Research Paper Biotechnology

Indirect Shoot Organogenesis in Jatropha Curcas (L) for in Vitro Propagation

Manali Gangwar	Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat-173234, Solan, Himachal Pradesh, India
Swati Sharma	Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat-173234, Solan, Himachal Pradesh, India
Rajinder Singh Chauhan	Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat-173234, Solan, Himachal Pradesh, India
Hemant Sood*	Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat-173234, Solan, Himachal Pradesh, India*

ABSTRACT

Jatropha curcas a monoecious perennial shrub belongs to family euphorbiaceae. Mainly known as a biodiesel crop its commercialization is elusive due to variations in seed yield, oil content, and slow germination rates. Optimization of in vitro conditions for callusing and rapid regeneration is of paramount importance due to its slow regeneration. We used different concentrations of growth hormones followed by optimizing the conditions that gave best results on MS medium supplemented with IBA (3mg/l) + KN (1mg/l). Callus regeneration was tried on different concentrations and combinations of BAP + KN + GA3. Regeneration of shoot primordial was highest in the calli cultured on MS + BA (2 mg/l) + KN (3 mg/l). Shoots were grown after 4-5 weeks of incubation under optimized conditions. This developed protocol would be utilized for long scale production of elite genotypes and for genetic interventions of Jatropha curcas. This in vitro protocols provides the usage for rapidly establishing high oil yielding plantlets.

KEYWORDS J. curcas, callusing, regeneration, shooting

INTRODUCTION

Jatropha curcas is a tropic perennial shrub belongs to family euphorbiaceae. *J. curcas* has gained much attention for both non-oil producers and oil producing countries around the world due to its high seed oil content, without competing with food production (Grimm 1996). *Jatropha curcas* is mainly cultivated as a biofuel crop on marginal land of poverty but its commercial success to be used as biofuel crop are mainly elusive due to variations in seed yield, oil content, low seed viability and slow germination rates. Therefore improvement in programmes of popularizing *J. curcas* by modern methods such as development of tissue culture protocols to facilitate large scale production of plants and genetic modifications is important (www.jatrophabiodiesal.org).

Various reports are available on tissue culture of *J. curcas* by using different explants such as leaves, shoot tips, nodes and axillary nodes, cotyledons and petiole cotyledons in MS media using cytokinin and auxin as growth hormones (Deore and Johnson 2008; Sujata et al, 2005). But the major issue with *J. curcas* is its slow regeneration through callus or other explants used for in vitro propagation. By taking in consideration this problem the present study was conducted to develop a protocol for rapid callusing and regeneration of *J. curcas* using leaf explant.

Material and Methods

Selection of plant material and establishment of axenic cultures

The *J. curcas* plant cuttings were procured from the National Botanical Research Institute, Lucknow, U.P., India and planted in pots in a polyhouse at the experimental area of the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, India.

Selection and culture of explants

Young leaves were taken from *in vitro* grown plantlets of *J. curcas* were surface sterilized with bavistin (0.5% for 2 mins) and HgCl2 (0.1% for 30 sec) and cultured on MS medium supplemented with different concentrations and combinations of growth hormones such as 2,4-D, IAA, NAA and IBA with sucrose 3% (w/v). The pH of the media was adjusted to 5.7 using 0.1 N HCl and 0.1 N NaOH and finally agar-agar 0.8% (w/v) was added as a gelling agent. The media were autoclaved at 121°C and 15 lb/in2 pressure for 15 - 20 min. The autoclaved media were kept in the Laminar Air Flow hood for 1 - 2 days before inoculations. The explants were excised aseptically and cultured on above mentioned media. The cultures were incubated at 16 h light /8 h dark cycle at 25 ± 2°C in plant tissue culture chamber. Data were recorded on days to initiation of callus formation, per cent explants forming calli, days to compete callus formation. The data were statistically analyzed for test of significance. The cultures were sub-cultured after every 15 – 20 days on callus induction media for 2 months so as to obtain good growth (Table 1).

Table1: Effect of different growth hormone supplemented in MS media on callus induction frequencies of *J. curcas*.

Name	Media composition	Callus Formed	% of callusing
M1	TDZ+24D (2:1)	40	No response
M2	TDZ+NAA+BAA (2:1:3)	25-30	50%-70%
M3	24D+NAA (3:1)	30-32	40%-50%
M4	BA + NAA (3:2)	20-25	70%-80%
M5	IBA+KN (3:1)	10-12	100%

Plantlet regeneration

After the explants were completely transformed into callus mass, the calli or parts thereof were transferred onto regeneration media consisting of MS salts supplemented with different concentrations of BA, KN and GA3 for differentiating into shoots. Calli of cream color originating from different explants were transferred onto regeneration media and incubated in the plant tissue culture chamber maintained at 16 h light/8 h dark photoperiod. Twenty calli from each explant were cultured on each regeneration medium for regeneration. The data were recorded on days to shoot primordial initiation, per cent calli forming shoot primordia, number of primordia/callus and number of shoots/callus. The regeneration frequency was determined by counting the number of calli forming shoot primordia and plantlets. The data were statistically analyzed for test of significance (Table 2).

Table 2: Effect of various media on shoot regeneration and shoot multiplication in calli- derived from explants of *J. curcas*.

Name	Media composition	% Calli formed into shoots after 3 weeks	No. of Shoots per explant
R1	BA+GA3 (2:1)	50-60%	2.8±0.8
R2	BA+KN (2:3)	80-90%	11.8±0.8
R3	BA (4)	No response	-
R4	BA+KN (4:3)	No response	-
R5	KN (3)	No response	-
R6	KN+GA3 (2:3)	40-60%	3.8±0.8
R7	KN+GA3 (0.5:1)	No response	-
R8	BA+IAA (1:1)	60-70%	7.8±1.3
R9	BA+NAA (1.5:0.5)	50-60%	11.8±0.8
R10	BA+NAA (1:1)	90-95%	24±1.3
R11	KN+GA3+BA (1:1.5:1)	No response	-
R12	KN+IBA (3:1)	No response	-
R13	BA+IBA+GA3 (1.5:0.25:0.5)	No response	-
R14	BA+GA3 (2.5:0.5)	No response	-
R15	KN+IBA+BAP (0.5:0.2:0.3)	No response	-

Results and Discussion

Establishment of callus cultures

Callus cultures were initiated from different explants such as leaf discs, nodal and root segments of *J. curcas*on MS salts supplemented with different concentrations of growth hormones as shown in Table 1. Callus formation initiated in all explants within 7 days at the cut surfaces in all test media combinations (Figure1).

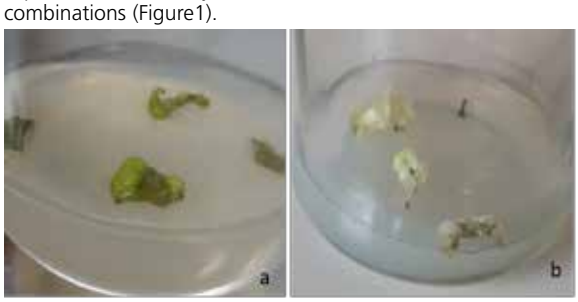

Figure 1: Callus initiation and proliferation from leaf explants of *J. curcas*; (a) Callus initiation (b) Callus growth.

All the explants were transformed into complete callus mass within 10-12 weeks of culture. Overall, MS medium supplemented with IBA (3 mg/l) + KN (1 mg/l) was found to be the best for callus induction with frequencies of 100%. Callus cultures derived from leaf explant were sub-cultured for 3-4 weeks so as to proliferate the calli on suitable callus induction media. Calli with a creamy and even green in appearance were taken for regeneration.

Regeneration of callus cultures into shoots

Proliferating callus cultures or parts thereof were subcultured onto regeneration media containing MS salts supplemented with different concentrations and combinations of BA, KN and IBA (Table 2). Media containing BA and KN induced shoot primordia formation in the form of green nodular structures. The MS + BA (2 mg/l) + KN (3 mg/l) was found to be the best for regeneration. (Figure 2).

Figure 2: Regeneration of callus cultures derived from leaf into shoots; (a) Emergence of shoot primordia (b) Shoot regeneration (c) Shoot elongation and growth.

The shoots regenerated from callus cultures were allowed to grow on regeneration medium for better growth. Shoot multiplication occurred in media consisting of MS + BA (1 mg/l) + NAA (1 mg/l) +GA3 (1.5 mg/l) and about 24±1.3 shoots were produced after 20-30 days.

Discussion

The overall objective of the current study was to develop an *in vitro* system for establishment of high frequency callusing and regeneration from leaf explants of *J. curcas.* Due to roadblocks in commercialization of *J. curcas* due to its low yield, improvement in various character such as flowering, seed oil content, no. of branches etc. genetic interventions is now been targeted. Therefore, rapid callusing regeneration is necessary which has been a major problem in *J. curcas*. According to the report by Soomro & Memon (2007) callus was induced after 15 days for leaf explant where with our hormone composition it was achieved within 7 days and complete callus mass as formed after 12-15 days with 99% of leaf explants responded. Callus produced was soft, very friable, compact, globular which is in accordance with Rao et al. (2006) expect callus was of cream color. Other interesting fact is that while growing callus on IBA + KN media darkening of callus did not occur even after 30-35 days.

Shooting was induced after 20-30 days of callus sub cultured on regeneration media with 80% frequency, which is quiet rapid. Induced shoots length was reached upto 4.5 cms after 20 days and shoots multiplied upto 24± 1.3 in number. However previous reports indicated that about 3.9 cm height was achieved after 6 weeks (Jeevan et al., 2013). Kumar et al. (2010) reported that using explants as leaf discs cultured in basal MS medium failed to give any response and turned yellow subsequently whereas our results were best using leaf discs as explants. In vitro propagation *of J. curcas* is important for homogeneity of the plant traits and varietal selection as in vitro raised plantlets has shown less variability than seed raised plantation or through miropropagation (Daudet et al., 2011)

Conclusion

The present study established that *J. curcas* plantlets were regenerated in vitro with in very short span of time through in-

direct organogenesis from leaf explant. These techniques will passed a way for future exploitation for large scale production and genetic interventions for high oil and high female flowering, which could be used for the production of high yielding variety of *J. curcas*.

Acknowledgements
The authors are thankful to the Department of Biotechnology, Govt. of India for providing financial support in the form of a R&D project *on Jatropha curcas*.

REFERENCES

1. Daudet, S., Mvé, M., Mergeai, G., Baudoin, J-P., Toussaint, A. (2011). Culture in vitro de Jatropha curcas L. Biotechnolie Agronomie Societe et Environment, 15(4), 567-574. | 2. Deore, C.A., & Johnson S.T. (2008). High frequency plant regeneration from leaf disc cultures of Jatropha Curcas.: An important biodiesel plant. Plant Biotechnololgy Reports, 2(1),7-11.doi: 10.1007/s11816-008-0042-y | 3. Grimm, C. (1996). The Jatropha project in Nicaragua. Bagani Tulu (Mali) 1: 10-14. | 4. Kumar, N., & Reddy, M, P. (2010). Plant regeneration through the direct induction of shoot buds from petiole explants of Jatropha curcas: a biofuel plant. Annals of Applied Biology, 156(3), 156: 367-375. doi: 10.1111/j.1744-7348.2010.00394.x | 5. Kumar, N., Anand ,V, K, G., & Reddy, P, M. (2010). In vitro plant regeneration of non toxic Jatropha Curcas L.: Direct Shoot Organogenesis from Cotyledonary Petiole Explants. Journal of Crop Science and Biotechnology, 13(3), 189-194. doi: 10.1007/s12892-010-0039-2 | 6. P, Jeevan., Rena, A, E., SubramanianS, S., & Nelson, R. In Vitro Culture of Jatropha Curcas L. – An Important Medicinal Plant. Journal of Microbiology and Biotechnology Research, 3(6), 44-48 | 7. Rao, A, Q., Hussnain S. Sarfraz, Shahzad M. Sadqib, Bokhari S. Yassir Abbas Hussain Tayyab and S. Rizuddin. (2006). Somatic embrogenesis in wild relatives of cotton (Gossipium spp.). Journal of Zhejiang University Science B, 7(4), 291-298. doi: 10.1631/jzus.2006.B0291 | 8. Soomro, Rashida., & Memom, Rabia.(2007). Establishment of callus and suspension culture in Jatropha Curcas. Pakistan Journal of Botany, 39(7), 2431-2441. | 9. Sujatha, M., & Mukta, N. (1996). Morphogenesis and plant regeneration from tissue cultures of Jatropha Curcas. Plant cell, Tissue and Organ Culture, 44 (2), 135-141. doi: 10.1007/BF00048191 |