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Regeneration of *Jatropha curcas* L.: An important biodiesel plant

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Abstract
Jatropha curcas plant has tremendous scope in contributing growing demand of energy resources. It can grow in any kind of soil; therefore, will not compete with conventional crop for cultivation. For the large scale propagation, several regeneration systems have been published using different explants such as cotyledons, hypocotyl, epicotyls, stem, leaf, shoot apex, petiole, node, axillary buds etc. Using these regeneration systems, a novel gene can be introduced in Jatropha plant for improving biodiesel quality.

Keywords: *Jatropha*, biodiesel, tissue culture

Introduction

Jatropha curcas is a multipurpose, drought resistant, perennial plant belonging to Euphorbiaceae family. It is cultivated in wide range of tropical and subtropical countries because of its hardiness, easy propagation, drought endurance, rapid growth and ability to adapt to wider agro-climatic conditions (Jones and Miller 1991; Kumar and Sharma 2008) [5, 7]. Jatropha grows almost anywhere and in any kind of soil, even on gravelly, sandy and saline soils. It can thrive on the poorest stony soil and crevices of the rocks. However, seed yield is sensitive to type of soil, soil fertility and moisture content. Its yield varies from 0.5- 12 tonnes / yr / ha depending upon soil fertility, rainfall and management of plant (Francis *et al.* 2005) [4].

Currently, more than 95% biodiesel production is carried out from edible oil. Diversion of edible oil for biodiesel production at large scale may cause food scarcity. Since, Jatropha produces non-edilble oil and has the ability to grow in non-cultivated/ wasteland, hence; it can solely be utilized for biodiesel production. Its seed contain 30-40% oil and is non-edible as its seed contain toxic compounds such as curcin, cyanic acid, trypsin inhibitor and phorbol ester (Nath and Dutta 1991; Adolf *et al.* 1984) [11, 1]. The major fatty acids found in *Jatropha curcas* oil are Oleic (40%), Linoleic (37%), Palmitic (15.63%) and Stearic (5.78%) acids. The oil contains approximately 21% saturated fatty acids and 77% unsaturated fatty acids (Nzikou *et al.*, 2009) [12]. Biodiesel with high content of saturated fatty esters gives higher viscosity to the fuel, decreasing its fluidity which causes clogging of fuel filter in winters. On the other hand, highly unsaturated fatty esters in biodiesel make it susceptible to auto-oxidation on storage. Genetic engineering techniques have been employed in large number of crops for crop improvement. Fatty acid compositions of Jatropha oil can also be modified by genetic engineering techniques for production of improved biodiesel quality. To manipulate the fatty acid synthetic pathway and large scale production of Jatropha plants an efficient regeneration and reproducible protocol is pre-requisite.

Regeneration of *Jatropha curcas*

Several regeneration system of *Jatropha curcas* have been reported by organogenesis. Organogenesis refers to the process of differentiation by which plants organ are formed adventitiously from an explant. Basically there are two mode of organogenesis: (1) Direct: direct formation of buds from excised explant and (2) indirect: regeneration of plant via callusing. For *Jatropha curcas* regeneration, both direct and indirect methods have been reported using different explants such as cotyledons, hypocotyl, epicotyls, stem, leaf, shoot apex, petiole, node, axillary buds etc (Table 1). All regeneration protocol has stated the use of Murashige and Skoog's (MS) medium with different phytohormones. The compositions of the medium, particularly phytohormones and media additives have profound effect in the regeneration of explants into well formed shoots. Proportionate ratio of auxins and cytokinins are required for efficient cell division and shoot bud induction. Most commonly used auxins for regeneration of *J. curcas* are IBA (Indole-3-butyric acid) and NAA (1-naphthaleneacetic acid) while cytokinins are BAP (6-Benzylaminopurine), Kn (Kinetin) and TDZ (Thidiazuron). These growth hormones were either used alone or in combination at varying concentration.

Sujatha and Mukta 1996 [19], for the first time reported regeneration of *Jatropha curcas* from explants such as hypocotyls, petiole, and leaf using a range of cytokinins like Zeatin, kinetin, Benzylaminopurine (BAP) either singly or in combination of Indole butyric acid (IBA). Independent of explants type, direct adventitious shoot bud induction was obtained highest on MS medium with 2.22 µM BA and 4.9 µM IBA. They also observed that explants from third expanding leaf showed higher regeneration frequency than fourth leaves. However, Thepsamran *et al.* 2008 [20] had reported callus mediated organogenesis from axillary bud using a combination of BAP and IBA. A combination of BAP and IBA along with growth additives (adenine sulphate, glutamine and L-arginine) was also found to be effective in inducing direct organogenesis from axillary node (Shrivastava

and Banerje 2008) [16]. Effectiveness of TDZ in inducing direct organogenesis from leaf explants was first reported by Deore and Johnson 2008 [3]. Later on, Kumar *et al.* 2010 [8]; Sharma *et al.* 2011 [15] and Panghal *et al.* 2012 [13] also reported direct shoot induction from different explants of *Jatropha curcas* using TDZ. Khurana-kaul *et al.* 2010 [6] reported that shoot bud induction frequency of TDZ was more than double as compared with BAP. ZhongGuang *et al.* 2012 [21] had also reported callus mediated shoot bud induction using epicotyl explants in TDZ supplemented medium.

The reports of regeneration of *Jatropha curcas* using different explants and media composition are summarized in Table1. Highest shoot bud induction frequency (91%) was reported by Singh *et al.* 2014 [17] using petiole explants. Other comparable shoot bud induction frequencies were reported by Rajore and Batra 2005 [14] (90%), Sharma *et al.* 2011 [15] (88.3%), Liu *et al.* 2016 [9] (88.42%), Khurana-kaul *et al.* 2010 [6] (88%) using shoot tip, hypocotyls, cotyledonary petiole and leaf explant respectively.

Table 1: Summary of most responsive regeneration media used in *Jatropha curcas*

Explant	Shoot regeneration media	Mode of regeneration	Percentage shoot formation frequency	Root induction media	Percentage rooting	Reference
Shoot tip	2.0 mg/l BAP + 0.5 mg/l IAA + 25mg/l Adenine sulphate, 100 mg/l Glutamine + 0.2% activated charcoal	-	90	-	-	Rajore and Batra 2005 [14]
Node	2.22 µM BAP + 55.6 µM Adenine sulphate; 2.3 µM Kn + 0.5 µM IBA + 27.8 µM Adenine sulphate	Direct	-	½ MS + 1 µM IBA	52	Datta *et al.* 2007 [2]
Axillary bud	2.22 µM BAP + 0.49 µM IBA	Indirect	-	½ MS + 2.46 µM IBA	50	Thepsamran *et al.* 2007 [20]
Leaf	2.27 µM TDZ, 2.22µM BAP, 0.49 µM IBA	Direct	53.5	Full MS + 0.5 µM IBA	80	Deore and Johnson 2008 [3]
Axillary node	3.0 mg/l BAP + 1.0 mg/l IBA + 25 mg/l Adenine sulphate + 50mg/l Glutamine + 15mg/l L-arginine + 25 mg/l Citric acid	Direct	-	½ MS + 3 mg/l IBA	-	Shrivastava and Banerjee 2008 [16]
Stem	1.0 mg/l BAP + 1.0 mg/l Kn; 0.5 mg/l BAP + 1.0 mg/l IAA	Direct	65.3	½ MS + 0.1 mg/l IBA	40	Singh *et al.* 2010 [18]
Leaf	0.90 µM TDZ + 0.98 µM IBA	Direct	88	½ MS + 2.46 µM IBA	-	Khurana-kaul *et al.* 2010 [6]
Hypocotyl	0.5 mg/l TDZ; 1 mg/l BAP + 2mg/l Kn; 0.5 mg/l BAP and 1.5 mg/l IAA	Direct	88.3	½ MS 3 mg/l IBA + 1 mg/l IAA + 1 mg/l NAA	62.6	Sharma *et al.* 2011 [15]
Petiole	2.27 µM TDZ; 10µM Kn + 4.5µM BAP + 5.5 µM NAA	Direct	58.35	½ MS + 15 µM IBA + 11.4 µM IAA + 5.5 µM NAA	19.34	Kumar *et al.* 2010 [8]
Petiole	0.52 mg/l TDZ + 50 mg/l Adenine sulphate + 100 mg/l Glutamine + 25 mg/l L-arginine + 0.0025 % Citric acid + 0.005% Ascorbic acid	Direct	64	½ MS + 1 mg/l IAA + 0.2 mg/l IBA	67.6	Panghal *et al.* 2012 [13]
Epicotyl	1 mg/l TDZ + 1 mg/l kin + 0.1 mg/l IBA	Indirect	80.5	½ MS + 0.1 mg/l IBA	90	ZhongGuang *et al.* 2012 [21]
Node	8 µM BAP + 2µM IBA+ 45µM adenine sulphate + 15 µM glutamine + 10 µM Proline	Indirect	40- 50	½ MS + 2 µM IBA + 2 µM NAA	80	Maharana *et al.* 2012 [10]
Petiole	8.88 µM BAP + 0.49 µM IBA + 1.9 µM ABA	Indirect	91%	½ MS + 2.45 µM IBA + 0.54 µM NAA + 0.02% activated charcoal	65%	Singh *et al*., 2014 [17]
Cotyledonary petiole	2o mg/L BAP	Direct	88.42	½ MS + 0.1 mg/l IBA	43.39	Liu *et al.* 2016 [9]

Conclusion

Jatropha curcas is a promising source for biodiesel production. A high efficiency and reproducible *in-vitro* regeneration system is required for genetic improvement of this elite plant. This review might be beneficial in understanding responses of different growth hormones on different explants and can further be applied in production of transgenic plants using genetic engineering techniques.

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