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Jatropha curcas L. and *Ricinus communis* L.: *In vitro* Plant Propagation from Shoot Tip Explants for Commercial Cultivation and Biofuel Production

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Authors' contributions

This work was carried out in collaboration between both authors. Author KN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author RSB managed the analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

In vitro propagation was achieved from shoot tip explants of 4 months old bioenergy crops, Jatropha (*Jatropha curcas* L.) and castor bean (*Ricinus communis*) plants. In both genotypes, propagation from shoot tip was evaluated on a range of concentrations of Benzyl adenine (BA) combined with indole-3-butyric acid (IBA) as 2.22 µM BA and 4.9 µM IBA, 1.11 µM BA and 0.48 µM IBA, 0.42 µM BA and 0.46 µM IBA and 0.44 µM BA and 0.44 µM IBA. Higher regeneration potential that direct adventitious shoot induction was recorded highest in Jatropha on MS medium

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with 2.22 µM BA and 4.9 µM IBA while in castor bean on MS medium with 1.11 µM BA and 0.48 µM IBA. Regenerated shoots, rooted on half strength MS medium supplemented with IBA (0.5 µM). Following simple hardening procedures, the *in vitro* raised plants were transferred to soil for commercial cultivation and grown to maturity for seed production from which crude oil were obtained.

Keywords: Jatropha curcas; Ricinus communis; shoot tip explants; plant regeneration; in vitro culture; bioenergy plant.

1. INTRODUCTION

The recent years due to high cost of conventional or fossil fuel energy, public attention has focused on non-conventional or renewable sources of fuel from plant based biomass. Thus, the growing of oil producing energy crops on large scale for biofuel to supplement fossil oil has become a lucrative agribusiness for both private and commercial farmers. The production of biofuel has the potential to reduce our dependency on fossil fuel and to create an environmental friendly environment all over the world**.**

Jatropha curcas L and *Ricinus communis* L the two important, oil bearing and second generation biomasss feedstocks have been considered as important bio energy crops [1,2]; that grows in almost all subtropical and tropical areas and gaining attention for producing biofuel as biodiesel in developed as well as in developing countries. Both the energy crops belong to the family Euphorbiaceae, produce unique seed oil with numerous industrial as well as medicinal applications [3-8]. They have been considered as the most promising alternatives for biofuel production and also identified as sustainable biodiesel feedstocks with high oil content of the seeds, rapid growth, stiffness of the plant, adaptable in harsh soil conditions, with minimum water and maintenance [9-12]. These plants can grow anywhere including soil considered infertile for food production, and can live for about 40-50 years [13,14]. As important oil bearing biomass feedstocks, they could ensure alternative source of energy all over the world.

The seed of Jatropha and castor bean contain 25-30% and 58-60% oil respectively highly viscous, non-edible, substitute of kerosene. The oil have been used in farming machineries and as lamp oil in some rural areas [15-17] of developing countries. As combustible oil which can be used without refining [11,18] and with good physicochemical properties make them commercially viable alternative to fossil fuel [19,20], used in the internal combustion engines in airplanes [21] and are efficient substitutes for diesel engines [22-26] and having high economic potential for large scale of industrial use [27-29].

After oil extraction, Jatropha and Castor bean oil cake could be used as rich source of organic fertilizer containing high content of nutrients especially Nitrogen, Phosphorus and Potassium and can be used for fish or animal feed (if detoxified), bio pesticide, to power electricity plants, or as biogas [30-35].

Also the oil contains a comparatively high percentage of unsaturated fatty acid (79.34%) resulting in characteristically low levels of free fatty acids, which improves storability. The high iodine value and the presence of unsaturated fatty acids enables it to remain fluid at lower temperatures. The low sulfur content equates to less harmful sulfur dioxide (SO2) emitted when the fuel is burnt. The crude oil could be converted to biodiesel by transesterification process with the byproducts oil/seedcake and glycerin. Biodiesel could be used in farming machineries, irrigation pumps, building generator and transportations with greater environmental and socioeconomic benefits than conventional carbon-based fossil fuels [36,37,20].

In spite of their high economic/industrial values and comparative advantage over food crops as renewable energy sources, oil producing crops have low seed viability making large scale commercial propagation difficult. The plants can be conveniently propagated from seeds, branch cuttings, grafting as well as by tissue culture. The alternative mode of propagation through *in vitro* tissue culture from plant explants is highly recommended for commercial cultivation. There have been reports of successful regeneration of both plant species through *in vitro* culture using seed, hypocotyl, shoot, nodal cuttings, peduncle, leaf and shoot explants, thus by embryogenesis or organogenesis [38-44] procedures.

In this context the *in vitro* tissue culture techniques have been performed recently for

mass clonal propagation [45,46] for commercialization.

Thus the regeneration and transformation of plant tissues can be used to develop an efficient protocol for propagation [47] especially shoot explants inoculated in media as they show faster morphological changes [48].

Plant regeneration by tissue culture technique would be a feasible alternative for improving the quality and production of the two mentioned biofuel plants to produce commercially. 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. *In vitro* propagation, therefore can be used to produce a large number of plants that are genetically identical to parent plant as well as to one another [49,50].

The purpose of the present study was to produce an optimized protocol for *in vitro* propagation of energy crops. So the main objective of the present study was to investigate the effect of different growth regulators on number of shoots, lengths of shoots, petioles and leaves to develop an effective in vitro method for plant propagation of Jatropha and castor bean.

2. MATERIALS AND METHODS

Shoot tip explants of *Jatropha curcas* and *Ricinus communis* were collected from earthen pot grown plant in early spring. Explants excised from 4 months old plants derived from seed 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. Further sterilization was done under aseptic conditions inside the laminar air flow hood. Explants cut into segments of approximate size 1 cm were surface sterilized with 70% ethanol for 1 minute followed by 3 minutes treatment with 0.01 $HgCl_2$ (W/V) 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 [51] containing 3.0% sucrose (W/V), 0.8% agar with BA (2.22 to 0.44 µM) and IBA (4.9 to 0.44 µM) at concentrations and combinations listed in Table 1. The pH of all the media was adjusted to 5.8 before autoclaving at 121°C, for 20 minutes. Cultures were incubated in a culture room at 25°C under 16/8 hr photoperiod by cool white fluorescent tubes (Phillips, India). The explants were placed in 12 culture vessels with 2-3 explants per vessel (3 vessels/ one media concentration). The explants were found to be swollen within 3 weeks of inoculation. The Initiation of the multiple shoots were observed within 6 weeks of culture and higher no of multiple shoot proliferation were found with better growth responses after 8 weeks of culture The shoot numbers, lengths including leaf, petiole lengths were measured.

Well-developed shoots were transferred for root induction on half strength MS medium supplemented with IBA (0.5 µM). For *ex vitro* establishment, well rooted plantlets were rinsed thoroughly with sterile water to remove residual agar medium from the plant body. The regenerated plantlets were then transferred to plastic cups containing sterile soil, sand and farm yard manure (1:1:1) and covered with polythene and maintained in tissue culture conditions. The well-developed plantlets were successfully hardened off and finally established in natural soil for commercial cultivation and seed production from which biofuel could be achieved.

3. RESULTS AND DISCUSSION

The callus formation including regeneration showed response within 6 weeks of culture in medium 2.22 µM BA and 4.9 µM IBA for Jatropha and 1.11 µM BA + 0.48 µM IBA for castor compared to other media combinations applied. Higher number of multiple shoots induction were observed in 8 weeks with higher shoot, petiole and leaf lengths of both the plant species (Table 2 & 3) in the above mentioned concentration. This result also confirm prior findings [3,40] 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 1. Different growth regulators combination with MS used for multiple shoot induction in *Jatropha curcas and Ricinus communis*

Media	Growth regulators combination
MS_1	2.22 µM BA + 4.9 µM IBA
MS_2	1.11 µM BA + 0.48 µM IBA
MS_3	0.42 µM BA + 0.46 µM IBA
MS_4	0.44 µM BA + 0.44 µM IBA

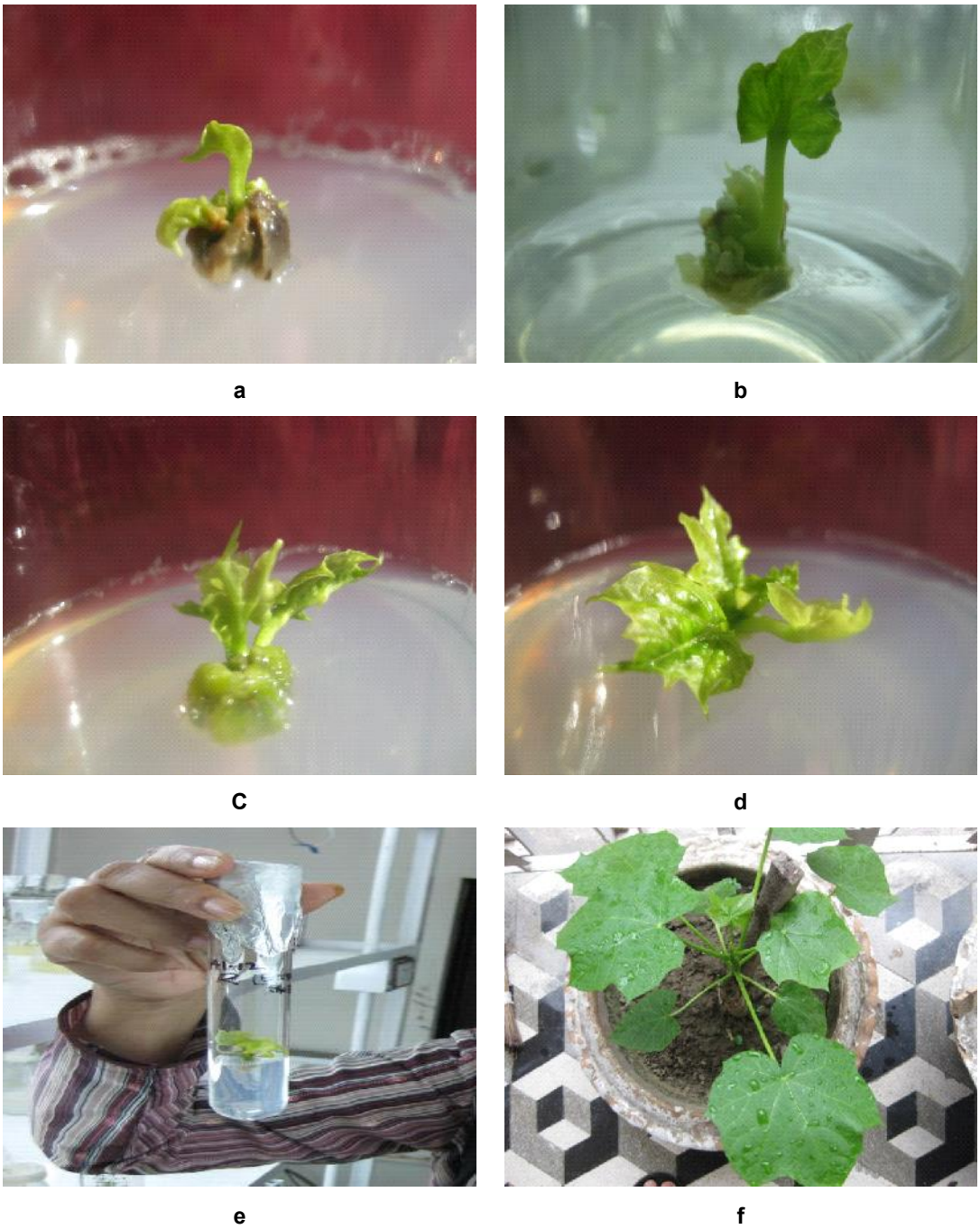

Fig. 1. Micropropagation of Jatropha at different stages of the transformation procedure. a-b. Multiple shoot initiation, c-d. Multiple shoots, e-f. Rooting and well established plant in the earthen pot

The results from the experiment confirms prior findings [52] that indicated successful regeneration of plantlets from shoot tip explants for future genetic transformation of the plants. The callus formation including plant regeneration confirm prior findings [3] who demonstrated the effect of growth regulators on multiple shoot formation from the callus of bioenergy plants. In the present study micro propagation protocol was established from shoot tip explants of the two mentioned bioenergy plants species. The rehabilitation micro propagation development

total process was completed in 8 weeks to 10 weeks. This efficient micro propagation protocol will be useful for conservation, and the improvement of *Ricinus communis* and *Jatropha curcas* using genetic transformation for commercialization and production of biofuel.

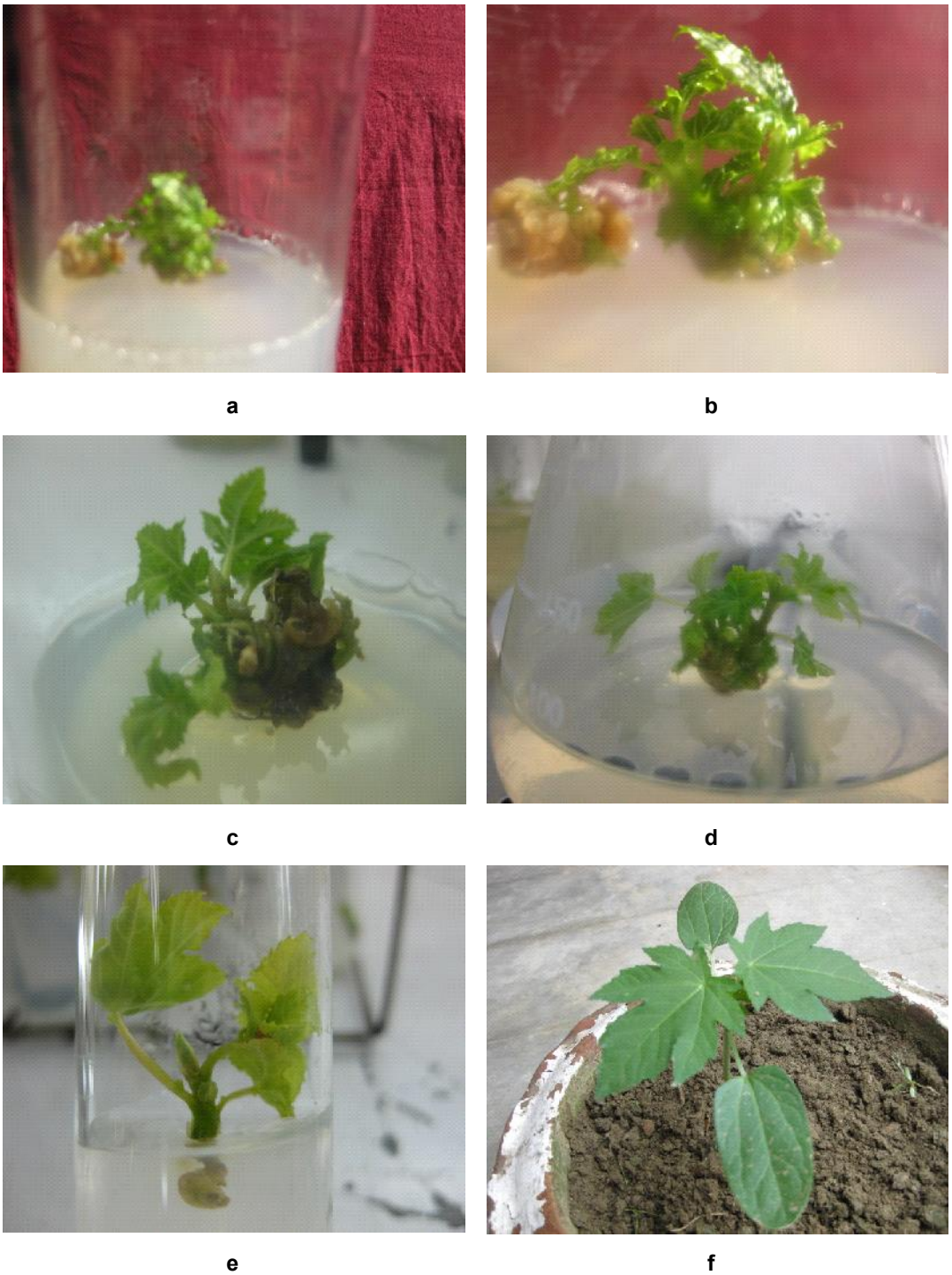

Fig. 2. Micropropagation of castor at different stages of the transformation procedure. a-b. Multiple shoot initiation, c-d. Multiple shoots, e-f. Rooting and well established plant in the earthen

Table 2. Response of multiple shooting of *Jatropha curcas* in different media after 8 weeks of culture

Media	Mean shoots/proliferated explants (n)	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

Table 3. Response of multiple shooting in different media of *Ricinus communis* after 8 weeks of culture

Media	Mean shoots/proliferated explants (n)	Shoot length (cm)	Leaf length (cm)	Petiole length (cm)
MS_1	4.0	1.0	0.9	0.5
MS_2	7.0	2.54	1.5	1.0
MS_3	5.0	1.5	0.99	0.6
MS_4	3.0	0.8	0.70	0.5

4. CONCLUSION

In conclusion the micropropagation protocol was established from shoot tip explants of *Jatropha curcas* and *Ricinnus communis*. The rehabilitation micropropagation 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 explants. The development of appropriate techniques by micropropagation of oilseed crops is necessary for germplasm collections, breeding program and mass propagation for commercialization. So the present protocol advocated the use of enhanced concentration of BA and IBA in MS medium to improve regeneration of *in vitro* oil producing plant species *Jatropha curcas* and *Ricinus communis* for production of biofuel as source of bioenergy or green energy.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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