resources can aggravate greenhouse gases, which are held responsible for global warming and ozone depletion. Biofuels and bioenergy encompass a wide range of alternative sources of energy of biological origin. These are pollution free, environmentally clean, and socially relevant. Many oil producing crops and plants have been considered for the purpose, among these *Jatropha curcas*, a member of Euphorbiaceae family with several attributes and considerable potential has evoked interest all over the tropics as a potential biofuel

plant (Openshaw 2000). Special interest has been shown in the cultivation of *J. curcas* as energy plantations because of its seed oil which could be easily converted into quality biodiesel (Ghosh et al. 2007), easy adaptability to semi-arid marginal lands and non-competitiveness with conventional crop for land (Francis et al. 2005).

Large-scale cultivation of *J. curcas* remains the single most important issue that will ultimately decide success. Low and unpredictable yields were reported from established plantations. Therefore, there is an urgent need for improvement of this species. Genetic engineering is a powerful tool to aid in the improvement of agronomic traits. Used with classical breeding methods, genetic engineering can accelerate the development of new cultivars with improved traits (Sharma et al. 2005; Lemaux 2008). Genetic engineering of plants relies on a tissue culture system to regenerate transformants. Despite the research efforts over the past few years in *J. curcas* tissue culture, no facile protocol of regeneration has been developed so far (Sujatha and Mukta 1996; Wei et al. 2004; Sujatha et al. 2005; Rajore and Batra 2007; Jha et al. 2007; Deore and Johnson 2008). It is also reported that regeneration in *J. curcas* is highly genotype dependent (da Camara Machado et al. 1997).

Keeping the economical importance of *J. curcas* and critical analysis of earlier reports, the objective of this study was to develop an in vitro plant regeneration method from cotyledonary leaf explants without intervening callus in *J. curcas* for subsequent use in genetic transformation. As far as we are aware, this is the first report on direct organogenesis from cotyledonary leaf explants of three genotypes. This study also compares the regeneration efficiency of in vitro and in vivo cotyledonary leaf explants.

Materials and methods

Plant material and source of explants

Seeds from three high yielding genotypes [CSMCRI-JC-1 (IC 5655713), CSMCRI-JC-2 (IC 5655716), and CSMCRI-JC-3 (IC 5655717)] of *J. curcas* were obtained from Central Salt and Marine Chemicals Research Institute (CSMCRI), experimental plantation established on wastelands at Chorvadla, India (21°75′N, 72°14′E) for the present study. Seed coats were removed and surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 15 min and rinsed five times in sterile distilled water. The sterilized decoated seeds were germinated on growth regulator-free MS liquid medium (Murashige and Skoog 1962) supported with filter paper boats. Two weeks after germination, cotyledonary leaf explants were collected from germinated seedlings and used as in vitro explants. For in vivo explants, seedlings were raised in the nursery, and cotyledonary leaf explants were collected from 2-week-old seedlings, and sterilized with 0.1% mercuric chloride ($HgCl_2$) for 4 min and rinsed five times in sterile distilled water and used as in vivo explants.

Shoot bud induction

The in vitro and in vivo cotyledonary leaf explants of all the three genotypes were cultured on MS medium supplemented with different concentrations of two cytokinins, i.e., thidiazuron (TDZ) or 6-benzyl aminopurine (BAP) to optimize their concentrations for direct organogenesis. Cotyledonary leaf explants were inoculated on medium surface in 200 × 38 mm culture tubes (Borosil, India). The percentage of explants with shoot bud induction and the number of shoot buds per explant were recorded after 6 weeks of culture.

Shoot proliferation and elongation of induced shoot buds

The induced shoot buds were transferred on MS medium supplemented with 10 μM kinetin (Kn), 4.5 μM BAP, and 5.5 μM α-naphthaleneacetic acid (NAA) for shoot proliferation (proliferation medium) (Reddy et al. 2008). Shoots were individually separated and the number of shoots per explant was recorded after 4 weeks. Individual shoots were further tested for elongation on MS medium supplemented with different concentrations and combinations of plant growth regulators (PGRs), i.e., BAP, indole-3-acetic acid (IAA), NAA, and indole-3-butyric acid (IBA). The length of elongated shoot was recorded after 6 weeks of culture.

Rooting and acclimatization

Green and healthy elongated shoots with 3–4 leaves were excised and cultured on half strength MS liquid medium supplemented with different concentrations and combinations of auxins, i.e., IBA, IAA, and NAA with the support of Whatman No. 44 filter paper for 4 days. These auxin-treated elongated shoots were transferred to growth regulator-free half strength MS solid medium supplemented with 0.25 mg l^{-1} activated charcoal. The percentage of shoots with root induction was recorded after 4 weeks. Rooted shoots were carefully taken out of the medium and washed thoroughly in sterilized distilled water to remove medium attached to the roots. The plants were transferred to plastic bags containing sterilized sand and soil in the ratio of 1:1 and wetted with 0.02% w/v carbendazim and covered with transparent plastic bags to maintain humidity.

After 3–4 weeks, the established plants were transplanted to polybags containing garden soil and farmyard manure and transferred to a greenhouse for further growth, and the percentage of surviving plants were recorded after 6–8 weeks.

Culture conditions and data analysis

Uniform culture conditions were applied in all experiments. The pH of the medium was adjusted to 5.7 using 1 N KOH or HCl, prior to autoclaving at 1.05 kg/cm^2 pressure at 121°C for 20 min. The cultures were maintained at 25 ± 2°C under a 16-h photoperiod with light intensity of 35–40 μmol m^{-2} s^{-1} (cool white fluorescent tubes).

All the experiments were set up in completely randomized design (CRD) and repeated three times with 25 replicates per treatment and one explant was cultured per test tube. Statistical difference among the means was analyzed by Duncan's multiple range test using the SPSS (version 7.5), and the results were expressed as the mean ± SE of three independent experiments. Data were also subjected to analysis of variance (ANOVA).

Results

Effect of cytokinins on direct organogenesis

Cytokinins in the medium significantly influenced shoot bud induction from cotyledonary leaf explants in all the genotypes studied. However, the regeneration efficiency was higher in TDZ containing medium as compared to BAP. On TDZ containing medium, regeneration efficiency varied from 23.6 to 93.4%, and the number of shoot buds per explant varied from 8.6 to 26.2, whereas on BAP containing medium, regeneration efficiency varied from 14.1 to 56.1%, and the number of shoot buds per explant varied from 2.8 to 6.9 (Tables 1, 2) among the genotypes studied. Of the different concentrations of TDZ tested, highest percentage of explants with shoot bud induction (93.4%), and highest number of shoot buds (26.2) per explant was obtained in the presence of 9.08 μM of TDZ (Tables 1, 2); however, further proliferation and elongation of shoot buds inhibited due to compact shoot bud induction. It was observed that 2.27 μM was found optimum for shoot bud induction and subsequent culture. At 2.27 μM TDZ, the percentage of explants with shoot bud induction varied from 79.6 to 87.1%, and the number of induced shoot buds per explant varied from 15.1 to 20.9 among the three genotypes. Of the different concentrations of BAP tested, highest percentage of explants with shoot bud induction (56.1%), and highest number of shoot buds (6.9) per explant was observed in the presence of 54 μM of BAP among the genotypes studied (Tables 1, 2).

Effect of explant sources on direct organogenesis

The sources of explant also influenced significantly the plant regeneration at tested concentration of cytokinins. In vitro explants responded more efficiently as compared to in vivo explants irrespective of genotypes studied. The percentage of explants with shoot bud induction varied from 27.4 to 93.4% in in vitro explants and 23.6–91.6% in in vivo explants (Table 1; Fig. 1a, b), and the number of induced shoot buds per explant varied from 8.6 to 26.2 in in vitro explants and 8.8–25 in in vivo explants (Table 2) at tested concentration of TDZ among the three genotypes. The percentage of explants with shoot bud induction varied from 22.1 to 56.1% in in vitro explants and 14.1–49.1% in in vivo explants (Table 1), and the number of induced shoot buds per explant varied from 3 to 6.9 in in vitro explants and 2.8–6.5 in in vivo explants (Table 2) at tested concentration of BAP among the three genotypes.

Effect of genotype on direct organogenesis

Significant differences in percent of explants with shoot bud induction and the number of induced shoot buds per explant was observed among the genotypes studied at tested concentration of cytokinins. CSMCRI-JC-2 performed best at tested concentrations of cytokinins both in percentage of explants with shoot buds induction and the number of shoot buds per explant followed by CSMCRI-JC-1. The percentage of explants with shoot bud induction in CSMCRI-JC-2 and CSMCRI-JC-1 varied from 19.1 to 93.4% and from 17.1 to 92.1%, respectively, and the number of induced shoot buds per explant varied from 3.9 to 26.2 and from 3.1 to 24.8, respectively at tested concentrations and combinations of PGRs. The percentage of explants with shoot bud induction in CSMCRI-JC-3 varied from 14.1 to 92.5%, and the number of induced shoot buds per explant varied from 2.8 to 23 at tested concentrations of cytokinins (Tables 1, 2).

Shoot proliferation and elongation of induced shoot buds

The transfer of induced shoot buds from 0.22 to 2.27 μM TDZ containing medium to proliferation medium resulted in 5 ± 0.5 to 13 ± 2.8 shoots per explant, whereas higher concentration of TDZ (4.54–9.08 μM) resulted in 3.5 ± 1.2 to 6.5 ± 1.8 shoots; however, 2 ± 0.1 to 6 ± 0.9 shoots produced when transfer of induced shoot buds from 13.5 to 54 μM BAP containing medium to proliferation medium in all genotypes studied (Fig. 1c).

Table 1 Effect of different concentrations of cytokinins [thidiazuron (TDZ) or 6-benzyl aminopurine (BAP)], source [in vitro or in vivo], and genotype on the percentage of explants with shoot bud induction from cotyledonary leaf explants of three genotypes of *J. curcas* after 6 weeks

Cytokinins		Explants with shoot bud induction (%)					
		In vitro			In vivo		
TDZ (μM)	BAP (μM)	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.22	0	27.4 ± 1.5^{a}	52.4 ± 3.8^{c}	36.7 ± 2.0^{c}	23.6 ± 1.0^{a}	50.4 ± 2.0^{c}	35.3 ± 1.4^{b}
0.45	0	57.2 ± 3.0^{d}	70.6 ± 4.3^{d}	44.3 ± 2.6^{d}	52.6 ± 2.1^{c}	69.1 ± 2.6^{d}	43.9 ± 1.4^{c}
0.90	0	62.4 ± 2.6^{d}	74.5 ± 4.3^{d}	71.6 ± 2.3^{e}	61.1 ± 2.5^{c}	73.6 ± 3.2^{d}	69.1 ± 2.8^{d}
2.27	0	85.4 ± 4.7^{e}	87.1 ± 4.6^{e}	81.3 ± 4.9^{f}	79.6 ± 3.0^{d}	86.1 ± 4.1^{e}	80 ± 3.5^{e}
4.54	0	91.7 ± 4.9^{e}	91.5 ± 2.6^{e}	90 ± 5.5^{g}	81.2 ± 3.4^{d}	90.9 ± 4.2^{e}	89 ± 4.0^{e}
9.08	0	92.1 ± 3.9^{c}	93.4 ± 3.6^{e}	92.5 ± 3.3^{g}	83 ± 3.3^{d}	91.1 ± 4.1^{e}	91.6 ± 3.9^{e}
0	13.5	26.3 ± 1.8^{a}	28.1 ± 1.7^{a}	22.1 ± 1.4^{a}	17.1 ± 1.5^{a}	19.1 ± 1.1^{a}	14.1 ± 0.8^{a}
0	27.0	32.1 ± 1.6^{b}	36.1 ± 2.1^{b}	29.6 ± 2.2^{b}	23 ± 1.7^{a}	29.1 ± 1.6^{b}	17.3 ± 1.0^{a}
0	40.5	41 ± 2.1^{c}	42.9 ± 2.2^{b}	43.1 ± 1.8^{d}	40.1 ± 2.1^{b}	44 ± 2.3^{c}	36 ± 2.2^{b}
0	54.0	45.1 ± 2.6^{c}	56.1 ± 2.7^{c}	50 ± 2.8^{d}	42.5 ± 2.6^{b}	49.1 ± 2.4^{c}	44.6 ± 2.5^{c}

Values represent mean ± SE of 25 explants per treatment in three repeated experiments; mean in each column followed by same letters are not significantly different according to DMRT at $\alpha = 0.05$

Table 2 Effect of different concentrations of cytokinins [thidiazuron (TDZ) or 6-benzyl aminopurine (BAP)], source [in vitro or in vivo], and genotype on the number of shoot buds induction per cotyledonary leaf explant of three genotypes of *J. curcas* after 6 weeks

Cytikinins		Number of shoot buds per cotyledonary leaf explant					
		In vitro			In vivo		
TDZ (μM)	BAP (μM)	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
0.22	0	8.6 ± 0.9^{c}	12.0 ± 0.9^{c}	9.1 ± 0.6^{c}	8.8 ± 1.2^{c}	12.4 ± 0.9^{c}	9.6 ± 1.2^{b}
0.45	0	11.4 ± 1.0^{d}	15.0 ± 0.8^{d}	13 ± 1.0^{d}	11.1 ± 1.3^{c}	13.1 ± 0.9^{c}	13.7 ± 0.7^{c}
0.90	0	17.6 ± 1.1^{e}	19.0 ± 0.8^{e}	14 ± 1.0^{d}	17.9 ± 1.3^{d}	19.1 ± 1.1^{d}	14.9 ± 0.7^{c}
2.27	0	20.9 ± 1.2^{e}	20.1 ± 4.4^{e}	15.1 ± 1.1^{d}	20.1 ± 1.2^{d}	20.1 ± 1.5^{d}	15.3 ± 0.8^{c}
4.54	0	22.7 ± 1.7^{e}	22.1 ± 1.4^{e}	18.6 ± 1.3^{e}	22.6 ± 1.5^{d}	22.3 ± 1.4^{de}	18.6 ± 1.0^{d}
9.08	0	24.8 ± 1.7^{c}	26.2 ± 2.1^{f}	23 ± 1.2^{f}	22.5 ± 1.5^{d}	25 ± 1.6^{e}	21.9 ± 1.1^{e}
0	13.5	3.6 ± 0.5^{a}	4.7 ± 0.6^{a}	3 ± 0.5^{a}	3.1 ± 0.3^{a}	3.9 ± 0.3^{a}	2.8 ± 0.4^{a}
0	27.0	3.9 ± 0.5^{a}	5.0 ± 0.6^{a}	4 ± 0.5^{b}	4.7 ± 0.3^{ab}	4.5 ± 0.5^{ab}	3.6 ± 0.5^{a}
0	40.5	5.5 ± 0.6^{b}	5.1 ± 1.0^{a}	5.1 ± 0.6^{ab}	4.9 ± 0.5^{ab}	4.9 ± 0.6^{ab}	4.9 ± 0.6^{a}
0	54.0	6.9 ± 1.0^{b}	6.9 ± 1.1^{a}	5.7 ± 0.9^{b}	4.9 ± 0.5^{b}	6.5 ± 1.0^{b}	4.9 ± 1.0^{a}

Values represent mean ± SE of 25 explants per treatment in three repeated experiments; mean in each column followed by same letters are not significantly different according to DMRT at $\alpha = 0.05$

Individual (0.3–0.5 cm) shoots were separated from the clump of proliferated shoots and transferred to medium containing different concentrations and combinations of PGRs, i.e., BAP, IAA, NAA, and IBA for elongation (Table 3). Significant differences in elongation were observed at different concentrations and combinations of PGRs. BAP and IAA combination was found best in all the genotypes studied. The best elongation (3.3–3.6 cm) was observed on medium containing 2.25 μM BAP and 8.5 μM IAA (Fig. 1d). The elongation ranged from 1.9 to 3.6 cm on medium containing BAP and IAA combinations (Table 3).The elongation ranged from 2.1 to 2.5 cm on medium containing BAP and IBA combinations and 4.5 μM BAP and 7.5 μM IBA gave the best elongation (2.3–2.5 cm). The BAP and NAA combinations give the least elongation and the elongation ranged from 1 to 1.9 cm. The best elongation was observed in CSMCRI-JC-1 genotype (1.1–3.6 cm) followed by CSMCRI-JC-2 (1–3.5 cm). The least elongation was observed in CSMCRI-JC-3 genotype (1.1–3.3 cm) (Table 3).

Rooting and acclimatization

Percentage of rooting significantly differed depending upon the concentrations and combinations of IBA, IAA, and NAA. Rooting increased with the increase in concentration

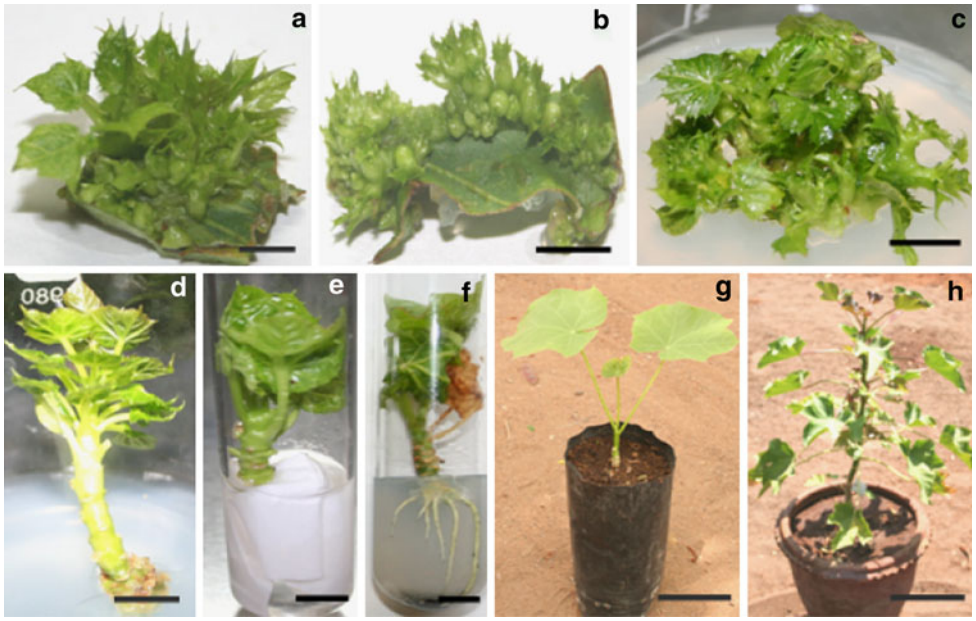

Fig. 1 Shoot regeneration from cotyledonary leaf explants of *J. curcas*. Direct organogenesis from **a** in vitro cotyledonary leaf explant (*bar* 5 mm), **b** in vivo cotyledonary leaf explant (*bar* 5 mm) on MS medium with 2.27 μM thidiazuron (TDZ) after 6 weeks. **c** Shoot proliferation of induced shoot buds on MS medium with 10 μM kinetin (Kn) + 4.5 μM 6-benzyl aminopurine (BAP) + 5.5 μM α-naphthaleneacetic acid (NAA) after 4 weeks (*bar* 100 mm). **d** Elongation of proliferated shoot on MS medium with 2.25 μM BAP + 8.5 μM indole-3-acetic acid (IAA) after 6 weeks (*bar* 5 mm). **e** Elongated shoot cultured on half strength basal MS liquid medium supplemented with 15 μM indole-3-butyric acid (IBA) + 5.7 μM IAA + 5.5 μM NAA for root induction (*bar* 5 mm). **f** Development of roots at the base of auxins treated elongated shoot on half strength basal MS medium with 0.25 mg/L activated charcoal after 4 weeks (*bar* 1 mm). **g** Regenerated plant in polybag after 4 weeks (*bar* 150 mm). **h** Regenerated plant in pot soil after 6 months under natural condition (*bar* 100 mm)

of IBA, and inclusion of IAA and NAA further increased the percentage of rooting (Table 4). The best rooting percent (49.8–51.9%) were observed when elongated shoots were incubated with half strength MS liquid medium containing 15 μM IBA, 5.7 μM IAA, and 5.5 μM NAA for 4 days, followed by transfer to growth regulator-free half strength MS solid medium supplemented with 0.25 mg l^{-1} activated charcoal among the genotypes studied (Fig. 1e, f). After 6–8 weeks, more than 90% of plants survived. No morphological abnormalities were observed in regenerated plants (Fig. 1g, h).

Discussion

Direct plant regeneration is the useful means of production of plantlets with a lower risk of genetic variability than by other routes. Optimum concentration of cytokinins for direct shoot bud induction irrespective of genotypes of *J. curcas* was determined using cotyledonary leaf explants. This is the first report on direct organogenesis from cotyledonary leaf explants and could be achieved on MS medium containing TDZ or BAP individually. However, in the present study, concentrations and type of cytokinins in the medium and source of explants and genotypes significantly influenced explants response on direct organogenesis. The cytokinins play an important key role in plant morphogenesis. Huetteman and Preece (1993) have reported that TDZ is a potent cytokinin for woody plant tissue culture. Responses of explants forming shoot bud increased with increase in the concentration of TDZ. Similar observations were made in *Alstromeria* species (Lin et al. 1997), *Camellia sinensis* (Mondal et al. 1998), *Solanum melongena* (Magioli et al. 1998), *Hagenia abyssinica* (Feyissa et al. 2005), *Embellia ribes* (Raghu et al. 2006) and *J. curcas* (Deore and Johnson 2008). Such response may be due to increase in the level of endogenous cytokinins by the effect of growth regulator used. Hare and Van Staden (1994) reported that TDZ has the capacity to inhibit the action of cytokinin oxidase, which in turns may increase the level of endogenous cytokinins. In the present investigation, higher than 2.27 μM TDZ induced high frequency compact shoot buds and could not elongate. Lower than 2.27 μM TDZ induced relatively fewer shoot buds and developed into harvestable shoots in subsequent culture. Nielsen et al. (1993) in *Miscanthus sinensis* and Hyde and Phillips (1996) in *Capsicum annuum* also observed similar results. In the present study, it was observed that TDZ was more effective than BAP. Similar observations were reported in *Camellia sinensis* (Mondal

Table 3 Effect of different concentration and combinations of plant growth regulators (PGRs) on elongation of proliferated shoots from cotyledonary leaf explants of three genotypes of *J. curcas* after 6 weeks

PGRs (μM)				Shoot length (cm)		
BAP	IAA	NAA	IBA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
2.25	2.8	0	0	3 ± 0.4^{ab}	2.9 ± 0.4^{b}	2.9 ± 0.6^{a}
4.5	2.8	0	0	2.7 ± 0.2^{b}	2.2 ± 0.4^{c}	1.9 ± 0.1^{d}
2.25	8.5	0	0	3.6 ± 0.2^{a}	3.5 ± 0.7^{a}	3.3 ± 0.9^{a}
4.5	8.5	0	0	2.9 ± 04^{b}	2.8 ± 0.4^{bc}	2.8 ± 0.6^{a}
2.25	0	2.75	0	1.1 ± 0.1^{e}	1.5 ± 0.5^{f}	1.9 ± 0.1^{d}
4.5	0	2.75	0	1.7 ± 0.2^{d}	1.4 ± 0.2^{f}	1.3 ± 0.1^{de}
2.25	0	8.25	0	1.9 ± 0.3^{d}	1.5 ± 0.4^{ef}	1.2 ± 0.2^{e}
4.5	0	8.25	0	1.1 ± 0.07^{e}	1 ± 0.1^{f}	1.3 ± 0.1^{e}
2.25	0	0	2.5	2.1 ± 0.4^{cd}	2.2 ± 0.3^{cd}	2.3 ± 0.2^{bc}
4.5	0	0	2.5	2.4 ± 0.4^{bc}	2.1 ± 0.4^{de}	2.2 ± 0.1^{cd}
2.25	0	0	7.5	2.2 ± 0.3^{c}	2.4 ± 0.3^{cd}	2.2 ± 0.2^{b}
4.5	0	0	7.5	2.3 ± 0.5^{d}	2.4 ± 0.4^{d}	2.5 ± 0.4^{ab}

Values represent mean ± SE of 25 proliferated shoots per treatment in three independent experiments. Mean in each column followed by same letters are not significantly different according to DMRT at $\alpha = 0.05$

BAP 6-benzylaminopurine; *NAA* α-naphthaleneacetic acid; *IAA* indole 3-acetic acid; *IBA* indole-3-butyric acid

Table 4 The percentage of shoots with root induction on growth regulator-free half strength MS medium after 4 days treatment of elongated shoots on various concentration and combination of auxins in half strength MS liquid medium of three genotypes of *J. curcas* after 4 weeks

Auxins (μM)			Shoots with root induction (%)		
IBA	IAA	NAA	CSMCRI-JC-1	CSMCRI-JC-2	CSMCRI-JC-3
5	0	0	10.4 ± 1.0^{a}	11.8 ± 0.6^{a}	10.5 ± 0.5^{a}
10	0	0	12.7 ± 0.8^{a}	12.1 ± 0.8^{a}	13 ± 0.5^{a}
15	0	0	24.6 ± 0.9^{b}	23.8 ± 1.2^{b}	23.9 ± 0.8^{b}
5	5.7	5.5	21.7 ± 1.0^{b}	22.8 ± 1.2^{b}	22 ± 0.9^{b}
10	5.7	5.5	39.3 ± 1.7^{c}	40.5 ± 1.7^{c}	40 ± 0.9^{c}
15	5.7	5.5	51.9 ± 2.1^{e}	49.8 ± 2.3^{e}	51.8 ± 0.9^{e}
15	11.4	5.5	46.9 ± 2.3^{de}	46.8 ± 1.7^{de}	45.9 ± 0.8^{d}
15	17.2	5.5	43.7 ± 2.2^{cd}	42.9 ± 1.7^{cd}	42.7 ± 0.8^{cd}
15	5.7	11	41.8 ± 2.2^{cd}	40.8 ± 1.6^{c}	40.9 ± 0.8^{cd}
15	5.7	16.5	41.7 ± 1.7^{cd}	41.8 ± 2.3^{cd}	42.8 ± 0.8^{cd}

Values represent mean ± SE of 25 elongated shoots per treatment in three independent experiments. Mean in each column followed by same letters are not significantly different according to DMRT at $\alpha = 0.05$

NAA α-naphthaleneacetic acid; *IAA* indole 3-acetic acid; *IBA* indole-3-butyric acid

et al. 1998), *Phellodendron amurense* (Azad et al. 2005), and *J. curcas* (Deore and Johnson 2008). Variation in the activity of different cytokinins can be explained by their differential uptake (Blakesey 1991), varied translocation rates to meristematic regions and metabolic processes (Kaminek 1992). Regeneration efficiency was also affected by source of explants. In vitro explants have higher rate and the number of shoot buds as compared to in vivo explants irrespective of genotype studied. This may be due to difference in endogenous growth regulators concentration and their metabolism. Similar results were observed in *Echinacea purpurea* (Guda et al. 2003), *Tomentosa steud* (Ozaslan et al. 2005), and *J. curcas* (Reddy et al. 2008). Genotype is one of the most important factors affecting regeneration (Feyissa et al. 2005). In our study, all the three genotypes showed differences in percentage of explants with shoot bud induction, the number of shoot buds per explant and elongation of regenerated shoot buds. Similar results were reported in *Morus alba* (Chitra and Padmaja 2005) and *Hagenia abyssinica* (Feyissa et al. 2005). Genotypic effect on shoot regeneration and elongation has been described in many species, and could be due, in part, to differences in the levels of endogenous growth regulators, particularly cytokinins levels during the induction period, although, the precise mechanism remains unclear (Pellegrineschi 1997; Schween and Schwenkel 2003). Henry et al. (1994) reported that varietal differences with respect to embryogenesis and regeneration results from quantitative or qualitative genetic differences. The inhibitory effect of high concentration of TDZ on shoot elongation has been reported and recommends that TDZ concentration must be reduced and/or other cytokinins or combinations of cytokinins and auxins must be used for further shoot elongation (Preece and Imel 1991; Feyissa et al. 2005; Raghu et al. 2006). The present results are in agreement with above findings. Therefore, the clump of induced shoot buds were transferred to a medium containing 10 μM Kn, 4.5 μM BAP, and 5.5 μM NAA for shoot proliferation, and proliferated shoots were separated and transferred to elongation medium. The elongation of individual shoots depended upon the concentrations and combinations of PGRs in the medium. The maximum elongation was obtained in BAP and IAA combinations as compared to BAP and IBA, and BAP and NAA combinations. The best elongation was obtained in medium containing 2.25 μM BAP and 8.5 μM IAA. Our results are consistent with earlier reports (Christopher and Rajam 1996; Venkataiah and Subhash 2003). Elongation was low in the medium containing BAP and IBA, may be due to proliferation of axillary buds. Similar observation was reported in *Eupatorium triplinerve* (Martin 2003). The least elongation observed in a medium containing BAP and NAA may be due to the profuse callusing at the basal end of proliferated shoots (Kumar et al. 2008; Koroch et al. 2002). The percentage of rooting was directly proportional to the concentration of IBA and a higher percentage was observed in combinations of auxins as compared to IBA alone. Similar observation has been reported in

Simmondsia chinensis (Singh et al. 2008). It is well reported that auxins are a potent hormones for rooting (Vuylasteker et al. 1998; Nandagopal and Ranjitha Kumari 2007). The acclimatization of the rooted shoots was accomplished and more than 90% of the plants were successfully transferred to polybags in greenhouse conditions.

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References

Azad MAK, Yokota S, Ohkubo T, Andoh Y, Yahara S, Yoshizawa N (2005) In vitro regeneration of the medicinal woody plant *Phellodendron amurense* Rupr. through excised leaves. Plant Cell Tiss Organ Cult 80:43–50

Blakesey D (1991) Uptake and metabolosm of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. Plant Cell Tiss Organ Cult 25:69–74

Chitra DS, Padmaja G (2005) Shoot regeneration via direct organogenesis from in vitro derived leaves of mulberry using thidiazuron and 6-benzylaminopurine. Sci Hortic 106:593–602

Christopher T, Rajam MV (1996) Effect of genotype, explant and medium on in vitro regeneration of red pepper. Plant Cell Tiss Organ Cult 46:245–250

da Camara Machado A, Frick NS, Kremen R, Katinger H, da Camara Machado ML (1997) Biotechnological approaches to the improvement of Jatropha curcas. In: Proceedings of the international symposium on jatropha, Nicaragua

Deore AC, Johnson TS (2008) High-frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L.: an important biodiesel plant. Plant Biotech Rep 2:10–15

Feyissa T, Welander M, Negash L (2005) In vitro regeneration of *Hagenia abyssinica* (Bruce) J.F. Gmel. (Rosaceae) from leaf explants. Plant Cell Rep 24:392–400

Francis G, Edingger R, Becker K (2005) A concept for simultaneous wasteland reclamation, fuel production, and socio-economic development in degraded areas in India. Need, potential and perspectives of Jatropha plantations. Nat Res Forum 29:12–24

Ghosh A, Chaudhary DR, Reddy MP, Rao SN, Chikara J, Pandya JB, Patolia JS, Gandhi MR, Adimurthy S, Vaghela N, Mishra S, Rathod MR, Prakash AR, Shethia BD, Upadhyay SC, Balakrishna V, Prakash CR, Ghosh PK (2007) Prospects for *Jatropha* methyl ester (biodiesel) in India. Int J Environ Stud 64:659–674

Guda CD, Castello S, Savona M, Farina E (2003) *Echinacea purpurea*—in vivo and in vitro propagation and field evaluation of ornamental clones. Col Prot 32:101–107

Hare PD, Van Staden J (1994) Inhibitory effect of thidiazuron on the activity of cytokinin oxidase isolated from soybean callus. Plant Cell Physiol 35:1121–1125

Henry Y, Vain P, Buyser JD (1994) Genetic analysis of in vitro plant tissue culture responses and regeneration capacities. Euphytica 79:45–58

Huetteman CA, Preece JE (1993) Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss Organ Cul 33:105–119

Hyde CL, Phillips GC (1996) Silver nitrate promotes shoot development and plant regeneration of Chilepepper (*Capsicum annuum* L.) via organogenesis. In Vitro Cell Dev Biol Plant 32:72–80

Jha T, Mukherjee P, Datta MM (2007) Somatic embryogenesis in *Jatropha curcas* Linn., an important biofuel plant. Plant Biotech Rep 1:135–140

Kaminek M (1992) Progress in cytokinin research. Trends Biotech 10:159–162

Koroch A, Juliani HR, Kapteyn J, Simon JE (2002) In vitro regeneration of *Echinacea purpurea* from leaf explants. Plant Cell Tiss Organ Cult 69:79–83

Kumar N, Pamidimarri SDVN, Kaur M, Boricha G, Reddy MP (2008) Effects of NaCl on growth, ion accumulation, protein, proline contents, and antioxidant enzymes activity in callus cultures of *Jatropha curcas*. Biologia 63:378–382

Lemaux PG (2008) Genetically engineered plants and foods: a scientist's analysis of the issues (Part I). Annu Rev Plant Biol 59:771–812

Lin HS, De Jeu MJ, Jacobsen E (1997) Direct shoot regeneration from excised leaf explants of in vitro grown seedlings of *Alstroemeria* L. Plant Cell Rep 16:770–774

Magioli C, Rocha APM, de Oliveira DE, Mansur E (1998) Efficient shoot organogenesis of eggplant (*Solanum melongena* L.) induced by thidiazuron. Plant Cell Rep 17:661–663

Martin KP (2003) Rapid axillary bud proliferation and ex vitro rooting of *Eupatorium triplinerve*. Biol Plant 47:589–591

Mondal TK, Bhattacharya A, Sood A, Ahuja PS (1998) Micropropagation of tea (*Camellia sinensis* (L.) O. Kuntze) using thidiazuron. Plant Growth Regul 26:57–61

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–479

Nandagopal S, Ranjitha Kumari BD (2007) Effectiveness of auxin induced in vitro root culture in chicory. J Cen Eur Agri 8:73–79

Nielsen JM, Kirsten B, Hansen J (1993) Long-term effects of thidiazuron are intermediate between benzyladenine, kinetin or isopentenyladenine in *Miscanthus sinensis*. Plant Cell Tiss Organ Cult 35:173–179

Openshaw K (2000) A reviews of *Jatropha curcas*: an oil plant of unfulfilled promise. Biomass Bioeng 19:1–5

Ozaslan M, Can C, Aytekin T (2005) Effect of explant source on in vitro propagation of *Paulownia tomentosa* Steud. Biotech Biotech Equ 19:20–26

Pellegrineschi A (1997) In vitro plant regeneration via organogenesis of cowpea [*Vigna unguiculata* (L.) Walp.]. Plant Cell Rep 17:89–95

Preece JE, Imel MR (1991) Plant regeneration from leaf explants of Rhododendron 'P.J.M. Hybrids'. Sci Hortic 48:159–170

Raghu AV, Geetha SP, Martin G, Balachandran I, Ravindran PN (2006) Direct organogenesis from leaf explants of *Embelia ribes* Burm.—a vulnerable medicinal plant. J For Res 11:57–60

Rajore S, Batra A (2007) An alternative source for regenerable organogenic callus induction in *Jatropha curcas*. Ind J Biotech 6:545–548

Reddy MP, Kumar N, Vijay Anand KG, Singh AH, Singh S (2008) Method for micropropagation of *Jatropha curcas* plants from leaf explants (Patent filed US and PCT, File No. 2537de2008).

Schween G, Schwenkel HG (2003) Effect of genotype on callus induction, shoot regeneration, and phenotypic stability of regenerated plants in greenhouse of *Primula* ssp. Plant Cell Tiss Organ Cult 72:53–61

Sharma KK, Bhatnagar-Mathur P, Thorpe TA (2005) Genetic transformation technology: status and problems. In Vitro Cell Dev Biol Plant 41:102–112

Singh A, Reddy MP, Patolia JS (2008) An improved protocol for micropropagation of elite genotypes of *Simmondsia chinensis* (Link) Schneider. Biol Plant 52:538–540

Sujatha M, Mukta N (1996) Morphogenesis and plant regeneration from tissue cultures of *Jatropha curcas*. Plant Cell Tissue Organ Cult 44:135–141

Sujatha M, Makkar HPS, Becker K (2005) Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Growth Regul 47:83–90

Venkataiah P, Subhash K (2003) Thidiazuron-induced adventitious shoot bud formation and plant regeneration in *Capsicum annuum* L. J Plant Biotech 5:245–250

Vuylasteker C, Dewaele S, Rambour S (1998) Auxin induced lateral root formation in chicory. Ann Bot 81:449–454

Wei Q, Wei-Da L, Liao Y, Shu-Lin P, Xu Y, Tang L, Fang C (2004) Plant regeneration from epicotyl explants of *Jatropha curcas*. J Plant Physiol Mol Biol 30:475–478