Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 1777-1780
Received: 22-01-2018
Accepted: 25-02-2018

Sweta Mishra
Biotechnology section, Central Instrumentation Laboratory, SDAU, S.K. Nagar
Dist. Banaskantha, Gujarat, India

Correspondence
Sweta Mishra
Biotechnology section, Central Instrumentation Laboratory, SDAU, S. K. Nagar
Dist. Banaskantha, Gujarat, India

In-vitro direct shoot organogenesis in *Jatropha curcas* L.

Sweta Mishra

Abstract
An efficient and reproducible plant regeneration protocol was developed from axillary bud explants derived from physiologically mature trees of *Jatropha curcas* L., a promising tree for production of biodiesel. Direct organogenesis yields true to type plantlets. PVP (1.0 g/l), ascorbic acid (150 mg/l) and citric acid (50 mg/l) was used to effectively counter the phenolics released from the explants of the mature tree. The additives like adenine sulphate (25 mg/l) and glutamine (50 mg/l) helped to enhance the growth of *Jatropha curcas* explants. 91.65 ± 0.31 % shoot induction was observed in the axillary bud explants on MS medium supplemented with BA (2.00 mg/l) + Kinetin (1.00 mg/l) + GA_3 (0.10 mg/l). The maximum number of shoots (10.24±0.07) was observed in the MS + BA (2.00 mg/l) + Kinetin (1.00 mg/l) + GA_3 (0.05 mg/l). Maximum shoot length of 07.21 ± 0.25 was obtained on the MS medium supplemented with BA (2.00 mg/l) + Kinetin (0.50 mg/l) + GA_3 (0.10 mg/l). Profuse rooting of the regenerated shoots were obtained from the on the MS medium supplemented with IBA (1.00 mg/l) + NAA (1.00 mg/l) having a root induction response of 89.67±0.23 %. The highest number of roots (06.46±0.28) was obtained on MS + IBA (1.50 mg/l) + NAA (0.10 mg/l) having root length (04.53±0.25 cm). The survival percentage of the plants in the field after acclimatization was 80.0%.

Keywords: *Jatropha curcas*, axillary bud, adenine sulphate, acclimatization survival

Introduction
Jatropha curcas L. belonging to Euphorbiaceae falimy is one of the most preferred tree bearing oil species that has been exploited for biodiesel production in tropical and subtropical regions. In order to meet huge biodiesel production requirement a large amount of the plant biomass of high quality need to be produced. *Jatropha* is normally propagated by seeds or by plant cuttings. However the plants produced from seeds are heterogeneous in nature and those developed from the plant cuttings develop shallow roots that render them prone to lodging in the poor and marginal soils. Plant tissue culture is the best suited technique for mass multiplication of disease-free clonal and homogenous plants of good quality and high production potential. Jatropha plant tissue culture has been attempted from varied explants like hypocotyls, leaves, petioles and cotyledons (Leela *et al*., 2011; Sujatha and Mukta 1996, Jyothi *et al*. 2000, Wei *et al*. 2004, Rajore and Batra 2005, Sujatha *et al*. 2005, Nannapat *et al*. 2006, Datta *et al*. 2007) [4, 14, 2, 17, 10, 14, 7, 1]. However, none of the protocols produced were economically viable. The present study was carried out to explore the culture conditions affecting direct organogenesis from axillary bud explants focusing on the most efficient hormonal concentration in combination with adenine sulfate, glutamine and others additives, to enhance the growth and development of *Jatropha curcas* and produce a large number of sturdy regenerated plantlets.

Material and Methods
Plant Material
Axillary buds of *Jatropha curcas* cv. SKJN 1, were collected from 4-5 year old trees grown in the Centre for Crop Improvement, SDAU, S.K.Nagar, Gujarat, India.

Collection of Explants and Sterilization
The axillary buds collected were washed in running water and were rinsed with distilled water 2-3 times in the laboratory. Axillary buds, used as explants were excised and prepared for culture in the laminar air flow hood. The explants were treated with 4.00% sodium hypochlorite for 2 minutes followed by treatment for 7 minutes with 0.1% (w/v) mercuric chloride adding 2 drops of tween-20 per 100 ml solution. Finally, the explants were rinsed 4-5 times with sterile distilled water.

Shoot bud induction and proliferation

Murashige and Skoog (MS) [6] was used along with various concentrations of plant growth regulators like 6-benzyl adenine (BA) (1.50-2.50 mg/l), kinetin (KIN) (0.50-3.00 mg/l) and GA_3 (0.05-0.10 mg/l) in combination for shoot bud induction along with adenine sulphate (25 mg/l), glutamine (50 mg/l), ascorbic acid (150 mg/l), citric acid (50 mg/l), PVP (1.0 g /l), sucrose (40 g/l), agar (7g/l).

***In-vitro* rooting**

The elongated shoots were subcultured on MS medium supplemented with IBA (0.50-2.00 mg/l) and NAA, (0.10-1.00 mg/l) for rooting.

Culture Conditions

The cultures were incubated in culture room maintained at 26±2° C, under a 16 h photoperiod with a light intensity of 35 μ m^{-2} s^{-1} from Philips cool white fluorescent tubes with 100% relative humidity.

Acclimatization and Hardening

In vitro raised plantlets with well developed roots were removed from the culture medium and roots were washed thoroughly under tap water. Plantlets were transferred to plastic cups containing potting-mixture of sterilized sand and soil (1:1:1). The plantlets were maintained inside the culture room covering them with polythene cover to maintain the relative humidity. The plantlets were watered with Hogland solution devoid of sucrose and myo-inositol supplemented with 1mg/l IBA at three days interval for a period of 15 days. The regenerated plantlets were shifted to polyhouse, the polythene cover was removed and the plantlets were transferred to big polythene bags containing soil and kept under shade for another 2 months before transferring to the field.

Statistical Analysis

A minimum of 40 cultures were taken for each treatment and all the experiments were repeated thrice. The cultures were examined periodically and the morphological changes were recorded on the basis of visual observations. All experiments were performed in triplicate and were submitted to the Student's t-test which was used to indicate significant differences ($p < 0.001$ or $p < 0.05$). Data represent means ± standard error for three replicates.

Results and Discussions

Aseptic cultures were established by treating with 4.00% sodium hypochlorite for 2 minutes followed by treatment for 7 minutes with 0.1% (w/v) mercuric chloride adding 2 drops of tween-20 per 100 ml solution. Finally, the explants were rinsed 4-5 times with sterile distilled water. As the explants are collected from the physiologically mature tree of 4-5 yeas age, the axillary buds produce a large amount of phenolic substances from their cut ends which result in browning of the culture media and the explants that become unresponsive under *In vitro* conditions. This condition of browning of the cultures was effectively controlled by adding polyvinyl pyrrolidone (PVP) (1.0 g/l), ascorbic acid (150 mg/l) and citric acid (50 mg/l) in the culture medium. Although plant regeneration was reported previously (Sujatha and Mukta 1996, Jyothi *et al*. 2000, Wei *et al*. 2004, Rajore and Batra 2005, Sujatha *et al*. 2005, Nannapat *et al*. 2006, Datta *et al*. 2007) [13, 2, 17, 10, 14, 7, 1], an attempt was made to achieve a higher frequency of regeneration from direct organogenesis.

Shoot Bud Induction and Proliferation

Murashige and Skoog (MS) [6] was used along with various concentrations of plant growth regulators like 6-benzyl adenine (BA) (1.50-2.50 mg/l), kinetin (KIN) (0.50-3.00 mg/l) and GA_3 (0.05-0.10 mg/l) in combination for shoot bud induction along with adenine sulphate (25 mg/l), glutamine (50 mg/l), ascorbic acid (150 mg/l), citric acid (50 mg/l), PVP (1.0 g /l), sucrose (40 g/l), agar (7g/l). The various concentrations of plant growth regulators were found to affect the *In vitro* shoot regeneration frequency in *Jatropha curcas*. Out of the twenty-four combinations of plant growth regulators in the media tested, the axillary bud explants showed the highest shoot induction frequency of 91.65±0.31 per cent observed on MS medium supplemented with 2.00 mg/l BA + 1.00 mg/l Kinetin + 0.10 mg/l GA_3 followed by 87.29±0.23 observed in MS medium supplemented with 2.00 mg/l BA + 1.00 mg/l Kinetin + 0.05 mg/l GA3. Among the cytokinins, BA is the best to stimulate the buds regeneration and proliferation from nodal explants of *J. curcas* (Datta *et al*., 2007) [1]. On increasing or decreasing the concentration of BA and Kinetin, the shoot induction frequency was found to reduce. The use of auxins was avoided in the culture media as it may have resulted in callus induction which generates variability in the cultures and the plantlets developed thereof, thus, affecting the clonal fidelity of the tissue culture raised plantlets. (Table 1, Fig 1A-F).

The choice of explant is a critical factor that determines the success of most tissue culture experiments. The axillary buds have a high totipotency to regenerate into plantlets and to produce multiple shoots in the artificial media combinations under controlled culture conditions. In the tree species the axillary bud explants are the most amenable to culture conditions and have always yielded good results. In the present study, the axillary bud explants produced 10.24±0.07 shoots per explant on an average on the MS medium supplemented with 2.00 mg/l BA + 1.00 mg/l Kinetin + 0.05 mg/l GA_3; followed by 10.05±0.14 shoots per axillary bud on the MS medium supplemented with 2.00 mg/l BA + 1.00 mg/l Kinetin + 0.10 mg/l GA_3.The MS medium supplemented with 2.00 mg/l BA + 0.50 mg/l Kinetin + 0.05 mg/l GA_3; 2.00 mg/l BA + 0.50 mg/l Kinetin + 0.10 mg/l GA_3; 2.00 mg/l BA + 2.00 mg/l Kinetin + 0.10 mg/l GA_3 also produced large number of shoots per explant.

The shoot length of 07.21±0.25 cm was obtained on the MS medium supplemented with 2.00 mg/l BA + 0.50 mg/l Kinetin + 0.10 mg/l GA3. MS media supplemented with 2.00 mg/l BA + 1.00-2.00 mg/l Kinetin + 0.05-0.10 mg/l GA3 also yielded high shoot length. The plants produced by the direct organogenesis method exhibit greater genetic stability than those produced via callus-mediated organogenesis (Piéron *et al*., 1993) [8]. Glutamine was shown to be indispensable to the formation of bud aggregates. Glutamine is frequently employed in the culture medium as an organic nitrogen source. Glutamine in combination with inorganic forms of nitrogen has generally been used *In vitro*. In bromeliads, glutamine was an excellent nitrogen source to improve gain of dry shoots mass of plantlets cultivated *In vitro*. The glutamine had a strong influence on shoot hormonal contents (cytokinin) and it has a great effect on the promotion of shoot-bud organogenesis from leaf base pineapple explants (Mercier and Kerbauy, 1998; Vasanth *et al*., 2006; Vasudevan *et al*., 2004) [5, 15, 16]. Glutamine also proved most effective to control leaf fall in multiple shoots (Sanjaya *et al*., 2005) [11]. Adenine sulfate has also been shown to have a synergistic effect with other cytokinins, it stimulates cell growth and concomitantly

enhances shoot formation (Raha and Roy, 2001) [9]. Effect of citric acid has also been reported to increase shoot length in a variety of plants (Sanjaya *et al*., 2005) [11].

Table 1: Direct shoot induction in axillary bud explants of *Jatropha curcas* L. on MS media supplemented with various concentrations of plant growth regulators

BA	KIN	GA_3	Shoot induction %	Average number of shoots per explant	Shoot length (cm)
1.50	0.50	0.05	38.43±0.33	00.00±0.00	00.00±0.00
1.50	0.50	0.10	29.67±0.26	02.54±0.16	02.31±0.22
1.50	1.00	0.05	32.67±0.24	03.16±0.17	02.58±0.34
1.50	1.00	0.10	32.53±0.24	04.26±0.25	03.63±0.31
1.50	2.00	0.05	43.52±0.17	04.81±0.32	04.38±0.26
1.50	2.00	0.10	39.38±0.16	06.29±0.23	04.57±0.15
1.50	3.00	0.05	42.71±0.19	04.77±0.16	03.38±0.29
1.50	3.00	0.10	46.58±0.22	07.58±0.24	04.72±0.36
2.00	0.50	0.05	58.31±0.26	09.68±0.29	05.45±0.21
2.00	0.50	0.10	60.46±0.23	09.57±0.24	07.21±0.25
2.00	1.00	0.05	87.29±0.23	10.24±0.07	06.76±0.24
2.00	1.00	0.10	91.65±0.31	10.05±0.14	06.53±0.22
2.00	2.00	0.05	79.72±0.24	08.36±0.27	06.06±0.35
2.00	2.00	0.10	76.21±0.28	09.22±0.06	06.03±0.32
2.00	3.00	0.05	51.44±0.35	08.42±0.18	03.87±0.24
2.00	3.00	0.10	50.38±0.28	03.25±0.29	04.32±0.16
2.50	0.50	0.05	65.57±0.34	06.43±0.32	04.12±0.23
2.50	0.50	0.10	58.31±0.21	07.71±0.14	05.03±0.16
2.50	1.00	0.05	45.62±0.25	06.53±0.18	04.61±0.28
2.50	1.00	0.10	40.07±0.18	04.72±0.26	03.22±0.25
2.50	2.00	0.05	24.83±0.31	03.38±0.18	02.03±0.21
2.50	2.00	0.10	21.53±0.37	02.45±0.02	02.17±0.24
2.50	3.00	0.05	21.27±0.29	00.00±0.00	00.00±0.00
2.50	3.00	0.10	18.21±0.34	00.00±0.00	00.00±0.00

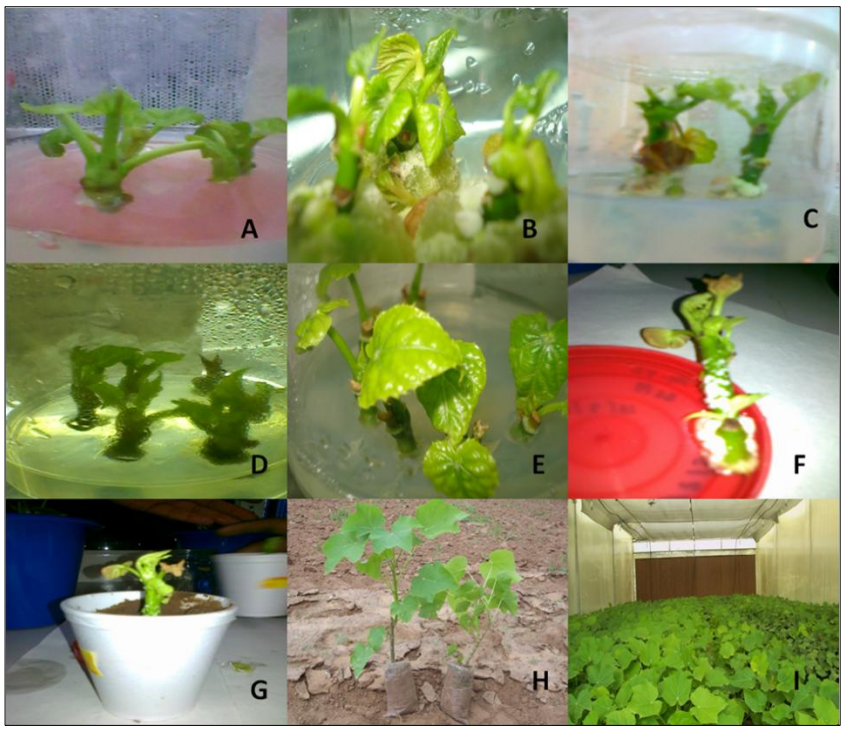

Fig 1: Direct organogenesis from *Jatropha curcas* axillary buds. (A) Shoot bud induction in axillary bud explants of *Jatropha curcas* (B-D) Shoot proliferation (E) Shoot elongation (F) Regenerated *Jatropha curcas* plantlet (G-I) Hardening.

In vitro Rooting

The multiple shoots regenerated were excised individually and were transferred to MS medium supplemented with IBA (0.50-2.0 mg/L) and NAA (0.10-1.0 mg/L). The shoots that were transferred to MS medium with IBA (1.00 mg/l) + NAA (1.00 mg/l) showed the highest rooting response (89.67±0.23 %) followed by 86.54±0.17 % obtained on the MS medium supplemented with IBA (1.00 mg/L) and NAA (0.50 mg/L). The highest number of roots (06.46±0.28) and root length (04.53±0.25 cm) was obtained on the MS medium containing IBA (1.50 mg/l) and NAA (0.10 mg/l) (Table 2). The role of auxins in root development is well established and reviewed (Scott, 1972) [12]. The high rate of rhizogenesis is therefore linked to the presence of IBA in the medium (Kumar *et al*., 2011) [3].

Table 2: *In vitro* rooting in regenerated shoots of *Jatropha curcas* L.

IBA	NAA	Root induction %	Average Number of roots per shoot	Root length (cm)
0.50	0.10	35.73±0.21	03.61±0.16	01.82±0.32
0.50	0.50	48.56±0.24	03.82±0.18	01.34±0.17
0.50	1.00	64.94±0.26	04.11±0.14	02.71±0.28
1.00	0.10	62.28±0.39	03.54±0.16	02.79±0.31
1.00	0.50	86.54±0.17	04.47±0.13	03.37±0.33
1.00	1.00	89.67±0.23	05.63±0.17	03.76±0.28
1.50	0.10	73.45±0.31	06.46±0.28	04.53±0.25
1.50	0.50	66.38±0.26	04.78±0.31	04.24±0.24
1.50	1.00	58.39±0.21	03.52±0.14	03.77±0.23
2.00	0.10	47.27±0.32	02.06±0.18	01.94±0.21
2.00	0.50	43.52±0.31	02.13±0.36	02.02±0.26
2.00	1.00	28.53±0.24	02.08±0.13	01.43±0.17

Acclimatization and hardening

The regenerated plantlets were shifted to plastic cups filled with potting mixture of sterilized sand: soil (1:1) and covered with transparent polythene cover punctured with holes to maintain the relative humidity. Initially these plantlets were kept in the culture room for 20 days and were later transferred to the poly-house for acclimatization for 3 months before transplanting to the field (Fig.1G-I). The survival percentage of the acclimatized plants in the field was 80.0%.

Conclusions

Direct organogenesis is the most efficient mode of *In vitro* regeneration in tree species. The axillary bud explants proved to be the most responsive explants that produced a large number of shoots on the MS medium supplemented with 2.00 mg/l BA + 1.00 mg/l 2ip + 0.05 mg/l GA_3. Profuse rooting was obtained on the MS medium supplemented with IBA (1.00 mg/l) + NAA (1.00 mg/l). The survival percentage of the plants in the field after acclimatization was 80.00%.

Acknowledgements

The financial support for this investigation by the National oilseed and vegetable oils developmental Board, Government of India is gratefully acknowledged

References

1. Datta MM, Mukherjee P, Ghosh B, Jha TB. *In vitro* clonal propagation of biodiesel plant (Jatropha curcas L.). Curr. Sci. 2007; 93:1438-1442.
2. Jyothi S, Batra A, Ali DJ. An expeditious method for regeneration of somatic embryos in Jatropha curcas L. Phytomorphol. 2000; 50(384):239-242.
3. Kumar D, Singh S, Sharma R, Kumar V, Chandra H, Malhotra K. Above-ground morphological predictors of rooting success in rooted cuttings of Jatropha curcas L. Biomass Bioenergy. 2011; 35(9):3891-5.
4. Leela T, Naresh B, Srikanth Reddy M, Madhusudhan NC, Cherku PD. Morphological, physico-chemical and micropropagation studies in Jatropha curcas L. and RAPD analysis of the regenerants. Appl Energy. 2011; 88(6):2071-9.
5. Mercier H, Kerbauy GB. Endogenous IAA and cytokinin levels in bromeliad shoots as influenced by glutamine and ammonium nitrate treatments. Rev Bras Fisiol Veg. 1998; 10:225-8.
6. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue culture, Physiol Plant. 1962; 15:473-497.
7. Nannapat T, Chockpisit T, Aree T. *In vitro* induction of shoots and roots from *Jatropha curcas* L. explants. J Horti Sci & Biotech. 2006; 83:106-112.
8. Piéron S, Belaizi M, Boxus P. Nodule culture, a possible morphogenetic pathway in *Cichorium intybus* L. propagation. Scientia Horticulturae. 1993; 53(1–2):1-11.
9. Raha S. and Roy S.C. A review of Jatropha curcas: An oil plant of unfulfilled promise. Biomass Bioenergy. 2001; 19:1-15.
10. Rajore S, Batra A. Efficient Plant regeneration via shoot tip explants in Jatropha curcas L. J Plant Biochem Biotech. 2005; 14:73-75.
11. Sanjay, *et al*. Micropropagation of Psedoxytenanthera stoskii Munro. *In vitro* Cell Dev. Biol. 2005; 41(3):333-337.
12. Scott TK. Auxin. roots. Ann. Res. Plant Physiol. 1972; 23:235-258.
13. Sujatha M, Mukta N. Morphogenesis and plant regeneration from tissue cultures of Jatropha curcas. Plant Cell Tiss. Org. Cult. 1996; 44:135-141.
14. Sujatha M, Makkar HPS, Becker K. Shoot bud proliferation from axillary nodes and leaf sections of non-toxic *Jatropha curcas* L. Plant Gr. Reg. 2005; 47:83-90.
15. Vasanth K, Lakshmiprabha A, Jayabalan N. Amino acids enhancing plant regeneration from cotyledon and embryonal axis of peanut (*Arachis hypogaea* L.). Indian J Crop Sci. 2006; 1(1-2):79-83.
16. Vasudevan A, Selvaraj N, Ganapathi A, Kasthurirengan S, Ramesh Anbazhagan V, Manickavasagam M. Glutamine: a suitable nitrogen source for enhanced shoot multiplication in Cucumis sativus L. Biologia Plantarum. 2004; 48(1):125-8.
17. Wei Q, Lu W, Da LY, Pan SL, Xu Y, Tang L, Chen F. Plant regeneration from epicotyl explant of Jatropha curcas. J Plant Physiol. Mol. Biol. 2004; 30(4):475-478.