The Bioscan 10(2): 563-566, 2015
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INTERACTION EFFECT OF EXPLANTS TYPES AND PHYTHORMONES ON TISSUE CULTURE OF *JATROPHA CURCAS* SEED EMBRYO

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KEYWORDS
Jatropha curcas
BAP
Kinetin
IBA

Received on :
24.12.2014

Accepted on :
17.05.2015

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ABSTRACT
Reserves of crude oil are rapidly diminishing and the reliability and security of oil supplies has been of global concern. Regenerated plantlets from embryos were separated and inoculated in MS medium supplemented with different combinations of BAP, Kinetin,IBA and 2,4 D. The results showed that 2,4 D(4.0 mg/L) was found to be more efficient for callus development in petiole(94 %) followed in leaf(90 %). The best shoot regeneration (11.5 shoots/explants) was observed in MS medium supplemented with IBA (0.4mg/L) and BAP (2.5mg/L). Root induction was successfully obtained (90-100%) in plane MS and MS fortified with auxins. Acclimatization and hardening was quite successful with survival rate of 80%.Developed regeneration protocol gives fair numbers of shoots per explants over existing protocols of jatropha.

INTRODUCTION

The current annual import bill of crude oil in terms of foreign exchange is around Rs. 60, 4000 crores. India produces only about 30 per cent of its annual crude oil requirement of 105 MT, relying on import to the tune of Rs 90,000 crores per annum for meeting the remaining requirement. Use of less expensive feedstock containing fatty acids such as inedible oils, animal fats and waste food oil and by products of the refining vegetables oils is one of the ways of reducing the biodiesel production costs. Vegetable oil is a promising alternative because it is renewable, environ-friendly, carbon neutral, safe for use in all conventional diesel engines, non-flammable and nontoxic reduces tailpipe emissions, visible smoke, noxious fumes, odors and offers the same performance and engine durability as petroleum diesel fuel [Pradhan *et al.,* 2009 and Makkar *et al.,* 1997]. Biofuel is renewable and benign to environment and has showed a great potential in coping with worldwide energy crisis and the increasingly serious environmental problems (Kumar, 2014).To meet the demand in future large amount of quality planting material will be needed. *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.).Vegetative propagation approach is considered to be most appropriate viable alternative in tree improvement programs, the greatest demand being for increased biomass production by fast-growing trees. Major bottlenecks for the same are availability of high quality planting material throughout the year. However, this is limited due to unavailability of quality planting material. Plant regeneration by tissue culture technique would be a feasible alternative for improving the quality and production of *Jatropha curcas* plant. Tissue culture is a proven means of producing millions of identical plants under *in vitro* condition independent of seasonal constraints and allows further augmentation of elite disease free plantlets. 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).*In vitro* regeneration techniques offer a powerful tool for germplasm conservation, mass multiplication of true-to-type plants and genetic transformation (Kumar and Reddy, 2010). 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 current study is to observe effect of various phytohormones on *in vitro* regeneration by using embryo as explants.

MATERIALS AND METHODS

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. The pH of the media was adjusted to 5.8 using

NaOH or HCl and supplemented with 7g/L agar. Media was autoclaved at 15 psi at 121°C for 20 minutes. Growth conditions were maintained at 26 + 2°C with 16 h photoperiod. Seeds were soaked overnight, washed in sterile distilled water containing Tween-20 for 10 mins. After rinsing with sterile distilled water four times seeds were washed in 70 % ethyl alcohol for 2 minutes followed by 2minutes rising with sodium hypochlorite solution. Finally they were surface sterilized with 0.1 % mercuric chloride for 10 min and then washed with sterile distilled water six times to remove all the traces of chemicals.

The phytohoromes used for callus induction were IBA/2,4 D (0.5-4.0mg/L) and BAP/kinetin (0.1-0.5mg/L).The number of callus and percentage of callus induction frequency was recorded after 30 days. Proliferated callus were sub cultured for shooting (IBA/2,4 D 0.1-0.5 mg/L + BAP/kinetin 1.0-3.0 mg/L). 3-4 leaf stage pantlets were transferred for rooting in plane MS medium and MS medium fortified with IBA/2,4 D. Rooted plantlets were successfully hardened and transferred to green house for commercial cultivation.

RESULTS AND DISCUSSION

Inoculated (80-85 days old) embryos were starting to germinate after 4-5 days and small plantlets were obtained after 15-20 days. A low percentage (4-5%) of embryos germinated normally. This was accompanied with elongation of greenish, thick hypocotyl and a short, white radicle with secondary roots. Radicle part did not elongate rapidly as in the normal conditions(Mohan *et al.,* 2011).Small plantlets were further divided into stem, leaf and petiole and inoculated in various combinations of IBA/2,4 D and BAP/Kinetin for callus induction (Table 1). Stem produced white compact callus, whereas petiole and leaf produced greenish color compact callus. 2,4 D alone and in combination with BAP was found to be optimum for obtaining high frequency of nodulated callus (Fig 1A)(Rao *et al.,* 2006). 2,4 D @ 4.0 mg/L was found to be optimum for callusing (94 %) in petiole and in leaf (90%). Callus induction frequency was found higher in petiole and leaf as compared to stem.2,4-D alone induced soft, friable, and light yellowish callus (Fig. 1) (Kaewpoo and Te-chato, 2009). It was necessary to subculture after every 15 days, otherwise the calli became dark and growth ceased. The darkening of callus was probably due to the production and oxidation of phenolic compounds released by explants (Monacilli *et al.*, 1995).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 (Table 2). Irrespective of the source, the calluses produced shoot clusters up on transfer to a medium with a higher cytokinin/auxin ratio. Shoot induction shown by different explants varied widely depending on the concentration of 2,4 D/IBA and BAP/kinetine (Table 2). Leaf and petiole responses11.5 and 7.5 shoots per explants in BAP (2.5 mg/L) and IBA (0.4 mg/L) (Sujatha *et al.,* 2005, Thepsamran *et al.*, 2008). Similar observations were also recorded in strawberry (Diengngan and Murthy, 2014). Leaf showed higher shoot induction efficiency as compared to stem and petioles (Table 2). 2.5-3.5 cm long(3-4 leaf stage) shoots were transferred in plane MS, ½ MS and MS fortified by IBA/2,4 D. Basal MS medium along with IBA/2,4 D (0.5 and 1.0 mg/L) produced good (90-100 per cent) root induction frequency(Verma *et al.,* 2008, Data not shown).Agar gelled full-strength MS and ½ MS medium were found to be the best for rooting (Sujata and Mukta, 1996, Verma and Nisha, 2013). 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 80 per cent (Martin, 2003).

In current study a simple, rapid and cost effective regeneration protocol has been developed for high frequency regeneration using embryo of elite *Jatroph* genotypes. Auxin independently and along with cytokinine can induce callus. Different explants shown differential ability for callus and shoot induction. These findings indicated that *J. curcas* responds to high auxin and high cytokinin primarily by stimulating cell division. Variations among the explants in regeneration frequencies presumably may be due to predisposition of

Table 1: Interaction effect of hormones and explant types on callus induction and shoot regeneration

Hormone combinations	Callus Induction frequency (%)			Hormone combinations	Callus Induction frequency (%)		
	Petiole	stem	Leaf		Petiole	stem	Leaf
2,4 $D_{0.5}$	33	30	25	$IBA_{0.5}$	30	23	20
2,4 $D_{1.0}$	74	70	70	$IBA_{1.0}$	70	66	63
2,4 $D_{2.0}$	85	80	82	$IBA_{2.0}$	80	75	74
2,4 $D_{3.0}$	89	88	86	$IBA_{3.0}$	84	84	79
2,4 $D_{4.0}$	94	80	90	$IBA_{4.0}$	87	71	80
2,4 $D_{0.5}BAP_{0.1}$	30	20	25	$IBA_{0.5}BAP_{0.1}$	24	15	10
2,4 $D_{1.0}$ $BAP_{0.2}$	78	68	72	$IBA_{1.0}$ $BAP_{0.2}$	71	60	60
2,4 $D_{2.0}$ $BAP_{0.3}$	87	74	80	$IBA_{2.0}$ $BAP_{0.3}$	81	65	70
2,4 $D_{3.0}$ $BAP_{0.4}$	84	85	83	$IBA_{3.0}$ $BAP_{0.4}$	78	80	74
2,4 $D_{4.0}$ $BAP_{0.5}$	88	80	85	$IBA_{4.0}$ $BAP_{0.5}$	80	74	77
2,4 $D_{0.5}Kinetin_{0.1}$	24	15	20	$IBA_{0.5}Kinetin_{0.1}$	17	10	10
2,4 $D_{1.0}$ $Kinetin_{0.2}$	50	40	45	$IBA_{1.0}$ $Kinetin_{0.2}$	44	30	35
2,4 $D_{2.0}$ $Kinetin_{0.3}$	65	55	60	$IBA_{2.0}$ $Kinetin_{0.3}$	60	45	50
2,4 $D_{3.0}$ $Kinetin_{0.4}$	76	62	70	$IBA_{3.0}$ $Kinetin_{0.4}$	73	55	55
2,4 $D_{4.0}$ $Kinetin_{0.5}$	79	50	75	$IBA_{4.0}$ $Kinetin_{0.5}$	72	43	60

* Data is mean of 5 replicates

Table 2: Interaction effect of hormones and explants types on shoot induction

Hormone combinations	No of shoots /explant			Hormone combinations	No of shoots /explant		
	Petiole	stem	Leaf		Petiole	stem	Leaf
$Kinetin_{1.0}$	0	0	2.2	$BAP_{1.0}$	0	0	2.1
$Kinetin_{1.5}$	1	1	6.1	$BAP_{1.5}$	2	2	6.5
$Kinetin_{2.0}$	2	2	6.4	$BAP_{2.0}$	2.1	2	8.2
$Kinetin_{2.5}$	3.7	2.2	8.2	$BAP_{2.5}$	4.6	3.3	9.8
$Kinetin_{3.0}$	3.3	3.1	7.6	$BAP_{3.0}$	3.3	4.5	9.2
$2,4\ D_{0.1}BAP_{1.0}$	0	0	3	$IBA_{0.1}BAP_{1.0}$	1.2	0	4
$2,4\ D_{0.2}\ BAP_{1.5}$	2.1	2.3	7.3	$IBA_{0.2}\ BAP_{1.5}$	3.6	2.2	8.3
$2,4\ D_{0.3}\ BAP_{2.0}$	5.5	2	8.6	$IBA_{0.3}\ BAP_{2.0}$	5.4	3.7	10.4
$2,4\ D_{0.4}\ BAP_{2.5}$	6.2	4.2	9.8	$IBA_{0.4}\ BAP_{2.5}$	7.5	5.2	11.5
$2,4\ D_{0.5}\ BAP_{3.0}$	5.7	5.4	9.5	$IBA_{0.5}\ BAP_{3.0}$	7.1	6.5	9.4
$2,4\ D_{0.1}Kinetin_{1.0}$	0	0	2	$IBA_{0.1}\ Kinetin_{1.0}$	1	0	2.1
$2,4\ D_{0.2}\ Kinetin_{1.5}$	2	0	4.2	$IBA_{0.2}\ Kinetin_{1.5}$	3.2	2.4	5.3
$2,4\ D_{0.3}\ Kinetin_{2.0}$	2.3	1	5.5	$IBA_{0.3}\ Kinetin_{2.0}$	4	2.3	6.3
$2,4\ D_{0.4}\ Kinetin_{2.5}$	5.4	3.2	8.6	$IBA_{0.4}\ Kinetin_{2.5}$	6.2	4.4	9.4
$2,4\ D_{0.5}\ Kinetin_{3.0}$	3	4.7	8.4	$IBA_{0.5}\ Kinetin_{3.0}$	5.3	5.8	7.1

* Data is mean of 5 replicates

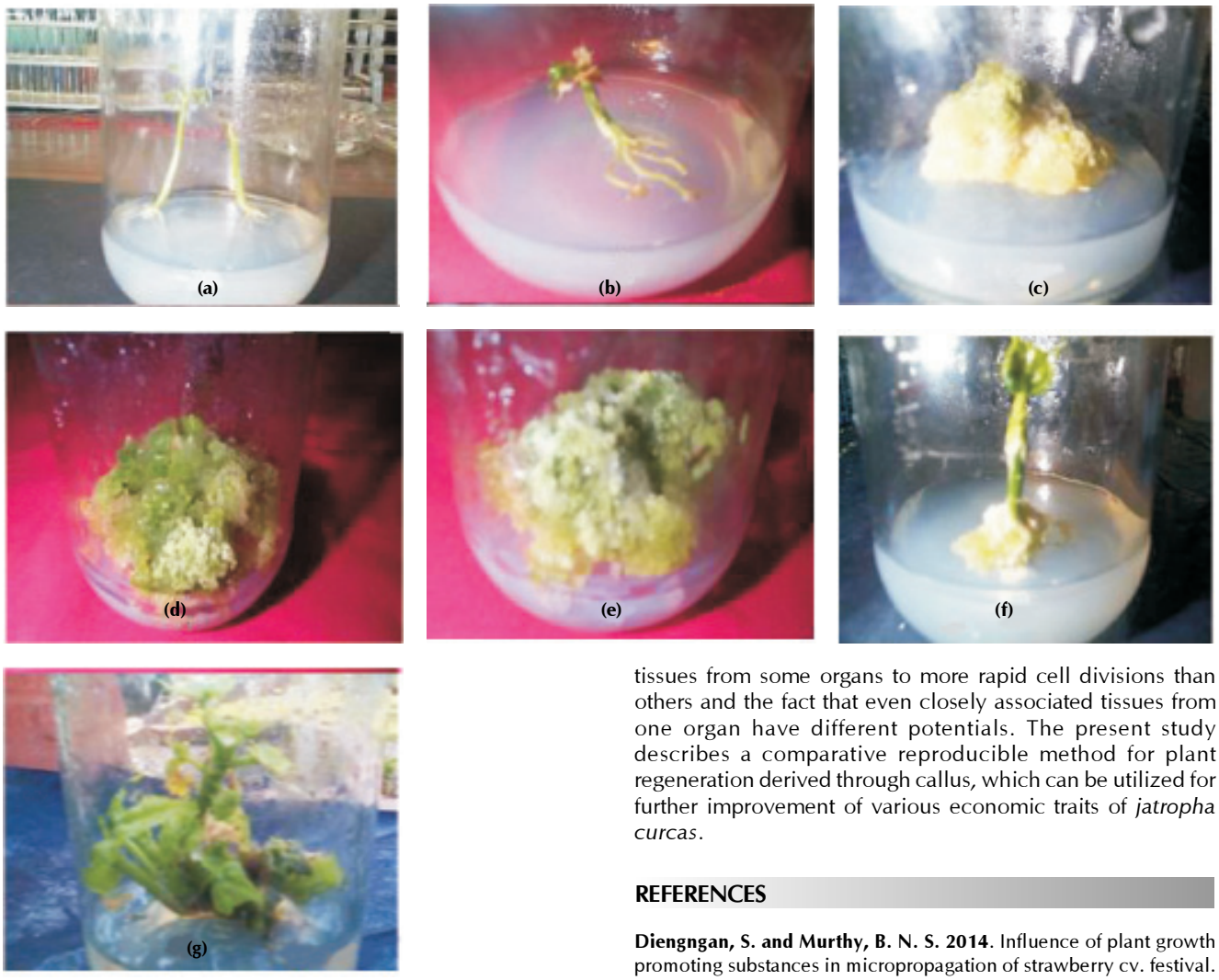

Figure 1: Regeneration of jatropha curcas from embryo (a) Normal embryo germination, (b) Abnormal embryo germination, (c) callus from stem, (d) callus from leaf, (e) callus from petide, (f) Single shoot formation, (g) Mutiple shoot formation

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 comparative reproducible method for plant regeneration derived through callus, which can be utilized for further improvement of various economic traits of *jatropha curcas*.

REFERENCES

Diengngan, S. and Murthy, B. N. S. 2014. Influence of plant growth promoting substances in micropropagation of strawberry cv. festival. *The Bioscan*. **9(4):** 1491-1493.

Kumar, N. and Reddy, M. P. 2010. Plant regeneration through the direct induction of shoot buds from petiole explants of *Jatropha curcas*: a biofuel plant. *Annals of Applied Biology*. **156:** 367-75.

Kumar, R. 2014. Biochemical characterization of jatropha (*Jatropha curcas* L.) accession in eastern himalaya, India. *The Bioscan.* **9(3):** 1131-1134.

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*. *J. Agriculture Food Chemistry*. **45(8):** 3152-3157.

Martin, K. P. 2003. Rapid in vitro multiplication and ex vitro rooting of *Rotula aquatica* Lour, a rare rhoeophytic woody medicinal plant. *Plant Cell Reporter.* **21:** 415-20.

Mayuree, K. and Sompong, Te-chato 2009. Influence of explant types and plant growth regulators on multiple shoot formation from *Jatropha curcas*. *Science Asia*. **35:** 353-57.

Monacelli, B., Pasqua, G., Cuteri, A., Varusio, A., Botta, B. and Monache, G. D. 1995. Histological study of callus formation and optimization of cell growth in *TaxusBaccata*. 159 *Cytobios*. **81:** 159-70.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*. **15:** 473-97.

Nandini, M., Saeid, N. and Singh, G. 2011. Studies on seed germination and embryo culture of *Jatropha curcas* L. under *in vitro* conditions. *Biotechnology, Bioinformatics and Bioengineering*. **1(2):** 187-94.

Pradhan, R. C., Naikl, S. N., Bhatnagar, N. and Vijay, V. K. 2009. Physical Properties of Tree Borne Oil Seed: Potential Biodiesel Feedstockin India. *Assam University J. Science and Technology*. **4(2):** 46-53.

Rao, A. Q., Hussnain, S., Sarfraz, Shahzad, M. Sadqib, Bokhari, S. Yassir Abbas Hussain Tayyab and Rizuddin, S. 2006. Somatic embrogenesis in wild relatives of cotton (*Gossipium* spp.). *J. Zhejiang University SCIENCE*. **B:** 291-98.

Sardana, J., Batra, A., Sharma, R. and Sardana, J. 1998. *In vitro* Plantlet formation and micropropagation of *Jatropha curcas* (L). *Advances in Plant Science*. **11(2):** 1627-69.

Sujatha, M. and Mukta, N. 1996. Morphogenesis and plant regeneration from tissue cultures of *Jatropha curcas*. *Plant Cell Tissue and Organ Culture*. **44(2):** 135-41.

Sujatha, M., Makkar, H. P. S. and Becker, K. 2005. Shoot bud proliferation from axillary nodes and leaf sections ofnon-toxic *J. curcas* L. *Plant Growth Regulator.* **47:** 83-90.

Thepsamran, N., Thepsithar, C. and Thongpukdee, A. 2008. In vitro induction of shoots and roots from *J. curcas* L. explants. *J. Horticulture Science and Biotechnology*. **83:** 106-12.

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 J. Plant Physiology* **13(3):** 231-37.

Verma, K. C. and Juneja, N. 2013. Biophysicochemical evaluation and micropropagation study of *Jatropha curcas* L. collections for biodiesel production: International Journal of Sustainable Energy. http://dx.doi.org/10.1080/14786451.2013.794141.