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In Vitro Plant Regeneration from Shoot Tip Explants Of Jatropha Curcas L: A Biodiesel Plant

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ABSTRACT

In vitro regeneration was achieved from shoot tip explants of 4 months old Jatropha curcas plant. Propagation from shoot tip was evaluated on a range of concentrations of Benzyl adenine (BA) with indole-3-butyric acid (IBA) as 2.22 µM/L BA and 4.9 µM/L IBA, 1.11 µM/M BA and 0.48 µM/L IBA, 0.42 µM/L BA and 0.46 µM/L IBA and 0.44 µM/L BA and 0.44 µM/L IBA. Higher regeneration potential that direct adventitious shoot induction was recorded highest on MS medium with 2.22 µM/L BA and 4.9 µM/L IBA. Regenerated shoots then rooted on half strength MS medium supplemented with IBA (0.5µM). Following simple hardening procedures, the in vitro raised plants were transferred to soil and grown to maturity in the field.

Keywords: *Jatropha curcas, Shoot tip explants, Plant regeneration, Bidiesel plant, in vitro culture.*

1. INTRODUCTION

Jatropha curcas Linn is an oil bearing tree or shrub that grows in almost all subtropical and tropical areas. It has been identified as sustainable biodiesel feedstock because it is a non food crop that can grow without much water. Jatropha belongs to the family Euphorbiaceous and has been considered one of the most promising alternatives for biofuel production and thus a relevant economic crop (Kumar and Reddy 2012). The genus Jatropha comprises over 175 native species, occurring in South to Central America (Mukherjee et al. 2011), Asia and Africa (Opens haw 2000, Kumar and Reddy 2012). The most important aspect of this species is its large potential for biofuel production, owing to high oil content of the seed, rapid growth and stiffness of the plant (Mukherjee et al. 2011).

It is a wild growing hardy plant that well adapted to harsh conditions of soil and climate (Katwal et al., 2003). Moreover, it can be conveniently propagated from seeds, branch cuttings, grafting as well as by tissue culture. Jatropha curcas seeds contain about 30-35 percent of non-edible oil, an efficient substitutes for diesel engines (Bhasubutra and Sutiponpeibun, 1982, Heller 1996, Gubitz et al., 1999,Henning, R. 2002 and Deng et al 2010).

As an oil bearing biomass feedstock, it can ensure an alternative source of energy and reduce our dependency on fossil fuel. This plant can grow anywhere including soil considered infertile for food production, and can live for about 50 years (Dager 2006, Henning, 2010).

The plant is important for climate change issues as a mature plant or tree absorbs around 18 lbs of carbon dioxide (CO_2) per year. So cultivating Jatropha in one hectare of land can sequester around 20 tons of CO_2 annually (Benard Muok, 2008)

Additionally, the oil derived from the plant seeds directly can be used as a replacement for kerosene cooking fuel, to light lamps and for small farming machineries in rural areas (Cerrate et al 2006). It can also be converted into biodiesel for use in engines. 1000 grams of Jatropha oil can produce 980 grams of pure biodiesel. However most optimized practical processes yield around 94% of biodiesel (Alkabbashi et al 2009), which emits 80% less CO_2 and 100% less SO_2 than fossil diesel (Tiwari et al., 2007).

Jatropha curcas L is as a sustainable source of second generation biodiesel feedstock species and the overall supply can be increased with different propagation technologies. In addition, the plant can grow in drought, and also in different types of soil (Nahar 2011).

Due to the increasing demand for biofuel, breeding programs of J. curcas have been established in distinct countries, for instance Brazil, India, Senegal and Cape Verde (Divakara et al. 2010). In this context, in vitro tissue culture techniques have mainly been performed for mass clonal propagation of J. curcas elite lines (Kalimuthu et al. 2007). For this purpose, in vitro regeneration of J. curcas plantlets has been achieved mainly through organogenesis (Rajore and Batra 2005; Jha et al. 2007; Kumar and Reddy 2012) and embryogenesis procedures.

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). The 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, Achten et. Al., 2008).

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Therefore specific objectives of the present study to produce optimize protocol in vitro propagation of Jatropha curcas. Plant regeneration by tissue culture technique would be a feasible alternative for improving the quality and production of Jatropha curcas plant. So in vitro plant regeneration is the best method available for the production of high quality plants which are free of any disease and pest ensuring the maximum production potential of varieties that are genetically identical to parent plant as well as to one another (Raven et al., 1999)

So the objective of the present study was to investigate the effect of different growth regulators on number of shoots, lengths of shoot/leaf and to develop an effective in vitro method for plant propagation of Jatropha curcas and the propagated plants were successfully established in field conditions.

2. MATERIAL AND METHODS

Shoot tip explants of Jatropha curcas were collected from earthen pot grown plant of author's roof top garden in early spring. Explants were excised from 4 months old plant were surface sterilized by cleaning thoroughly under running tap water for 20 minutes and washed with commercial detergent followed by running tap water and rinse with distilled water.

The plant materials were washed with 70% ethanol, 0.1% $Hgcl_2$ (W/V) for 3 minutes and rinse with sterile distilled water for 3 to four times under aseptic condition to remove traces of $Hgcl_2$. The explants were cultured on MS containing 3.0% sucrose (W/V), 0.8% agar and BA (2.22 to 0.44 µM/L) an IBA (4.9 to o.44 µM/L) for multiplication of plants (Table 1). The P^H of all the media was adjusted to 5.8 before autoclaving at 121^o centigrade, 15lb pressure for 20 minutes. Cultures were incubated in a culture room at 25^o centigrade under 16/8 hr photoperiod by cool white fluorescent tubes (Phillips, India). The shoot numbers, lengths including leaf, petiole lengths were measured.

Table 1: Different growth regulators combination with MS used for multiple shoot induction

Media	Growth Regulators Combination
MS_1	2.22 µM/L BA + 4.9 µM/L IBA
MS_2	1.11 µM/L BA + 0.48 µM/L IBA
MS_3	0.42 µM/L BA + 0.46 µM/L IBA
MS_4	0.44 µM/L BA + 0.44 µM/L IBA

Well-developed shoots were transferred for root induction on half strength MS medium supplemented with IBA (0.5µM). For ex vitro establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual nutrient from the plant body. The regenerated plantlets were then transferred to plastic cups containing sterile soil, sand, compost (1:1:1) and covered with polythene and maintained in tissue culture conditions. The well-developed plantlets were transferred to bigger earthen pot, kept in greenhouse and finally transferred to the field.

3. RESULT AND DISCUSSION

Shoot tip explants of 2 cm each were cultured on the MS media supplemented with different concentrations of BA (2.22 to 0.44) and IBA (4.9 to o.44). The callus formation including Plant regeneration showed better response within 6 weeks of culture in medium 2.22 µM/L BA and 4.9 µM/L IBA compared to other media combinations applied. Higher number of multiple shoot induction was observed in 8 weeks with higher shoot, petiole and leaf length (Table 2) in the above mentioned concentration. This result also confirm the findings of Kumari et al., 2008, Nahar & Borna, 2012 and Rajore et al., 2007,those who demonstrated the effect of growth regulators on the callus formation and also multiple shoot formation from the callus of Ricinus communis and Jatropha curcas respectively.

Table 2: Response of multiple shooting in different media

Media	No of shoot per explants (av)	Shoot length (cm)	Leaf Length (cm)	Petiole Length (cm)
MS_1	4.0	2.21	1.6	1.3
MS_2	2.0	1.54	1.0	0.9
MS_3	1.0	0.9	0.72	0.50
MS_4	1.0	0.8	0.70	0.52

Well-developed shoots were then transferred for root induction. The regenerated plantlets were successfully hardened off and finally established in natural soil.

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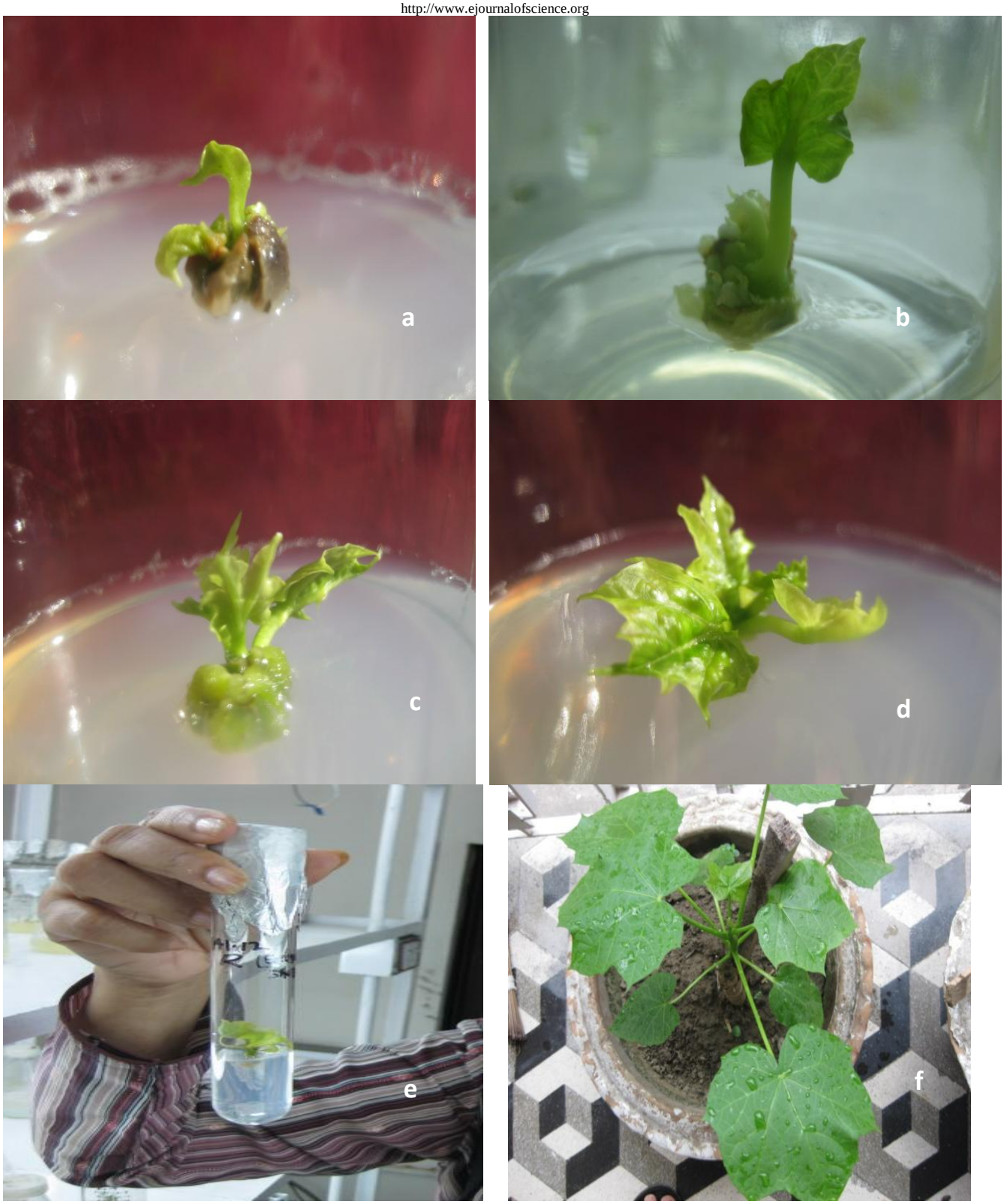

Fig 1: Plant regeneration at different stages of the transformation procedure. a-b. Multiple shoot initiation, c-d. Multiple shoots, e-f. Rooting and well established plant in the earthen pot

In conclusion the micro propagation protocol was established from shoot tip explants of Jatropha curcas. The rehabilitation micro propagation development total process was completed in 60 days. The procedure demonstrated the appropriate culture medium for in vitro induction of multiple shoots from shoot tip. The development of appropriate techniques for in vitro culture and micro propagation of oil crops is necessary for germplasm collections, breeding program and mass propagation. So the present protocol advocated

the use of enhanced concentration of BA and IBA in MS Basal medium to improve regeneration of in vitro oil producing plant species Jatropha curcas for production of Biofuel.

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