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IN VITRO CALLUS AND SHOOT INDUCTION IN JATROPHA CURCAS (LINN.)

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ABSTRACT
During present studies carried out on *Jatropha curcas*, initiate role of BAP and KIN on multiple shoot formation, callus induction using apical shoot tips and axillary leaf as an explant. These explant were inoculated on MS medium with supplemented various growth regulators viz. KIN, BAP and IAA. However, multiple shoots were observed, 6 mg/l BAP with combination of 3 mg/l IAA to produce callus. Quantity of callus was further increased in sub culturing on same medium in addition of various concentration of charcoal or ascorbic acid like 20 mg/l, 25 mg/l and 30 mg/l. Shoot tip explant inoculated on MS medium supplemented with 3.0 mg/l BAP + 3.0 mg/l KIN and ascorbic acid two shoots were observed. Average percentage of shoots and calli were recorded in 3.5 mg/l, 4.0 mg/l KIN, 4.5 mg/l, 5.0 mg/l, 5.5 mg/l BAP and various concentration of IAA.

KEY WORDS: *Jatropha curcas*, *in vitro* propagation, explant.

INTRODUCTION

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. Today it 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. 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 J*atropha 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 asafoetida 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

Source of Explants:

Seeds of *Jatropha curcas* were collected from locally previous grown plant of Omerga and grow in green house, Botanical garden, Shiv Chatrapati college omerga. Apical shoot and axillary leaf of *Jatropha curcas* were collected from three month old plants grown in the greenhouse of Botanical garden. These explants were used from donor plants during present study. The explants were washed carefully in running tap water for 5 minute and followed by distilled water for 5 minutes. For surface sterilization, chemical such as 70% ethanol, Hgcl2 (0.3 %) were used. Explants were surface sterilized for 5 minute by 0.3% mercuric chloride followed by three subsequent rinses with sterilized double distilled water in a laminar flow. All these explants were dissected into small pieces and treaded to media maximum part can be exposed.

Culture medium and conditions

MS medium (Murashige and Skoog, 1962) was used for multiple shooting and callus initiation for using apical shoots and axillary leaf explants of *Jatropha curcas*. These explant examined using MS medium variously supplemented with BA, KIN, and IAA. MS medium contains with 3% sucrose and gelled with 6 gm/L solidified agent agar, and the pH was adjusted to 5.8 after adding the growth regulators. The media were steam sterilized in a autoclave under 15 psi and 121° C. 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 record

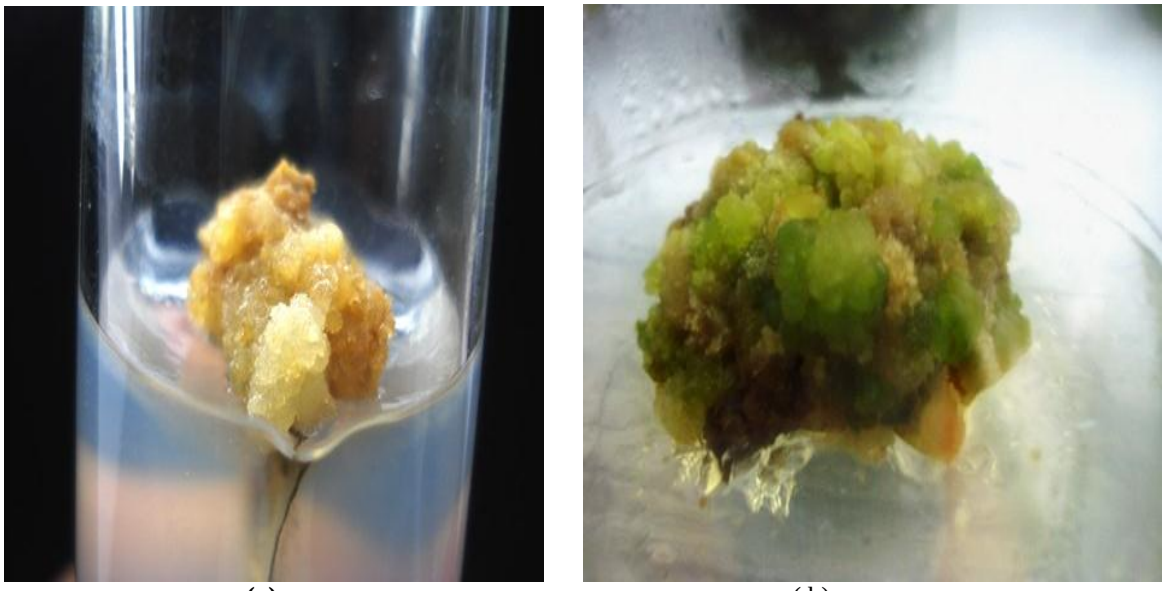
Data were measured after 30 days of five replicate for shoot multiplication and shoot length Mean (µ) values with the standard error (S.E.).

RESULTS AND DISCUSSION

When surface sterilizing plant parts it is necessary to disinfect tissues with a minimum amount of cellular damage to the host tissue (Conger 1987). Therefore these sterilized explants cut outline of both ends in proper size and shape and aseptically inoculated on MS medium with supplemented 3% sucrose, 6 gm/l agar and different concentration with growth regulators like, 3.0 mg/l KIN, 3.5 mg/l KIN and 3.0 mg/l BAP, 3.4 mg/l BAP, 3.6 mg/l BAP, 0.4 mg/l, 0.8, mg/l, 1.2 mg/l IAA, either alone combination or separately.

Callus induction

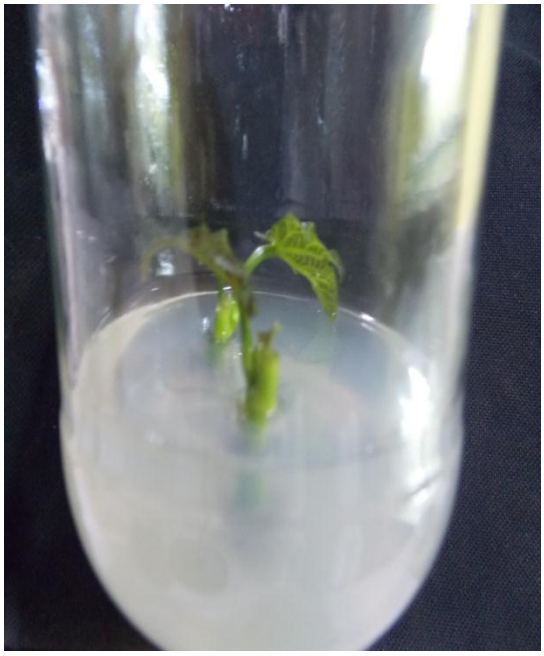
The various growth regulators and concentrations used vary accurately to the culture purpose
(Kumar U. 2001). There are several types of plant growth regulators, each having a well-defined effect on growth and development. Callus initiation appeared, using axillary leaf, axillary shoot explant. These explants were aseptically inoculated on MS medium with supplemented of various concentrations of BAP, KIN and IAA either alone or with combination. After 15–20 days calli grown significantly higher number of frequencies was achieved using 6 mg/l BAP with combination of 3 mg/l IAA. Average number callusing frequencies were 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 (Table 1). These induced callus was excreted some secondary metabolites in medium it problematic to maintaining the fresh calli and resulting media turn to change colored into brownish and black. Addition of different concentration in charcoal or ascorbic acid like 20 mg/l, 25 mg/l, 30 mg/l subsequently overcome this problem.



(a) (b)

Photo plate, showing (a) callus from leaf explant, (b) callus from axillary shoots explant

Photo plate, showing (a) callus from leaf explant, (b) callus from auxiliary shoots explants



(c) (d)

Photo plate, showing (c) and (d) formation of apical Shoot

Table 1 effect of various growth regulators on callus initiation

explant	Concentration of growth regulator (mg/l)				Frequency of Callus formation	Number of Shoot/ callus
	BAP	KIN	IAA	IBA		
Axillary leaf	4.0	3.0	0.5	-	+	-
	4.5	3.5	1.0	-	+++	-
	5.0	4.0	1.5	-	++	-
	5.5	-	2.0	-	+++++	-
	6.0	-	2.5	-	++++++	-
Apical shoot	4.0	-	-	0.5	+	1
	4.5	-	-	1.0	+++	1
	5.0	-	-	1.5	+++	-
	5.5	-	-	2.0	++++	-
	6.0	-	-	2.5	++++	-

***Callus formation less or more**

In vitro shoot induction

Growth regulators must be added selectively to culture media for the shoot initiation, via 3.0 mg/l KIN, 4.0 mg/l KIN, 4.0 mg/l BAP, 5.0 mg/l BAP, 6.0 mg/l BAP either alone or with combination of separately 0.4 mg/l IAA, 0.8 mg/l IAA. Significantly higher number of shoots regeneration were recorded in various concentration of BAP like, 3.5 mg/l, 4.0 mg/l followed by different combination of BAP and KIN 3.0 mg/l + 3.2 mg/l respectively. Addition of IAA in combination of BAP and KIN either alone or with combination separately, there was no significant effect of IAA with any combination of BAP and KIN on shoot formation (Table 02). Average numbers of shoots proliferation were found in combination of BAP and KIN like, 3.5 mg/l + 3.0 mg/l, 4.0 mg/l + 3.5 mg/l respectively. After 20-25 days proliferation of shoot were excrete some phenolic component resulting media get turn change its colour, so addition of various concentration in charcoal or ascorbic acid viz, 20 mg/l, 25 mg/l, 30 mg/l subsequently overcome this problem.

Table 2: Effect of BAP, KIN and IAA concentrations on shoot formation of J. curcas

Explant	Concentration of growth hormones (Mg/L)			% of shoot formation	Shoot length (Mean± SE) (cm)
	BAP	KIN	IAA		
Apical shoot	3.0	3.0	-	20	2.80 ± 0.300
	3.5	3.2	-	40	3.64 ± 0.256
	4.0	3.4	-	30	4.10 ± 0.113
	4.5	3.6	-	15	6.64 ± 0.129
	5.0	3.8	-	10	4.12 ± 0.115
	3.0	-	0.2	20	2.64 ± 0.129
	3.5	-	0.4	30	2.52 ± 0.122
	4.0	-	0.6	40	2.62 ± 0.139
	4.5	-	0.8	35	1.88 ± 0.073
	5.0	-	1.0	30	1.70 ± 0.130

***After 25 days mean ± SE of 5 replicate**

After proliferation of shoot, these were transfer into fresh MS medium containing 3% sucrose, 6 gm/l agar with supplemented various concentration of BAP, KIN and IAA. Use of IAA in the MS medium no significant effect of shoot regeneration was observed. In *Jatropha curcas*, cytokinin mainly BAP and KIN was required for callus initiation and shoot proliferation. The results obtained from the present investigations revealed to the previous studied in Jatropha curcas KIN is important to enhance shoot proliferation while increased levels (2 mg/land 3mgL/1) of BA inhibited shoot proliferation, There was no significant effect of NAA level on shoot proliferation, Therefore the effect of other auxins (IAA and IBA) and lower concentrations of NAA on shoot proliferation need to be further examined, (P.S. Warakagoda and S. Subasinghe 2009).

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