

DIRECT SOMATIC EMBRYOGENESIS OF A BIODIESAL PLANT *JATROPHA CURCAS* (LINN.)

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ABSTRACT

Present research work was initiated on induction of direct somatic embryo genic callus from cotyledon explants in important biodiesel and medicinal plant *Jatropha curcas*. These explant inoculated on MS medium supplemented with various concentration of growth hormone like, BAP, IAA, and GA₃ addition with proline and PEG. MS media containing 2, 4- D in combination of BAP were able to initiation of callus. MS medium containing BAP in various concentrations viz 0.2, 0.4, 0.6, 0.8 mg/l with combination different concentrations of GA₃ 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 observations were also recorded. Induced callus from cotyledon explants were using Murashige and Skoog (MS) medium supplemented with 0.5 mg/l BAP, 0.6 mg/l gibberellic acid (GA₃), and proline was able to produce direct somatic embryo genic callus. This method regeneration of plant was more effective as compare to other methods because regeneration percentage 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.

KEY WORDS: *in vitro* *Jatropha curcas*, somatic embryo, cotyledon explants.

INTRODUCTION

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 Portuguese 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, 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, 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*.

MATERIALS AND METHODS

Preparation of explant and surface sterilization

Seeds of *Jatropha curcas* were collected from Botanical garden, Shri Chatrapati Shivaji College, Omerga, Dist. Osmanabad, (M.S.). 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 HgCl₂ (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 embryogenesis by using cotyledon is an explant of *Jatropha curcas*. These explant examined using MS medium with supplemented different concentration and combination of growth regulators like, BAP, KIN, GA₃, 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 the surface sterilization it is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue or plant parts (Conger *et. al.*, 1987). However these sterilized seeds of *Jatropha curcas* is necessary to remove seed coat carefully and cotyledons were aseptically inoculated on MS medium supplemented with 3% sucrose, 3 gm/l Clerigel and different concentration of growth regulators like, BAP viz 0.2, 0.4, 0.6, 0.8 mg/l in combination with different concentrations of GA₃ 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 somatic embryos from cotyledons explant

Various concentrations of 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 supplemented with 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).

Table 1:Effect of various growth regulators on callus and somatic embryonic callus induction.

Explant	Concentration of growth regulator (mg/l)				Frequency of Callus formation	Direct Somatic embryonic Callus formation	Nature of Callus/ Somatic embryonic
	BAP	KIN	IAA	2, 4- D			
Cotyledons	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	

* + formation of callus and direct somatic embryonic callus

These induced calli was excreted for secondary metabolites in medium. It is problematic to maintain the fresh calli and subculture is necessary as colour 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. Sterilized cotyledons were inoculated on MS medium supplemented with 2.0 mg/l 2, 4-D, 0.5 mg/l BAP and various concentration of PEG after the 25 days these cotyledons produce direct Somatic embryonic callus was observed. Average number of induction of

direct Somatic embryonic callus were recorded in 1.5, 2.0, 2.5, 3.0 mg/l 2, 4-D with combination of 0.5, 1.0, 1.5, 2.0 mg/l BAP and addition of excess amount of PEG as shown in the Figure 1. C and D.

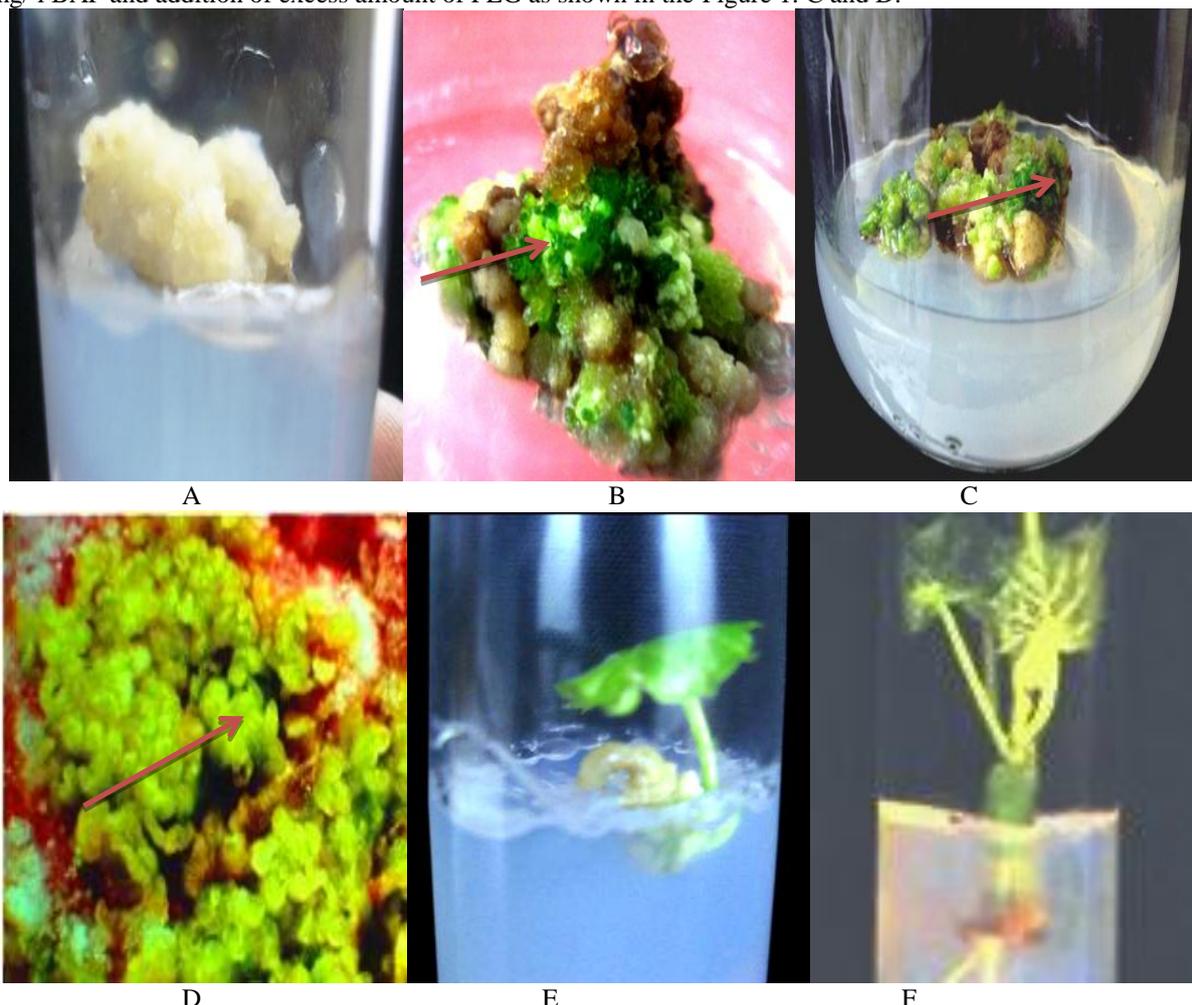


Figure 1. Showing, A) Callus from Cotyledons B) Callus convert into somatic embryos C) Direct somatic embryonic callus from cotyledons D) Somatic embryos E) Initiation of Axillary leaf from Somatic embryo F) Embryos converted into *in vitro* plantlet.

Regeneration of plant from somatic embryos

Greenish somatic embryos were mature after 3-4 weeks. These mature embryos were sub cultured on MS medium containing 3% sucrose 3 gm/l clorigel supplemented with various concentration of PEG and various concentrations of ABA viz. 0.2, 0.4, 0.6, 0.8 and 1.0 mg/l. After two weeks these embryos produce greenish leaflets were observed (as showing in the Figure 1. E). 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 for regeneration 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 concentration of 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 2, 4-D, BAP, GA3 and constant 3% sugar have effects on the growth 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/l 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/l ABA, after two weeks matured somatic embryos were transferred to growth hormone free medium for development of plant in *Jatropha carcus*.

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