Organogenesis and Somatic Embryogenesis of *Jatropha curcas* L.

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Abstract

Jatropha curcas is a small bush who has attracted the attention as an energy crop for the biodiesel production. A number of reports have been published regarding to the toxic *J. curcas* but there is little information relating to non-toxic *J. curcas*. A simple, high-frequency protocol for plant regeneration through organogenesis and somatic embryogenesis has been developed in cultured explants of non-toxic *Jatropha curcas* with different plant growth regulators. When Thidiazuron was used at concentrations of 3.5 and 4.5 µM, leaf explants showed the best response, forming a highly organogenic callus from which 78 and 64 shoots/explant were obtained. However, when Benzyladenine was combined with Indoleacteic acid, the hypocotyl explants showed a greater capacity for organogenesis compared with the leaf explant, both in indirect organogenesis (112 ± 1.8 shoots/explant) and in direct organogenesis (82 ±1.6 shoots/explant). Somatic embryogenesis was induced directly from leaf explants which were exposed to 8.9 µM of Benzyladenine + 5.6 µM of 2,4-Dichlorophenoxyactic acid. After 12 weeks of culture, the embryos began to turn green and eventually germinated. The shoots were elongated (73%) in the presence of 2.8 µM of Giberellic acid.

Keywords : Regeneration, biodiesel, plant growth regulators, somatic embryos, adventitious shoots, shoot elongation.

Introduction

The search of new sources for the alternative energy production is one of the main objectives of the international community. One subject that has been receiving attention is the search of non-edible crops that facilitate the biofuel production in an efficient manner (Kumar *et al* 2010a, Kumar *et al* 2010b). Among the existing biofuels we found the biodiesel which can be obtained from several sources such as rapeseed, sunflower, soybean, oil palm, linseed, cotton seed and beef tallow (Achten *et al* 2010). One of the candidate crops is *Jatropha curcas* (*J. curcas*) which is a multipurpose perennial shrub from the tropics and subtropics of South America Achten *et al* 2010). *J. curcas* (Euphobiaceace) has attracted the attention from various developmental agencies due its medicinal properties, resistance to various stresses and recently as an oilseed crop for the biodiesel production given its high oilseed content (40%) Martínez-Herrera *et al* 2010). One of the advantages of this species is the fact that its seed is not consumed due its toxicity and it can grow in low moisture soils (Martínez-Herrera *et al* 2010). Mexico is considered the center of origin of this species and it is the only place were non-toxic cultivars can be found, although they are not particularly abundant (Sujatha *et al* 2005, Achten *et al* 2010, Martínez-Herrera *et al* 2010). The byproducts of non toxic cultivars can be used animal fodder given its high protein content (Martínez-Herrera *et al* 2010).

A number of studies regarding the morphogenesis have been carried out in toxic cultivars of *J. curcas* reporting organogenesis. Varshney *et al* 2010, Wei *et al* 2011) and somatic embryogenesis. Cai *et al* 2011). It is known that the morphogenic processes are genotype dependent (George *et al* 2008), in the case of non-toxic *J. curcas* there are only two reports regarding this cultivars (Kumar *et al* 2010) both studies reports the organogenic pathway (adventitious shoots) and there is no

reports regarding the somatic embryogenesis pathway. The aim of this work is to evaluate the morphogenic potential of non-toxic *J. curcas* using different plant growth regulators (PGRs) in order to establish different systems for the *in vitro* regeneration in non-toxic cultivars as a basic for the genetic improvement and clonal propagation through biotechnological methods.

Materials and Methods

Source of vegetative material

Non-toxic *J. curcas* seeds were washed with soap and rinsed with abundant water. Seeds were exposed to ethanol (80% v/v, 5 min) after which they were submerged in a commercial solution of bleach (Clorox, 30% v/v, 15 min). The seeds were rinsed 3 – 4 times in sterile distilled water, under aseptic conditions. Mature zygotic embryos were extracted from seeds by dissection and placed vertically in germination media for 5 d, after which they were transferred to photo period of 16 he light. The germination media consisted on MS salts (Murashige and Skoog 1962) supplemented with 30 (g x dm^{-3}) sucrose and 2.2 (g x dm^{-3}) Gelrite. The pH was adjusted to 5.7 ± 2 before sterilization and was autoclaved at 121°C for 15 min at 1.01 kg x cm^{-2}.

Effect of BA and TDZ on the response of different explants

Different types of explants were used such as: mature zygotic embryos (MZE), cotyledons (Co) and leaves (Lf). The MZE were divided in four sections of equal size, the Co and Leaf explants were cut into 1 cm^2 segments. All the explants were placed horizontally (adaxial side for Co and Lf) in contact with the culture media. The media consisted in MS salts and individually supplemented with 0, 3.4, 4.5, 5.5 µM of Thidiazuron (TDZ) and 0, 4.4, 6.6, 8.9 µM of Benzyladenine (BA). Subcultures were carried out on the same media every 4 weeks on three occasions. Shoots of 0.5 – 1.0 cm length were isolated and subcultured in MS media supplemented with 0, 1.4, 2.8 and 4.2 µM of Giberellic acid (GA_3) for elongation with two subcultures every 4 weeks. The culture conditions for the induction and elongation of shoots were under photoperiod conditions of 16 h light at 25 ± 2 °C.

Effect of BA and IAA on the response of different explants

Hypocotyls (Hy) and Leaf explants were used. The segments were divided in fragments of 1 cm length and the Leaf explants were cut in 1 cm^2 fragments. The Hypocoty were then placed horizontally and the Leaf explants were placed with the adaxial side in contact with the culture medium. The culture medium consisted in MS salts supplemented with 0, 4.4, 8.9, 13.3, 17.8 µM BA in combination with 0.3 µM of indoleacetic acid (IAA). The cultures were maintained in a photoperiod of 16 hours light at a temperature of 25 ± 2 °C.

Effect of Kinetin and BA combined with 2,4-D on leaf explants

Leaf segments (1 cm^2), from 10 – 12 week old aseptic plants, were used as explants to induce the formation of somatic embryos. The explants were placed with the adaxial of the Leaf in contact with the culture medium. MS salts were used, Kinetin (Kin) and BA, combined with 2,4 dichlorophenoxyacetic acid (2,4-D) were evaluated (Ref. Table 3). The cultures were maintained in a photoperiod of 16 hours light at a temperature of 25 ± 2 °C.

Statistical Analysis

The experiments were set up in a completely randomized design. Each experiment consisted in 10 replicates and each replicate had three explants. The data collected were submitted to an Analysis of Variance (ANOVA) to detect significant statistical differences. The comparison of means are represented by standard error (S.E) and it was performed by Tukey´s test ($p \leq 0.05$), using the SPSS 16.0.0 software.

Result and Discussion

Effect of BA and TDZ on different types of explants

The response analysis of the different type of explants (MZE, Co and Lf) showed that the Leaf explant responded more effectively in the presence of both BA and TDZ with no significant differences observed between the treatments (Ref. Table 1).

Table 1. Response of different types of explants to different concentrations of BA and TDZ.

Treatments	Response (%)			Shoots / Explant		
	MZE	Co	Lf	MZE	Co	Lf
Control	----	----	----	----	----	----
4.4 µM BA	33 ± 14^{bc}	56± 5^{bc}	100 ± 0^{a}	6.9 ± 0.85^{de}	6.9 ± 0.44^{de}	7.3 ± 0.47^{de}
6.6 µM BA	40 ± 16^{bc}	70 ± 12^{ab}	90 ± 10^{ab}	7.3 ± 0.67^{de}	7.0 ± 0.56^{de}	8.1 ± 0.49^{de}
8.9 µM BA	56 ± 14^{bc}	36 ± 12^{bc}	70 ± 15^{ab}	6.6 ± 0.59^{de}	4.9 ± 0.78^{e}	5.6 ± 0.56^{de}
3.4 µM TDZ	16 ± 8.9^{cd}	13± 7.1^{cd}	100 ± 0^{a}	17 ± 2.2^{bc}	12 ± 3.7^{cd}	78 ± 1.5^{a}
4.5 µM TDZ	10 ± 10^{cd}	10± 1.3^{cd}	100 ± 0^{a}	17 ± 4.8^{bc}	18 ± 1.7^{bc}	64 ± 1.6^{ab}
5.5 µM TDZ	13 ± 1.3^{cd}	30± 5.3^{bc}	100 ± 0^{a}	15 ± 1.4^{cd}	16 ± 2.0^{cd}	22 ± 2.0^{c}

Mean ± S.E. MZE: Mature Zygotic Embryo, Co: Cotyledon, Lf: Leaf. Different letters indicate significant statistical differences (n = 10 repetitions, p≤ 0.05).

The TDZ showed the formation of a highly morphogenic callus of a greenish-white colour (Fig. 1A), from which organogenic structures (monopolar) were formed form the explant with no modification of the culture media and subsequently developed into diminutive shoots (Fig. 1 B).

These organogenic structures after two weeks of culture developed into robust, bright green shoots with vascular connection with the callus (Fig. 1 C), the development of the shoots were asynchronous, probably due the competition of the high frequency of induced regenerants (Fig. 1 D). The best results relating to the shoot formation were obtained with 3.4 and 4.5µM TDZ, with average numbers of 78 and 64 shoots per explant respectively, with no significant differences between treatments. The morphogenic potential of MZE and Co explants treated with TDZ was significantly inferior both in the number of responsive explants and in the number of shoots formed per explant. The BA showed the formation of organogenic structures but the number of shoots per explant was significantly lower in comparison with Leaf segments treated with TDZ.

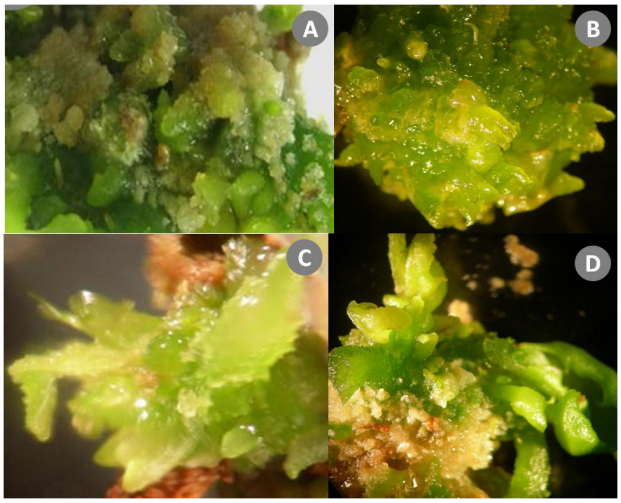

Fig. 1. Indirect organogenesis from leaf segments of *J. curcas:* **A)** Formation of morphogenic callus in medium with 3.4 µM TDZ, at 8 weeks of culture, **B)** Formation of diminutive morphogenetic structures from the callus formed (12 weeks of culture), **C** and **D)** Development of shoots with vascular connection to the callus (14 weeks of culture).

Effect of BA combined with IAA on hypocotyls and leaf segments

The Hypocoty and Leaf explants were cultured with different concentrations of BA and IAA. Among the explants used the Hypocoty were more responsive when compared to Leaf (Table 2), also the number of organogenic structures was significantly higher in Hypocoty than Leaf During the culture of Hypocoty we observed the formation of direct and indirect organs with significant differences in the numbers of shoots generated, were the indirect organogenesis had 112 ± 1.8 shoots/explant compared to the 82 ± 1.6 shoots/explant generated directly.

Table 2. Effect of different concentrations of BA combined with IAA on hypocotyl and leaf segments.

Treatments	Response (%)		Shoots / Explant	
	Hy	Lf	Hy	Lf
Control	----	----	----	----
4.4 µM BA + 0.3 µM IAA	86 ± 4.6^{a}	13 ± 7.1^{cd}	33 ± 1.2^{d}	6.9 ± 0.84^{bc}
8.9 µM BA + 0.3 µM IAA	92 ± 3.8^{a}	54 ± 2.6^{b}	61 ± 1.5^{c}	7.25± .67^{bc}
13.3 µM BA + 0.3 µM IAA	97 ± 4.4^{a}	79 ± 2.0^{a}	112 ± 1.8^{a}	6.6±0.59^{bc}
17.8 µM BA + 0.3 µM M IAA	96 ± 3.9^{a}	10 ± 1.3^{cd}	82 ± 1.6^{b}	27 ± 2.2^{a}

Mean ± S.E. Hy: Hypocotyl, Lf: Leaf. Different letters indicate significant statistical differences (n = 10 repetitions, p≤ 0.05).

The Hypocoty response to different treatments is illustrated in Figure 2, were the Hypocoty cultured in presence of 13.3 µM BA + 0.3 µM IAA showed the formation of greenish-white callus on which was possible to observe the shoot-apex formation around the periphery (Fig. 2 A) The organogenic structures were easily distinguishable due its bright green colour (Fig. 2 B) also an increase in the BA concentration up to 17.8 µM BA while maintaining the IAA concentration showed an intense proliferation of organogenic structures (Fig. 2 C and Fig. 2 D).

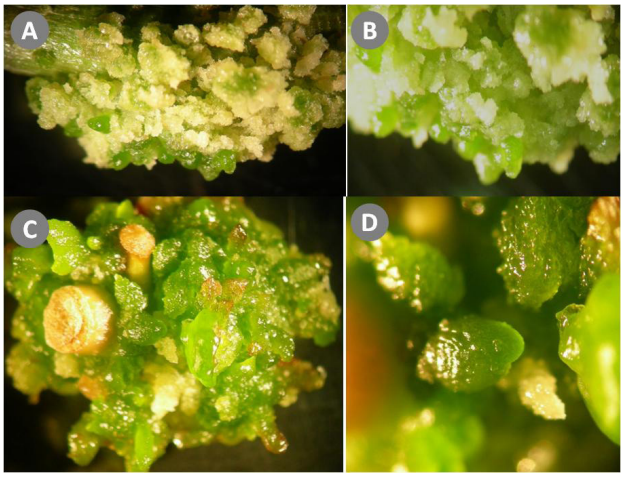

Fig. 2. A) *Indirect organogenesis* induced from hypocotyl segments of *J. curcas* in MS medium + 13.3 µM of BA + 0.3 µM IAA, at 4 weeks of culture, **B)** Differentiation of the organogenic structures, at 8 weeks of culture, **C)** and **D)** A*dventitious shoot-buds* formed directly from hypocotyl segments of *J. curcas* in MS medium + 17.8 µM of BA + 0.3 µM IAA, at 12 weeks of culture.

Shoot elongation

Table 3. Elongation of *J. curcas* shoots in the presence of GA_3, at 8 weeks of culture.

Treatments	Response (%)	Length (cm)
Control	31 ± 6.9^{b}	0.67 ± 0.1^{c}
1.4 µM GA_3	50 ± 7.2^{ab}	0.92 ± 0.^{5b}
2.8 µM GA_3	73 ± 7.1^{a}	1.35 ± 0.1^{a}
4.2 µM GA_3	30 ± 6.9^{b}	0.42 ± 0.41b

Mean ± S.E. Different letters indicate significant statistical differences (n = 10 repetitions, p≤ 0.05).

The organogenic structures formed in previous treatments were cultured in elongation media (Ref. Table 3).

The highest response to elongation and development was observed when the shoots were cultured in media containing 2.8 µM GA_3, (Fig. 3 C) with 73 ± 7.1 (%) of developed shoots presenting an average stem length of 1.35 ± 0.1 (cm), a significant differences in comparison with all other treatments, including the control (Fig. 3 A, Fig. 3 B and Fig. 3 D).

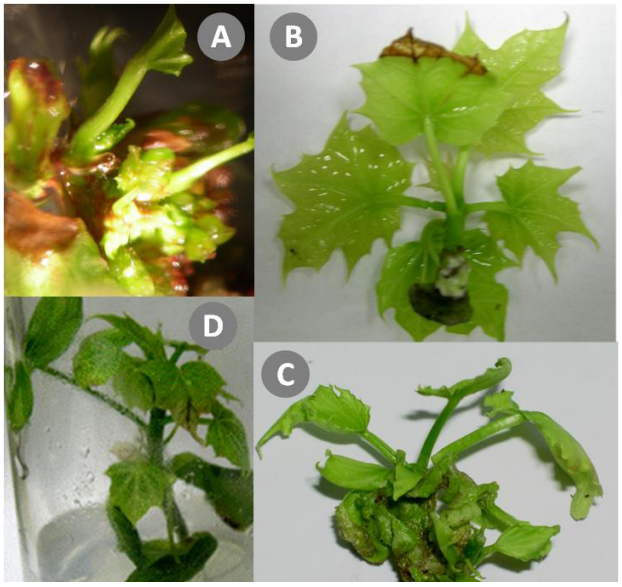

Fig. 3. Shoot elongation of *J. curcas* in different treatments with GA_3, at 8 weeks of culture:

A) Medium without GA_3, **B)** Medium with 1.4 µM GA_3, **C)** Medium with 2.8 µM AG_3 and **D)** Medium with 4.2 µM GA_3.

Effect of Kin and BA combined with 2,4-D on leaf explants

The explants treated with Kin, BA and Kin + 2,4-D (Ref. Table 4) showed callus formation, this callus did not form morphogenic structures during the 8 weeks of culture.

Table 4. Effect of Kin and BAP, combined respectively with different concentrations of 2,4-D, on somatic embryogenesis of *J. curcas.*

Plant Growth Regulators (µM)			Type of Response	Number of SEs/Explant
Kn	BA	2,4-D		
9.3	**0**	**0**	NR	0
9.3	**0**	**5.6**	Callus	0
9.3	**0**	**6.7**	Callus	0
9.3	**0**	**7.9**	Callus	0
0	**8.9**	**0**	Callus	0
0	**8.9**	**5.6**	DSE	12 ± 1.5^{a}
0	**8.9**	**6.7**	DSE	18 ± 1.9^{a}
0	**8.9**	**7.9**	ISE	17 ± 2.3^{a}

Mean ± S.E. NR: Non Responsive, DSE: Direct Somatic Embryogenesis. ISE: Indirect Somatic Embryogenesis.

Different letters indicate significant statistical differences (n = 10 repetitions, $p \leq 0.05$).

When BA was combined with 2,4-D it was possible to observe embryogenic structures at different stages of development at 8 weeks of culture in all the treatments evaluated (Fig. 4 A, Fig. 4 B and Fig. 4 C), the embryos developed and germinated at 12 weeks of culture (Fig. 3 D). Although the number of embryos did not differ significantly among the different treatments (Ref. Table 4) only the explants exposed to 8.9 µM BA + 5.6 – 6.7 µM 2,4-D formed direct somatic embryos.

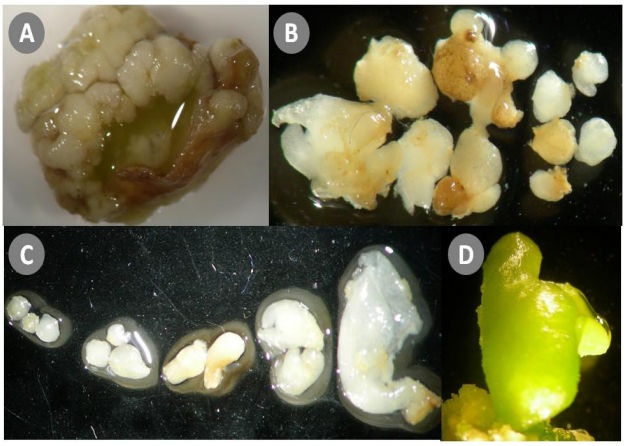

Fig. 4. Somatic embryos of *Jatropha curcas* L. **A)** Embryogenic callus at 4 weeks of culture, **B-C)** Somatic embryos in different stages of development, at 8 weeks of culture, **D)** Germination of somatic embryo, at 12 weeks of culture.

Discussion

The use of biotechnological tools to produce *J. curcas* plants with desirable traits is an immediate need, tissue culture protocols have been reported by various researchers (Sujtaha *et al* 2000).

Different PGRs, such as BA and TDZ, have proven to be effective in the organogenesis of *J. curcas* (Kumar *et al* 2010b, Kumar *et al* 2010c). The process of indirect organogenesis from leaf explants has been reported earlier in toxic and non-toxic cultivars using BA and IAA (Sujatha *et al* 2005) and the direct organogenesis process has been obtained using BA and TDZ alone or in combination with other PGRs using other type of explants (Khurana-Kaul *et al* 2010, Kumar *et al* 2010a). It is known that the morphogenic response it is a combination of different factor such as the culture media, the synergism between the PGRs (auxin and cytokine mainly), the tissue used and the genotype of the donor plant (Kumar *et al* 2010b).

In this report we observed that the TDZ as a sole regulator in the culture media promoted the indirect organogenesis process in Leaf explants with 3 – 6 fold increase in the number of shoots obtained when compared to previous reports (Kumar *et al* 2010c). Recently a similar amount of shoots (62 shoots/explant) were obtained using higher concentrations of TDZ (9.08 µM) (Kumar *et a*l 2010b), we observed that higher concentrations of TDZ resulted inhibitory for the organogenic process, this differences could be attributed to physiology or the genotype of the explant (Kumar *et al* 2010b, Kumar *et al* 2010c). We also observed that the synergism between PGRs it is important because, the use of BA and IAA increased the amount of shoots per explant using Hypocoty as a source of explant, previous reports in *J. curcas* have used different combinations of PGRs were the effect was beneficial for the amount of shoots generated, in this study the combination of PGRs increased 2 – 10 fold the amount of shoots generated (Kumar *et al* 2010c). During the elongation phase we observed the concentration effect of the GA_3 on the organogenic structures, as reported high concentrations of GA_3 inhibited the shoot elongation (Purkayastha *et al* 2010). Also other reports have used combinations of GA_3 and other PGRs with large amounts of elongated shoots (over 80%) this effect may be attributed to the synergism between the different PGRs in the media (Kumar *et al* 2010c). In the current study the concentration of 2.8 µM GA_3 produced 73% of elongated shoots compared with the 46% reported in (Purkayastha *et al* 2010) using a similar concentration of GA_3. This difference could be related to the genotype because it has been reported that the response could change in varieties of the same species (George *et al* 2008, Kumar *et al* 2010b).

The somatic embryogenesis process in *J. curcas* has been achieved directly or indirectly (Jha *et al* 2007, Cai *et al* 2011) in this protocols the use of

auxins and cytokinines are needed to induce this process. The role of cytokinines and auxins in the different stages of somatic embryogenesis is well established However, what is now important is to determine the triggering combination and concentrations of PGRs, besides other factors that vary from cell to cell even within a particular type of tissue of a plant species. In this report we managed to increase the embryogenic response during the induction phase (from 50% to 100%) and reduce the amount of time required for the embryo maturation (from 12 weeks to 8 weeks) compared with previous reports (Jha *et al* 2007, Cai *et al* 2011). Also this is the first report were the somatic embryogenesis process is achieved for non-toxic *J. curcas*.

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References:

[1] Achten W.M.J., Nielsen L.R., Aert R., Lengkeek A.G., Kjaer E.D., Trabucco A., Hansen J.K., Maes W.H., Graudal L., Akinnifesti F. K. and Myus B., Towards domestication of *Jatropha curcas*. *Biofuels,* 1(1), (2010) 91 – 107.

[2] Basiron Y. ,Palm oil production through sustainable plantations. *Eur. J. Lipid Sci. Technol.,* 109, (2007) 289 – 295.

[3] Cai L., Fu L., and Ji L., Regeneration of *Jatropha curcas* through efficient somatic embryogenesis and suspension culture. *GM Crops,* 2 (2), (2011), 110 – 117.

[4] Deore A. and Johnson S., High frequency plant regeneration of leaf-disc cultures of *Jatropha curcas* L.: an important biodiesel plant. *Plant Biotechnol. Rep.,* 2, (2008) 7 – 11.

[5] Francis G., Edinger R. and Becker K., A concept of simultaneous wasteland reclamation, fuel production and socio-economic development in degraded areas in India: Need, potential and perspectives of *Jatropha* plantations. *Nat. Res. Forum,* 29, (2005) 12 – 24.

[6] Gao S., Ouyang S., Wang S., Xu, Y., Tang L. and Chen F., Effects of salt stress on growth, antioxidant enzyme and phenylalaline-lyase activities in *Jatropha curcas* L. seedlings. *Plant Soil Environ.,* 9, (2008) 374 – 381.

[7] George E.F., Hall M.A. and De-Klerk G.J., Plant Propagation by Tissue Culture. Vol. 1 The Background. *Springer.* (2008) 477.

[8] Jha T.B., Mukherjee P. and Datta M.M., Somatic embryogenesis in *Jatropha curcas* Linn., an important biofuel plant. *Plant Biotech. Rep.,* 1, (2007) 135 – 140.

[9] Kalimuthu K., Paulsamy S., Senthilkumar R. and Sathya M., *In vitro* Propagation of the Biodiesel Plant *Jatropha curcas* L. *Plant Tiss. Cult. Biotech.,* 17 (2), (2007) 137 – 147.

[10] Khurana-Kaul V., Kachwaha S. and Kothari S.L., Direct shoot regeneration from leaf explants of *Jatropha curcas* in response in thidiazuron and high copper contents in the medium. *Biologia Plantarum*, 54 (2), (2010) 369 – 372.

[11] Kumar N. and Reddy M .P., Plant regeneration through the direct induction of shoot buds from petiole explants of *Jatropha curcas*: a biofuel plant. *Ann. Appl. Biol.,* 1, (2010)1 – 9.

[12] Kumar N., Vijay-Anand K.G. and Reddy M.P., *In Vitro* Plant Regeneration of Non-Toxic *Jatropha curcas* L.: Direct Shoot Organogenesis from Cotyledonary Petiole Explants. *J. Crop Sci. Biotechnol.,* 13 (3), (2010) 189 – 194.

[13] Kumar S., Kumaria S. and Pramod T., Efficient *In Vitro* Plant Regenerarion Protocol from Leaf Explant of *Jatropha curcas* – A Promising Biofuel, *Plant. J. Plant Biochem. Biotechnol.,* 19 (2), (2010) 1 – 4.

[14] Li M., Li H., Jinag H., Pan X. and Wu G., Establishment of an *Agrobacterium*-mediated Cotyledon Disc Transformation Method for *Jatropha curcas*. *Plant Cell Tiss. Org. Cult.,* (2008) 92, 173 – 181.

[15] Martinez-Herrera J., Martinez-Ayala A.L, Makkar H., Francis G. and Becker K.,

Agroclimatic Conditions, Chemical and Nutritional Characterization of Different Provenances of *Jatropha Curcas* L. from Mexico. *Eur. J. Sci. Res.,* 39 (3), (2010) 396 – 407.

[16] Milinsk M.C., Visentainer J.V., Martin C.A., Arrabal-Arias C.A., Matsushita M. and de Sousza N.E., Proximate composition and fatty acid profile of brazilian conventional and transgenic soybean (*Glycine max* (L.) Merryl.) cultivars. *Electronic J. Environ. Ag. Food Chem.,* 6 (3), (2007)1905 – 1911.

[17] Misra P., Gupta N., Toppo D.D., Pandey V., Mishra M.K. and Tuli R., Establishment of long-term proliferating shoot cultures of elite *Jatropha curcas* L. by controlling endophytic bacterial contamination. *Plant Cell Tiss. Org. Cult.,* 100, (2010) 189 – 197.

[18] Murashige T. and Skoog F., A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiol. Plant.,* 15 (3), (1962)473 – 497.

[19] Openshaw K., A review of *Jatropha curcas*: an oil plant of unfulfilled promise. *Biomass and Bioenergy,* 19, (2000) 1 – 15.

[20] Purkayastha J., Sugla T., Paul A., Solleti S.K., Mazumdar P., Basu A., Mohommad A., Ahmed Z. and Sahoo L., Efficient *in vitro* plant regeneration from shoot apices and gene transfer by particle bombardment in *Jatropha curcas*. *Biologia Plantarum,* 54 (1), (2010) 13 – 20.

[21] Qin W.L., Yi W.D., Shu-Lin P., Ying X., Lin T. and Fang C., Plant regeneration form epicotyls explants of *Jatropha curcas*. *J. Plant. Physiol. Mol. Biol.,* 30, (2004) 475 – 478.

[22] Rajore S. and Batra A., Efficent plant regeneration via shoot tip explant in *Jatropha curcas* L. J. Plant Biochem. *Biotechnol.,* 14, (2005) 73 – 75.

[23] Srivastava P.S. and Johri B.M., Morphogenesis in Mature Endosperm Cultures of *Jatropha panduraefolia* Beitr. *Biol. Pflanz.,* 50, (1974)255 – 268.

[24] Sujatha M., Makkar H.P.S. and Becker K., Shoot Bud Proliferation from Axilary Nodes and Leaf Sections of non toxic *Jatropha curcas* L. *Plant Growth Reg.,* 47, (2005) 83 – 90.

[25] Sujatha M. and Reddy T. P., Morphogenic responses of *Jatropha intergrrina* explants to cytokinins. *Biologia,* 55, (2000) 99-104.

[26] Varshney A. and Johnson T. S., Efficient plant regeneration from immature embryo cultures of *Jatropha curcas*, a biodiesel plant. *Plant Biotech. Rep.,* 4, (2010) 139 – 148.

[27] Weber S., Friedt W., Landes N., Molinier J., Himber C., Rousselin P., Hahne G. and Horn R., Improved *Agrobacterium*-mediated transformation of sunflower (*Heliantus annuus* L.): assessment of macerating enzymes and sonication. *Plant Cell Rep.,* 21, (2007) 475 – 482.

[28] Wei Q., Lu W.D., Llao Y., Pan S.H., Xu Y., Tang L. and Chen F., Plant Regeneration from Epicotyl Explant form *Jatropha curcas*. *J. Plant Physiol. Mol. Biol.,* 30 (4), (2004) 475 – 478.