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Micropropagation of *Jatropha curcas* L. through *in vitro* culture of shoot tips

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ABSTRACT

Jatropha curcas L. is a promising source of biodiesel. An efficient protocol was developed for induction of multiple shoots via *in vitro* culture of shoot tip explants of the *Jatropha curcas* following inoculation on MS (Murashige and Skoog, 1962) medium supplemented with different concentrations and combinations of cytokinins (BAP/Kinetin) and auxin. The best response achieved on MS medium fortified with BAP 2.0 mg/l + IBA 0.5 mg/l. Maximum rooting was observed on half-strength MS medium with 2.0 mg/l IBA. After successful acclimatization, 70% of the *in vitro* derived plants survived.

1. Introduction

In a country reeling under the burden of a large scale import bill and spiraling oil prices, biodiesel is a promising indigenous and renewable source of energy. The demand for plant based feedstock for biodiesel production has received much attention in recent years due to green energy policy vis-a-vis blending requirement of diesel adopted by many countries (Rajgopal and Zilberman, 2007). European Union and India have set targets of 10% and 20% replacement of transport fossils fuel with biodiesel respectively by 2020 (Biswas *et al*., 2010; Rosch and Skarka, 2009). In different countries, major sources of biodiesel include rapeseed in USA, sunflower in Italy and Southern France, soybean in Brazil and USA, oil palm in Malaysia, linseed in Spain, cottonseed in Greece, beef tallow in Ireland and *Jatropha* in Nicaragua and South America (Jaysingh, 2004). Among the potential crops, *J. curcas* has attracted the interest of various developmental agencies in the tropics and subtropics due to its easy adaptability to semiarid marginal sites, use of the oil as a diesel fuel substitute and its use in erosion control (Sujatha *et al., 2005*)

Jatropha curcas (Euphobiaceae) is a drought resistant, perennial plant yielding 5-12 tons of oil seeds per hectare and produces 2-4 tones of biodiesel. It may also transform the poorest people and the use of most marginalized land into a source for encasing energy (Kochar *et al*., 2005). It is a shrub and starts producing fruits from the second year, which is stabilized by the fourth year.High density plantation of *Jatropha curcas* as energy crop may provide energy on a regular basis, annually, for a period of 40-50 years.Even in the infertile soils without replanting, unlike other fuel wood crops, it gives more seeds (Dagar, 2006; Hinning, 2002).

Seeds of *Jatropha curcas* contain 40-50% semidrying oil called as curcas oil. This oil possesses numerous medicinal and economic properties. It is an efficient substitute fuel for diesel engines and forms an essential ingredient in

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various soaps, dyes and wood industries. Moreover, the plant is very reliable in curing several diseases like rheumatism, leprosy, scabies, eczema, ringworm, chronic dysentery, abdominal complaints, anemia, fistula and diseases of heart (Rajore and Batra, 2005). The oil is high in octane value and can be used directly in diesel engines which is aclean fuel reducing green house gas emissions. It has greater lubricity and reduces engine wear. Pure *jatropha* oil is also non-toxic in nature.

India as well as the whole world has got growing demand of energy and transport fuel, where *Jatropha curcas* has the potential to become one of the world's key energy crops. However, inexpensive biodiesel can be producedfrom India's vast agro biotechnological resources offering a clean substitute for expensive fossil fuel imports, thus enabling the countries to meet the objectives of economic growth, fuel security and cleaner air. *J. curcas* is not only the most primitive species of the genus, but also it forms artificial and natural hybrid complexes posing a serious problem to the genetic fidelity (Pravakaran and Sujatha, 1999). On the other hand conventional agricultural practices using seeds and cuttings of stem partsfor its propagation are not feasible. In the species, as seeds areheterozygous, seed setting has been reported to be low.The alternative process of propagation through vegetative cuttings are not deep rooted which are easily uprooted as they do not form a taproot system (Sujatha *et al*., 2005). Hence tissue culture techniques offer rapid and continuous supply of the planting materials. Protocols for high frequency shoot regeneration from various explants of *J. curcas* have been developed by various workers (Sujatha and Mukta, 1996; Sujatha *et al.,* 2005; Jha *et al*., 2007). The present investigation has been undertaken to develop *in vitro* propagation techniques for non toxic *J. curcas* through shoot tip explants.

2. Materials and methods

2.1 Explant preparation and culture condition

The seed materials of the non-toxic variety of *J. curcas* was obtained from an elite tree (6 yr) growing in the experimental garden of Utkal University, Vanivihar, Bhubaneswar (Position - Lat. 20Ú40'00"N, Long. 085Ú50'00"E). Shoot tip explants from the apex were collected from 3-month old plantlets (juvenile shoots) and from the mature plant. The explants were surface sterilized with 0.1% mercuric chloride for 10 minutes followed by five rinses in sterile distilled water. The shoot tip explants were trimmed (1-1.5 cm) at the base and cultured with the cut surface in contact with the medium surface. Culture medium consisted of MS basal medium (Murashige and Skoog, 1962), with 30gm/l sucrose and 8gm/l agar. All the cultures were incubated at 26±2ÚC on a 16h photoperiod of 30 µmol/m^2 s irradiance level provided with coal white fluorescent tubes (Philips, India) and with 55-60% relative humidity.

2.2 Initiation of shoot bud proliferation

For shoot bud proliferation shoot tip explants were cultured on agarised MS basal medium supplemented with Kinetin (0.5-2.5Mg/l), BAP (0.5-2.5mg/l) individually and with combination of IBA(0.5 mg/l). The pH of media was adjusted to 5.8 with 0.1 N NaOH or 0.1 N HCl prior to addition of agar. The responding explants were transferred to medium supplemented with combination of BAP+IBA. Sub culturing of the induced explants was carried out with a regular interval of 20-25 days. For each treatment, 10 replicates were taken.

2.3 In vitro root induction and acclimatization of regenerants

For root induction on shoots regenerated from shoot tip, half strength MS medium containing 30 g/l sucrose and 8 g/l agar were used. The medium was further supplemented with IBA (0.5-2.0 mg/l), NAA (0.5-2.0 mg/l). After 5 weeks, the rooted plantlets having well developed roots were taken out from culture tubes, washed thoroughly with sterile water to remove the adhering medium and transferred to plastic cups (60x10mm) containing sterile vermiculite saturated with micronutrients and incubated in the green house under temperature 28 $\pm 1^0$ C. After 4 weeks of acclimatization, they were transferred into poly bags containing soil + sand + farm yard manure (FYM; 1:2:1) and kept for 3 weeks in the shade house and finally transplanted to the field.

3. Results and discussion

3.1 In vitro shoot proliferation

The results obtained in the shoot tip cultures clearly indicated that the response was dependent on the origin of the explants. Shoot tip explants obtained from juvenile shoots were more responsive than the explants obtained from the mature shoots. Lack of response in the explants of the mature shoot is due to the exudation of phenolic compounds from the cultured explants.This is inaccordance with the results obtained in species like *Eucalyptus tereticornis, E. camaldulensis* and *Tectona grandis* (Das and Mitra, 1990; Gill and Gill, 1994; Devi *et al*.,1994). Shoot tips cultured on MS medium containing cytokinin (BAP/Kinetin) singly as well as in combination with IBA showed varied response with respect to number of shoot buds obtained per explants. However, BAP with IBA showed the best result with maximum response (75%) than only BAP supplemented

medium where maximum response was 60%. Kinetin, alone and along with IBA did not show significant result as compared to BAP. This shows close resemblance with work done on shoot tip of *J. curcas* (Nahar and Brona, 2013; Dutta *et al*., 2007). Depending on the media composition shoots ranging from 2-7 in numbers were developed within 10-12 days from the day of inoculation.

3.2 Regeneration of plantlets from shoot tip explants

Shoot tips cultured on MS basal medium without growth regulators did not induce any morphogenetic changes and exhibited elongation of the existing shoot tip into a single shoot. Shoot tips cultured on MS basal medium with BAP 2 mg/l + IBA 0.5 mg/l showed better response where average of 3.4±0.5 shoots produced per explant (Table 1). Somehow it was varied from the results obtained in the shoot tip culture of *J. curcas* where BAP combined with IAA showed better response (Rajore and Batra, 2005). This may be attributed to the concentration of auxins for shoot proliferation (Fig.1). Higher number of multiple shoot induction was observed in 6 weeks with increase in shoot length. This result also confirms with the effect of growth regulators on the callus formation and also multiple shoot formation from the callus of *Ricinus communis* and *Jatropha curcas* respectively (Kumari *et al*., 2008; Nahar and Borna, 2012 and Rajore *et al.,* 2007*).*

3.3 In vitro root induction and acclimatization

In vitro root induction was obtained when elongated shoots were transferred to the half strength MS medium containing different concentrations of NAA or IBA (Table 2). Auxins had a stimulatory influence on root induction. Depending on the type and concentration of auxin, root number ranged between 2-7 per shoot. Roots were initiated between 10-12 days of culture. Shoots cultured on NAA supplemented medium showed a relatively low rooting

Table 1

Effect of plant growth regulators on multiple shoot inductionfrom shoot tips of *Jatropha curcas*

Plant growth regulators (mg/l)			Percentage of response	Days to shoot bud initiation	Mean shoot no.±S.E.	Mean shoot length (cm)±S.E.
BAP	Kin	IBA				
0.5			40	10-12	1.5±0.5	1.8±0.2
1.0			50	10-12	2.1±3.1	2.0±0.1
1.5			40	10-12	2.2±0.2	2.0±0.2
2.0			60	10-12	2.8±0.3	2.2±0.1
2.5			40	10-12	2.2±0.2	2.0±0.2
	0.5		NR	NR	NR	NR
	1.0		40	14-16	2.0±0.6	2.2±0.1
	1.5		40	14-16	1.8±0.2	2.0±0.2
	2.0		40	14-16	1.5±0.5	1.5±00
	2.5		20	14-16	1.0±0.0	1.0±0.0
0.5		0.5	40	10-12	2.0±0.4	2.0±0.2
1.0		0.5	50	10-12	2.8±0.3	2.2±0.1
1.5		0.5	50	10-12	2.5±0.5	2.2±0.2
2.0		0.5	75	10-12	3.4±0.5	2.5±0.2
2.5		0.5	40	10-12	2.0±0.0	1.8±0.2
	0.5	0.5	NR	NR	NR	NR
	1.0	0.5	40	14-16	2.2±0.2	2.0±0.2
	1.5	0.5	40	14-16	2.0±0.4	1.8±0.2
	2.0	0.5	40	14-16	1.8±0.2	1.8±0.2
	2.5	0.5	20	14-16	1.3±0.3	1.5±0.0

NR: No Response

Table 2

Effect of auxins on root induction in regenerated shoots of *Jatropha curcas* L.

Plant growth regulators (mg/l)		Percentage of Response	Days of root initiation	Mean root number ±SE	Mean root length(cm)±SE
IBA	NAA				
0.5		40	12-14	2.0±0.2	1.7±0.2
1.0		40	12-14	2.3±0.3	2.0±0.2
1.5		60	12-14	2.2±0.2	2.0±0.2
2.0		75	12-14	3.0±0.2	2.4±0.4
	0.5		NR	NR	NR
	1.0		14-16	2.0±0.2	1.7±0.2
	1.5		14-16	1.8±0.1	1.5±0.2
	2.0		14-16	1.5±0.5	1.2±0.2

NR: No Response

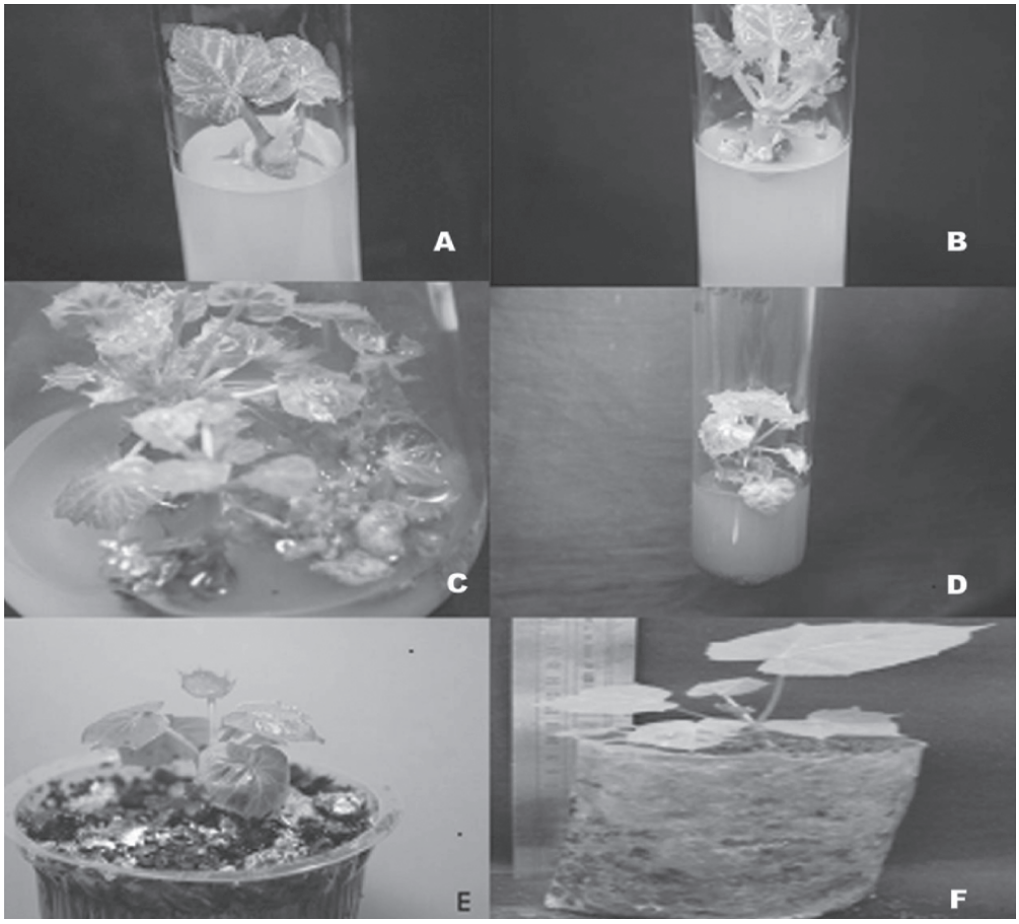

Fig. 1 **A.** Shoot tip culture on MS +2mg./l BAP. **B.** Shoot tip cultured on MS + 2.0 mg /I BAP+0.5 mg/l IBA showing shoot formation (2 week old). **C.** Shoot elongation cultured on MS + 2.0 mg/l BAP + 0.5 mg /I IBA (After 3 weeks of culture). **D.** Rooting of microshoot in K MS + 2.0 mg /I IBA (5 weeks old). **E.** Rooted plantlet replanted in the vermiculite medium in the green house (After 2 weeks of transplantation) **F.** Hardened plant showing new shoots (After 4 weeks of transplantation).

response.Root formation was accompanied by heavy callusing at the basal portion of the shoots. However, the maximum rooting response was observed on ½ MS+IBA (2 mg/l) which produced maximum number of roots (3.0±0.2). The superiority of IBA over NAA and other auxins in rooting of micro shoots has been discussed in a no .of tree and shrub species (Pradhan *et al.,*1998; Ndoye *et al*., 2003; Hansdah *et al*., 2011; Rajore and Batra, 2005).

Plantlets with well developed roots were carefully removed from the culture flasks and placed in plastic cups filled with vermiculite and diluted MS basal salts and kept for 4 weeks, following their transfer to polybags and kept in shade-net house containing a mixture of soil, sandand farm yard manure (FYM). New shoots formation was observed in the plants after second week of transfer with 70% survival rate. The present study has established a simple and efficient protocol which has the potentiality for rapid propagation of elite *Jatropha* plants for raising large-scale plantation.

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