International Journal of Basic and Applied Research ISSN - 2249-3352
Vol. 02 Issue-2 (December 2012) (18-23)

Induction of Somatic Embryos From Cotyledon Explant in Jatropha Curcas(LINN.)

Sanjay R. Biradar, Vishwanath Waghmare, Narayan Pandhure*

Department of Botany Shri Chhatrapati Shivaji College Omerga-413606, Dist. Osmanabad
**Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad-431001*

Email : sanjaybiradar2006@rediffmail.com

Key-words:
Jatropha curcas, somatic embryo, cotyledon explants.

Abstract: During present investigations induction of embryogenic callus from cotyledon explants *was* achieved from important biodiesel and medicinal plant *Jatropha curcas*. Explants inoculated on MS (Murashige and Skoog, 1962) medium supplemented with various concentration of growth hormone like, BAP 6-Benzyl amino purine), IAA (Indole 3- acetic acid), GA_3(Gibberellic acid) with addition proline and PEG (Polyethylene Glycol).MS media containing (2, 4- D-Dichlorophenoxy acetic acid) in combination of BAP were able to initiation of callus. MS medium containing BAP at various concentrations viz 0.2, 04, 0.6, 0.8 mg/l with combination different concentrations of GA_3 like 0.2, 0.4, 0.6, 0.8 and1.0 mg/l,IAA 0.1, 0.2, 0.3 mg/l and proline observations were also recorded. Induced callus from cotyledon explants were using MS medium with supplemented 0.5 mg/l BAP, 0.6 mg/1 GA_3, and proline was able to produce direct somatic embryogenic callus. This method of regeneration of plant was more effective as compare to other methods because regeneration percentage was 70% -80% respectively. The percentage of embryo maturation was observed after three weeks and the matured somatic embryos were subculture on hormone free MS medium for plant regeneration.

INTRODUCTION

Today *Jatropha* is found in almost all the tropical and sub-tropical regions of the world and known by nearly 200 different names, which indicate its significance and various possibilities of its use. Distribution of *Jatropha* beyond the tropical America was likely by the Portugese who transported *Jatropha* to Africa and Asia where it has since become more known by many local names. *Jatropha curcas* (Linn.) belongs to the family Euphorbiaceae and is closely related to some other important cultivated plants like rubber trees, castor etc. *J. curcas* is a small ever-green, nearly glabrous tree or soft wooded shrub, 3 to 4 meters high. In India, *Jatropha curcas* is found in almost all the states and is generally grown as a live fence for protection of agricultural fields against damage by livestock as unpalatable to cattle and goats, it grows in semi wild condition in the vicinity of villages. India has about 75 million hectares of waste lands, which need re-vegetation. *Jatropha curcas* is a wild growing hardy plant well adapted to harsh conditions of soil and climate

(Katwal et al., 2003).

Moreover, it can be conveniently propagated from seeds as well as branch cuttings.

It is profitable *Jatropha curcas* seeds contain semi-drying oil, an efficient substitutes for diesel engines (Bhasubutra and Sutiponpeibun, 1982). The importance of *Jatropha* are varied range from serving as a cultivated hedge, *J. curcas* oil finds wide usage and has high economic potential for large scale of industrial use (Raina, 1987). Additional known uses of *Jatropha* are based on exploiting the plant's poisonous and toxic effects. The leaves are used as a fumigant for bed bugs and a mixture of seeds and palm oil are used as rat poison; the latex apparently has properties which inhibit the growth of mosaic virus.

It has been mentioned that leaves are used as a feed for silk worms in Assam, young branches as mulch for coconut trees and the oil or pulp in the manufacturing of paper umbrellas. The latex, bark and roots have been employed as dyes and marking ink on cloth. In a few localized areas the seeds and leaves are roasted and cooked to enhance local dishes, suggesting that when cooked its toxicity is lost. J. Curcas oil has been used as a lamp oil in some rural areas (Makkar, H.P.S. 1997). Most important Jatropha oil is an environmentally safe, cost-effective renewable source of non-conventional energy and a promising substitute for diesel, kerosene and other fuel oils. Various part of Jatropha use in medicinally viz., latex, oil, twigs, wood and leaves are all reportedly used externally for healing wounds, to stop bleeding, and to treat skin disease and rheumatism (Dalziel J.M. 1955) . Other medicinal uses of the plant are as a laxative, cough remedy antidote for poisoning, relief for tooth-aches and to strengthen gums. The latex of *Jatropha curcas* contains an alkaloid known as Jatrophine which is believed to be having anti-cancerous properties. In bark is rubbed with asafetida and buttermilk and its paste used for the cure of dyspepsia and diarrhoea. Therefore specific objectives of the present study to produce optimize protocol in vitro propagation of Jatropha curcas.

MATERIAL AND METHOD :

Preparation of explant and surface sterilization

Seeds of *Jatropha curcas* were collected from Botanical garden, Shri Chhatrapati Shivaji

College Omerga. These seeds were used as explants from donor plants during present study. The seeds were washed carefully in running tap water for 10 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol and $Hgcl_2$ (0.3 %) were used. seeds were surface sterilized for 1 minute in 70% ethanol after the one minute these seeds are also sterilizing with 0.3% mercuric chloride for 3 minute followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these seeds were dissected and removing the seed coat carefully. Each seeds were containing two cotyledons and an embryo. These cotyledons were cut into small pieces and aseptically inoculated in test tube as well as culture vessels containing MS medium with various concentration of growth hormones.

Culture medium and conditions

MS medium (Murashige and Skoog, 1962) was used for formation of somatic embryogenesisby using cotyledon as an explant of *Jatropha curcas*. These explant examined using MS medium with supplemented different concentration and combination of growth regulators like, BAP, KIN, GA_3, IAA and various concentration of amino acid. MS medium containing with 3% sucrose and gelled 6 gm/L solidified agent agar and after the adding growth regulators the pH was adjusted to 5.8. The media were steam sterilized in a autoclave under 15 psi and 121° C. then these media was transfer to laminar air flow solidified and inoculation of explant after the inoculation culture tubes and culture vessels were transfers to culture room under a 16 h photoperiod supplied by cool white fluorescent cool tubes

light and temperature 25 ± ^{0}C. Data were measured after 30 days five replicate for formation of somatic embryogenesis Mean (μ) values.

RESULTS AND DISCUSSION

During surface sterilization it is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissueor plant parts (Conger et, al, 1987). However these sterilized seeds of *Jatropha curcas* is necessary to remove seed coat carefully and aseptically

inoculated on MS medium supplemented with 3% sucrose, 6 gm/l agar and different concentration of growth regulators like,BAP viz 0.2, 04, 0.6, 0.8 mg/l in combination with different concentrations of GA_3 like 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l, IAA 0.1, 0.2, 0.3 mg/l and proline, either alone or combination.

Induction of callus from cotyledon

The concentrationsof various growth regulators were used vary accurately for the culture purpose. There are several types of plant growth regulators, each having a well-defined effect on growth and development (Kumar 2001). Callus initiation appearing, use of axillary leaf, axillary shoot, root, nodal explants but present study was initiated for induction of somatic embryonic callus using cotyledon explant. These cotyledons were aseptically inoculated on MS medium with supplemented of various concentrations of BAP, KIN, 2, 4- D and IAA either alone or with combination. After 15–20 days callus was appeared and significantly higher number of frequencies of callus formation was achieved using 6 mg/l BAP with combination of 2.5 mg/l IAA and 1.0 mg/l BAP in combination 1.5 mg/l 2, 4- D**.**

Average number frequencies of callus induction was found in 4.5 mg/l BAP, 5.0 mg/l BAP and 3.5 mg/l KIN, 4.0 mg/l KIN either alone or with combination 0.8 mg/l IAA, 1.2 mg/l IAA and 0.5 mg/l, 1.0 mg/l, 1.5 mg/l 2, 4- D as showing (Table 1). These induced calli was excreted for secondary metabolites in medium.It is problematic to maintain the fresh calli and subculture is necessary as colour

Table 1 effect of various growth regulators on callus initiation

Explant	Concentration of growth regulator (mg/l)				Frequency of Callus formation	Nature of callus
	BAP	KIN	IAA	2, 4- D		
Cotyledon	4.0	3.0	0.5	-	+	White
	4.5	3.5	1.0	-	+++	White
	5.0	4.0	1.5	-	++	White
	5.5	-	2.0	-	+++++	Pale Yellow
	6.0	-	2.5	-	++++++	Pale Yellow
	0.0	-	-	0.5	++	White
	0.5	-	-	1.0	+++	White
	1.0	-	-	1.5	++++++	Pale Yellow
	1.5	-	-	2.0	++++	Pale Yellow
	2.0	-	-	2.5	+++	White

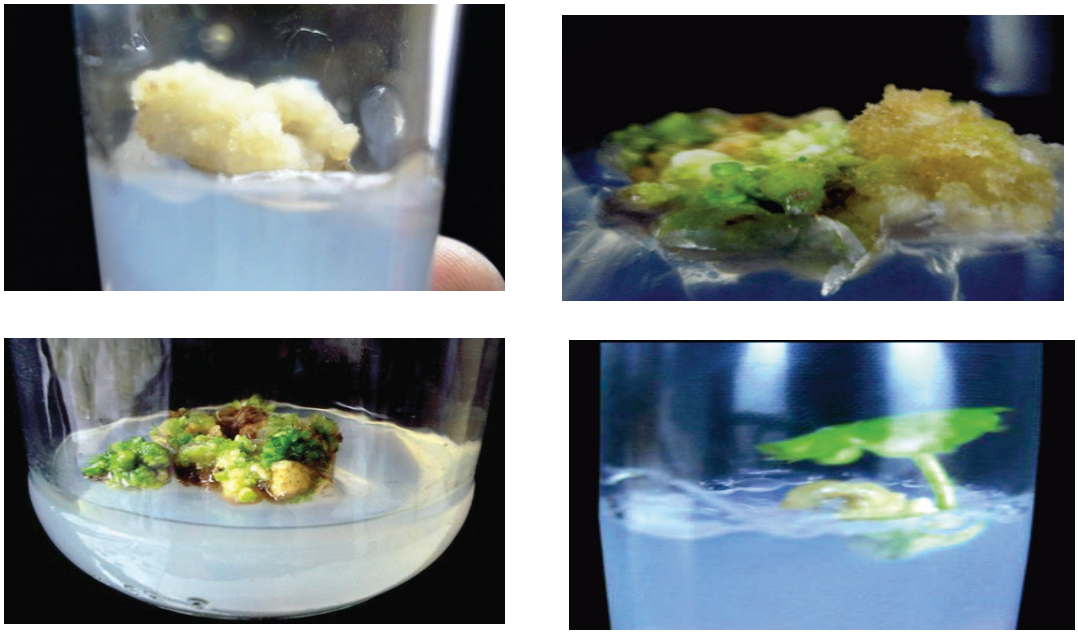

Plate 1 showing a. whitish callus from cotyledon b. immature embryos c. mature embryos d. shoot from mature embryos.

Table 2. Effect of different growth regulator on induction somatic embryos

Source for culture	Concentration growth Regulator mg/l			percentage of somatic embryos	% of plant from embryos
	BAP	IAA	GA_3		
Callus From Cotyled on explant	0.4	0.2	-	0.1	0.0
	0.8	0.4	-	0.5	0.0
	1.2	0.2	-	1	0.6
	1.6	0.4	-	1	0.5
	0.2	0.1	0.1	10	1
	0.4	0.1	0.2	25	3
	0.6	0.1	0.3	40	5
	0.8	0.1	0.4	30	4
	1.0	0.1	0.5	45	7

changes brownish and black. Addition of different concentration of charcoal or ascorbic acid in media viz. 20 mg/l, 25 mg/l, 30 mg/l subsequently overcome this problem.

Formation of somatic embryos

Callus inducedfrom cotyledon explants were subculture on MS medium containing 3% sucrose 5 gm/l agar and various concentration of growth hormone like BAP, IAA, 2, 4-D, GA3 and proline. This newly grown callus was turned to embryogenic after two weeks. Significant highest number of somatic embryonic callus was form MS medium with supplemented 0.5 mg/l BAP, 0.6 mg/1 gibberellic acid (GA3), and proline.These calluswas cultured onhalf strength MS medium supplemented with various concentration of BAP like 0.2, 04, 0.6, 0.8 mg/l with combination GA_3 like 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l, IAA 0.1, 0.2, 0.3 mg/l addition proline no significant effect of formation of somatic embryos. But calli were cultured on full strength MSmedium supplemented with various concentration of BAP like 0.2, 04, 0.6, 0.8 mg/l with combination GA3 like 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l, IAA 0.1, 0.2, 0.3 mg/l addition proline average number of somatic embryos form and these somatic embryos were greenish in colour observed. This callus was inoculated on MS medium supplemented with BAP and IAA without addition GA_3 no significant effect on formation of somatic embryos was noted.

Regeneration of plant through somatic embryos

Greenish somatic embryos were mature after three weeks. These mature embryoswere sub cultured on MS medium containing 3% sucrose 5 gm/l agar supplemented withvarious concentration of PEGand various concentrationsof ABA viz. 0.2, 0.4, 0.6,0.8and 1.0 mg/1. After two weeks these embryos produce greenishleaflets were observed. The maximum percentage of embryo maturation was found in MS media containing PEG,3%(w/v) and 0.6 mg/l ABA, after two weeks matured somatic embryos were transferred to growth hormone free MS medium for development of plant or regeneration of plant. In the present research work it was found that cotyledon explant is most suitable for callus induction and for the formation of somatic embryos. It was also noticed that IAA has no significant effect for development of leaflets but
with GA3 and BAP significantly affected to on somatic embryos and maturation respectively. PEG, sucrose and ABA were more expensively affected on regeneration of leaflets and plant development of plantlets.

Various similar results had been found forregeneration of plant through somatic embryonic callus in different plant *in vitro*. It was reported that somatic embryogenesis of *J. curcas* from leaf tissues using single cytokinin, kinetin (Jha et al. 2007). In present research work, somatic embryos of *J. curcas* were formed from cotyledon explant derived callus on MS medium containing phyto-hormones BAP and GA3. In some species, embryo development can be regulated by changing the sugar content of the medium. Lowering the concentrationof sucrose by 2% (58mM) early germination of immature pea embryos was achieved, while higher sugar content stopped embryo development (Cook et al., 1988). In contrast, low sucrose concentration inhibited the maturation of globular and heart-shaped embryos in *Citrus aurantium* embryos (Carimi et al., 1998).

Present research work demonstrates that the use of various concentration of BAP, GA3 and constant 3% sugar have effects on thegrowth of somatic embryos in *J. curcas.* Callus formed on nodal explants that had been cultured on MS medium supplemented with indole-3-acetic acid (IAA) did not produce somatic embryos upon transfer to MS basal medium. Somatic embryos were developed into plantlets and subsequently grown to maturity. These results indicate that nodal explants have high competence for somatic embryogenesis in *Ecliptaalba*(Devendra et al 2011). It was also recorded addition of IBA to the culture medium generally induced higher percentages of

complete plantlets as compared to NAA concentration. The optimum treatment that maximized the percentage of complete plant formation (86%) consisted of half-strength MS medium containing 0.2 to 0.4 mg/1 IBA. Somatic embryos that formed only shoots ranged from 2 to 26% and were associated with NAA-containing treatments in*Phoenix dactylifera*L. (Jameel M. Al-Khayri2003).In present study MS media containing PEG and 0.6 mg/lABA, after two
to growth hormone free medium for development of plant in *Jatropha carcus.*

ACKNOWLEDGEMENT

Authors are grateful to Principal of Department of Botany Shri Chhatrapati Shivaji College Omerga-413606, Dist. Omarabad. (M.S.), India for providing all necessary facilities and encouragement for the present researchwork.

REFERENCES

[1] **Basubutra R. and Sutiponpeibum, S. (1982)** Renewable energy. Review Journal. (4): 56-70.

[2] **Carimi F, D. E.Pasquale F. and Puglia A. M. (1998).**In vitro rescue of zygotic embryos of sour orange, Citrus aurantium L., and their detection based on RFLP analysis. Plant Breeding 117:261–266.

[3] **Conger B.V. (1987)** Cloning agricultural plants viz. in-vitro techniques. CRS Press, Florida. 1987.

[4] **Cook S.K., Adams H., Hedley C. L., Ambrose M. J., and Wang T. L. (1988).**an analysis of seed development in pisumsativum embryo development and precocious germination in vitro. Plant cell, tissue and organ culture 14: 89–101.

[5] **Dalziel J.M. (1955).** The Useful Plants of West-Tropical Africa. Crown Agents for Oversea Governments and Administration,London, p. 147.

[6] **DevendraSrinivas B. and Sandeep Reddy (2011).**High frequency somatic embryogenesis and plant regeneration in nodal explant cultures of Ecliptaalba L. Hassk Annals of Biological Research, 2 (3): 143-149.

[7] **Jameel M. Al-Khayri (2003)** In vitro germination of somatic embryos in date palm: Effect of auxin concentration and strength of MS salts, current 680 sciences, vol. 84, no. 5, 10.

[8] **Jha TB, Mukherjee P, Datta MM (2007).**Somatic embryogenesis in Jatropha curcas Linn.an important biofuel plant. Plant biotechnology.Rep. 1: 135-140.

[9] **Katwal R.P.S. and Soni P.L.**
140.

[9] **Katwal R.P.S. and Soni P.L. (2003).**Biofuels: an opportunity for socioeconomic development and cleaner environment. Indian Forester, 129(8), 939-949.

[10] **Kumar U. (2001)** Methods in plant tissue culture.Ag-robios, India 1-25 pp.2001.

[11] **Makkar, H.P.S., Becker, K., Sporer, F., Wink, M., (1997).** Studies on nutritive potential and toxic constituents of different provenanaces of Jatropha curcas. J Agric. Food Chem. 45,3152–3157.

[12] **Murashige T and Skoog F (1962)** A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497

[13] **Raina, A.K. and Gaikwad, B.R. (1987)** Oil JOTAI. Oct-Dec. 81-85.

[14] **Warakagoda P.S. and S. Subasinghe (2009).** In vitro culture establishment and shoot proliferation of Jatropha curcas L. Tropical Agricultural Research & Extension 12(2): 2009.