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Micropropagation Study of *Jatropha curcas* for Enhancing Shoot Induction Frequency

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Abstract

Jatropha curcas, is an upcoming energy source, which promises to mitigate energy crisis and environmental pollution. Jatropha seeds (0.4-12 tons/ha/yr) contain oil (30-40%) which is non edible due to the toxins such as phorbol esters, trypsin inhibitors, lectins and phylates. Various combinations of auxins with cytokinins were used for regeneration study. The best shoot regeneration (80%) was observed in MS medium supplemented with NAA (0.125ppm) and BAP (1.5ppm). Root induction was successfully obtained in plane MS/MS with auxins. Acclimatization and hardening was quite successful with survival rate of 60%.

Highlights

1. Biotype of plant and various plant parts shows variation in regeneration during tissue culture.
2. Best shoot regeneration (80%) was observed in MS medium supplemented with NAA (0.125ppm) and BAP (1.5ppm)
3. Root induction (90%) was successfully obtained in plane MS with survival rate of 70%

Keywords: Biodiesel, *jatropha curcas,* micropropagation, regeneration

Jatropha curcas, commonly known as Ratanjyot, a native of Central America, is remarkable multipurpose tree species belonging to family Euphorbiaceae, containing approximately 175 succulents, shrubs and trees (some are deciduous like *Jatropha curcas* L.). Vegetable oil is a promising alternative because it is renewable, eco-friendly and produced easily in rural areas (Pradhan *et al.,* 2009). As the demand for vegetable oil has increased tremendously, it is impossible to use it for biodiesel production. Whereas, non edible oil sources (Jatropha oil) may be use as an efficient substitute for diesel (Makkar *et al.,* 1997). Semi-drying property of Jatropha oil owes to abundance of linoleic acid and monounsaturated oleic acid. This semi-drying oil could be a proficient substitute for diesel fuel. To meet the demand in future large amount of quality planting material will be needed. Vegetative propagation approach is considered to be most appropriate viable alternative in tree improvement programs. Successful application of the method in Jatropha may help to produce genetically uniform, high oil containing, disease free planting materials throughout the year in spite of seasonal fluctuations. Despite the multifarious potentiality of Jatropha, there are some limitations in propagation of this plant species, as it is a latex containing shrub that makes it recalcitrant for tissue culture (Sardana *et al.*, 1998). Clonal multiplication and deployment studies need to be scaled up, gaps in knowledge need to be plugged and technological package need to be tested widely. Therefore, focus of the

current study is to regenerate elite jatropha with higher shoot regeneration frequency.

Materials and methods

Ten accessions of Jatropha were collected from different geographical locations of India. Among those best accession was selected on the basis of morphological (Seed size, Seed Weight) and biochemical parameters (Oil content, free fatty acid content, saturated fatty acid content etc.) for micropropagation study. The basal MS (Murashige and Skoog, 1962) medium with PVP (chelating agent bonding to ions responsible for activating polyphenol oxidative enzymes) was used with derived supplementation of phytoregulators for callus, shoot and root induction.

Fresh Leaves, stems and apical buds were surface sterilized using bavistin (0.5%), mercuric chloride (0.1%) and 70% ethanol. The medium used for callus induction was MS medium supplemented with NAA/2,4 D (1.0-3.0 mg/L), BAP (0.1-0.5 mg/L). The number of callus and percentage of callus induction frequency was recorded after 35 days. Proliferated callus were sub cultured for shooting (NAA/ 2,4 D: 0.125-0.5 mg/L + BAP: 0.5-4.0 mg/L). The elongated shoots (3-4 leaves, 2.5-3.0 cm height) were excised out from the callus and transferred in root induction medium.

The rooted shoots were removed out from jam bottles and washed with sterile water to remove traces of agar sticking on the roots and dipped in 0.2 per cent (W/V) bavistin solution for 20 min. Shoots were transferred to plastic pots filled with sterile soil and kept in glass house.

Reesults and discussion

Different combinations of NAA/2,4 D and BAP were used for callus induction (Table 1 and 2). White compact callus were obtained by several combinations of NAA/2,4 D and BAP was found to be optimum for obtaining high frequency of nodulated callus (Figure 1A). Callus induction frequency was found higher in petiole and apical buds as compared to leaf. The presence of callus of a segment of initial callus was necessary for callus mediated shoot regeneration (Sujata and Mukta, 1996). Adventitious shoots were induced on calli derived from nodulated callus in different phytohormone combinations (NAA/2,4 D: 0.125-1.0 mg/ L and BAP 0.5-5.0 mg/L)(Table 3,4). Irrespective of the source, the calluses produced shoot clusters up on transfer to a medium with a higher cytokinin/auxin ratio than that used for callus induction. 0.125 mg/L NAA along with 1.5 mg/L BAP was found to be optimum for maximum adventitious shoots regeneration frequency (Figure 1B). Shoot induction shown by different explants varied widely depending on the concentration of NAA/2,4 D and BAP. 2.5-3.5 cm long(3-4 leaf stage) shoots were transferred in plane MS and MS fortified by NAA/2,4 D. Basal MS medium along with NAA/2,4 D (0.5 and 1.0 mg/L) produced good (90 per cent) root induction (Verma *et al.* 2008). Agar gelled full-strength MS and ½ MS medium were found to be the best for rooting (Sujata and Mukta, 1996). This may be due to the higher level of endogenous auxins in *Jatropha curcas*. After 3-4 weeks of culture of shoots on rooting medium, the plantlets were transferred to pots for hardening and acclimatization. Plants were acclimatized with a survival rate of 65 per cent.

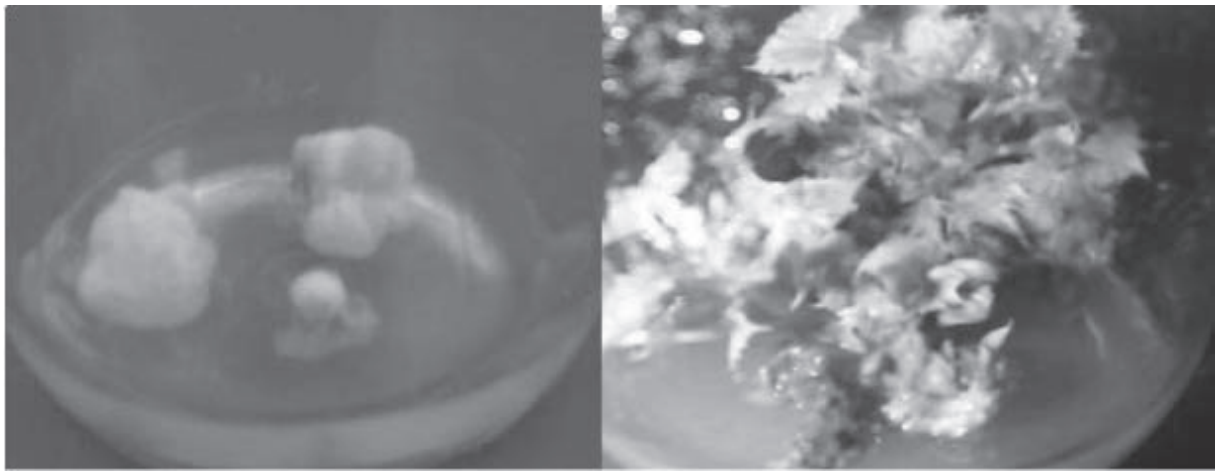

Figure 1: Callus induction(A) and shoot regeneration from *Jatropha Curcas*

Table 1: Callus induction from different explants of *J.Curcas* under different NAA and BAP combinations*

Combination	Days for callus initiation			Callus induction frequency (%)			Texture of Callus		
	P	AB	L	P	AB	L	P	AB	L
$N_{1.0}B_{0.3}$	9-12	7-9	-	75	85	-	White compact	White compact	-
$N_{1.0}B_{0.4}$	9-12	7-9	15	80	85	60	White compact	White compact	Green compact
$N_{1.0}B_{0.5}$	9-12	7-9	15	80	85	50	White compact	White compact	Green compact
$N_{1.5}B_{0.1}$	9-12	7-9	-	85	80	-	White compact	White compact	-
$N_{1.5}B_{0.2}$	9-12	7-9	-	85	80	-	White compact	White compact	-
$N_{1.5}B_{0.3}$	10-13	8-10	-	85	85	-	White compact	White compact	-
$N_{1.5}B_{0.4}$	10-13	8-10	-	80	85	-	White compact	White compact	-
$N_{1.5}B_{0.5}$	10-13	8-10	-	75	65	-	White compact	White compact	-
$N_{2.0}B_{0.1}$	10-13	8-10	-	65	65	-	White compact	White compact	-
$N_{2.0}B_{0.2}$	10-13	8-10	-	70	65	-	White compact	White compact	-
$N_{2.0}B_{0.3}$	10-13	8-10	-	65	55	-	White compact	White compact	-
$N_{2.0}B_{0.4}$	10-13	-	-	60	-	-	White compact	-	-
$N_{2.0}B_{0.5}$	10-15	-	-	55	-	-	White compact	-	-
$N_{2.5}B_{0.1}$	10-15	-	-	55	-	-	White compact	-	-
$N_{2.5}B_{0.2}$	10-15	-	-	55	-	-	White compact	-	-

* Non productive combinations are not shown

P - Petiole AB - Apical bud L - Leaf

C.D. at 1% PGR(A) : 3.18

Explants : 1.42

Interaction(A*B) : 7.11

Table 2: Influence of different concentrations of IAA and BAP on callusing from different explants of *J. curcas**

Combination	Days for callus initiation			Callus induction frequency (%)			Texture of Callus		
	P	AB	L	P	AB	L	P	AB	L
2,4 D-$_{1.0}$B$_{0.3}$	-	10-12	12-15	-	75	50	-	White compact	Green compact
2,4 D$_{1.0}$B$_{0.4}$	-	10-12	12-15	-	75	50	-	White compact	Green compact
2,4 D$_{1.0}$B$_{0.5}$	10-12	10-12	12-15	75	70	50	White compact	White compact	Green compact
2,4 D$_{1.5}$B$_{0.1}$	10-12	10-12	12-15	75	85	45	White compact	White compact	Green compact
2,4 D$_{1.5}$B$_{0.2}$	10-12	10-12	12-15	80	85	45	White compact	White compact	Green compact
2,4 D$_{1.5}$B$_{0.3}$	10-12	12-15	12-15	80	85	50	White compact	White compact	Green compact
2,4 D$_{1.5}$B$_{0.4}$	10-12	12-15	12-15	75	80	50	White compact	White compact	Green compact
2,4 D$_{1.5}$B$_{0.5}$	10-12	12-15	12-15	80	80	55	White compact	White compact	Green compact
2,4 D$_{2.0}$B$_{0.1}$	12-15	10-12	14-18	75	85	50	White compact	White compact	Green compact
2,4 D$_{2.0}$B$_{0.2}$	12-15	10-12	14-18	75	85	50	White compact	White compact	Green compact
2,4 D$_{2.0}$B$_{0.3}$	12-15	12-15	14-18	80	75	55	White compact	White compact	Green compact
2,4 D$_{2.0}$B$_{0.4}$	12-15	14-16	14-18	80	70	60	White compact	White compact	Green compact
2,4 D$_{2.0}$B$_{0.5}$	14-16	14-16	14-18	80	65	50	White compact	White compact	Green compact
2,4 D$_{2.5}$B$_{0.1}$	14-16	14-16	14-18	70	70	45	White compact	White compact	Green compact
2,4 D$_{2.5}$B$_{0.2}$	14-16	14-16	-	70	70	-	White compact	White compact	-
2,4 D$_{2.5}$B$_{0.3}$	14-16	-	-	60	-	-	White compact	-	-
2,4 D$_{2.5}$B$_{0.4}$	14-16	-	-	60	-	-	White compact	-	-
2,4 D$_{2.5}$B$_{0.5}$	14-16	-	-	55	-	-	White compact	-	-

* Non productive combinations are not show

P - Petiole AB - Apical bud L - Leaf

C.D. at 1% PGR(A): 7.33

Explants: 3.28

Interaction(A*B):16.40

Table 3: Effect of NAA and BAP on shoot formation from callus (derived from petiole and apical bud) of *J. curcas*

Combination	Average No. of shoots/callus		Regeneration frequency (%)	
	P	AB	P	AB
$N_{0.125}B_{1.0}$	3	1	70	60
$N_{0.125}B_{1.5}$	4	2	80	60
$N_{0.125}B_{2.0}$	2	1	60	50
$N_{0.125}$-B-$_{2.5}$	2	-	50	-
$N_{0.50}B_{0.5}$	1	-	50	-
$N_{0.50}$-$B_{1.0}$	1	1	50	60
$N_{0.50}$B-$_{1.5}$	2	2	60	70
$N_{0.50}$--$B_{2.0}$	1	-	50	-

P - Petiole AB - Apical bud L - Leaf
C.D. at 1% PGR(A) : 4.47
Explants: : 1.58
Interaction(AXB) : 6.33

Table 4: Effect of IBA and BAP on shoot formation from callus (derived from petiole and apical bud) of *J. Curcas*.

Combination	Average No. of shoots/callus		Regeneration frequency (%)	
	P	AB	P	AB
	P	AB	P	AB
2,4 $D_{0.125}B_{0.5}$	2	-	40	-
2,4 $D_{0.125}B_{1.0}$	3	2	50	60
2,4 $D_{0.125}B_{1.5}$	4	2	70	75
2,4 $D_{0.125}B_{2.0}$	3	1	65	70
2,4 $D_{0.125}B_{2.5}$	2	-	50	-
2,4 $D_{0.125}B_{3.0}$	2	-	40	-
2,4 $D_{0.125}B_{3.5}$	1	-	30	-
2,4 $D_{0.50}B_{1.0}$	1	1	50	40
2,4 $D_{0.50}B_{1.5}$	3	1	60	60

P - Petiole AB - Apical bud L - Leaf
C.D. at 1% PGR(A) : 4.47
Explants : 1.58
Interaction(A*B) : 6.32

Variations among the explants in regeneration frequencies presumably may be due to predisposition of tissues from some organs to more rapid cell divisions than others and the fact that even closely associated tissues from one organ have different potentials. The present study describes a reproducible method for the differentiation of adventitious shoots through callus (derived from vegetative explants) of *J. curcas,* which can be utilized for further improvement for the economic traits of this plant.

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References

Pradhan, R.C., Naikl S.N., Bhatnagar N. and Vijay V.K. 2009. Physical Properties of Tree Borne Oil Seed: Potential Biodiesel Feedstock in India. *Assam University Journal Science and Technology* **4**:46-53.

Makkar, H.P.S., Becker K., Sporer F. and Wink M. 1997. Studies on nutritive potential and toxic constituents of different provenances of *Jatropha curcas*. *Journal Agriculture Food Chemistry* **45**:3152-3157.

Murashige, T. and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiology Plant* **15**:473-497.

Sardana, J., Batra A., Sharma R. and Sardana J. 1998. *In vitro* Plantlet formation and micropropagation of *Jatropha curcas*(L). *Advances in Plant Sciences* **11**: 1627-169.

Sujatha, M and Mukta N. 1996. Morphogenesis and plant regeneration from tissue cultures of *Jatropha curcas*. *Plant Cell Tissue Organ Culture* **44** :135-141.

Verma, K.C. ,Gaur A.K. and Singh U.S. 2008. Evaluation of *In vitro* responses from different explants of elite *Jatroha curcas* L. *Indian Journal of Plant Physiology* **13**:231-237.

