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In vitro Regeneration of *Ricinus communis* L. and *Jatropha curcas* L. for Biofuel Production

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Abstract: The growing of oil producing tree crops for biodiesel on large scale to supplement fossil fuel has become lucrative agribusiness for farmers due to high cost of crude oil. In spite of their high economic value and comparative advantage over food crops as energy sources, oil producing tree crops have low seed viability making large scale commercial propagation difficult. Thus alternative mode of propagation via *in vitro* culture is highly recommended. In this study, attempts were made to regenerate *Ricinus communis* and *Jatropha curcas*, oil producing tree crops belonging to the family Euphorbiaceae. The addition of cytokinin (BAP, kinetin or 2iP) in the culture medium significantly increased the viability of zygotic embryos of *Ricinus* over the controls depending on the stage at which the fruits were collected but conversely in *Jatropha* the increase was not significantly different. Of the three cytokinins used 2iP enhanced the highest shoot regeneration with the optimal concentration ranging from 0.5 mg L^{-1} in *Ricinus* and 1.5 or 2.0 mg L^{-1} in *Jatropha*, indicating genotypic difference between the species. However, excessive callus formation and browning in *Ricinus* led to the loss of regenerants. Plant growth regulators also influenced regeneration from meristem explant with 2iP again being the best. The successful regeneration of plantlets from shoot tip explants of *Ricinus* and *Jatropha* augurs well for future genetic transformation of the crop for biofuel production.

Key words: Zygotic embryo, oil content, meristem explants, cytokinin, plant regeneration

INTRODUCTION

The recent high cost of fossil fuel energy on the international market has necessitated the search for alternative sources of fuel preferentially from plant species. Thus, the growing of oil producing tree 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 the economic burden of many developing countries. For instance, the blending of ethanol produced from sugarcane with oil has reduced total dependence of Brazil on fossil fuel (Islee and Hendriks, 2007). Besides sugarcane, several crops including cassava and corn (carbohydrate based crops) and *Jatropha* and *Ricinus* (oil producing tree crops) have been identified as potential sources for biofuel production.

Both *Jatropha* and *Ricinus* are oil producing plants belonging to the family Euphorbiaceae. The seed of *Jatropha* contains 25-30% highly viscous, non-edible combustible oil which can be used without refining (Openshaw, 2000). According to Ahmed and Salimon (2009), the seed oil belongs to the oleic or linoleic group with 21% saturated fatty acids and 79% unsaturated fatty acids. For *Ricinus*, the kernel contains 58-66% oil (Ahmed and Salimon, 2009). *Ricinus* oil is characterized by high specific gravity, viscosity, optimal rotation and high acetyl value. It is soluble in alcohol but insoluble in light petroleum and other mineral oils thus making it industrially useful. The good physicochemical properties of these oils make them commercially viable alternative to fossil fuel (Lele, 2006). Thus, oil obtained from *Ricinus communis* has been used as a lubricant in the internal combustion engines in aeroplanes (Alam *et al*., 2010).

Besides being used as a source of biodiesel, the oil can also be used for manufacturing candles, soaps and cosmetics and for treating several skin diseases (Deore and Johnson, 2008). After oil extraction, *Jatropha* and *Ricinus* oil cake could be used as a rich source of organic fertilizer in place of chemical fertilizer in the production of food crops.

In spite of these advantages, conventional propagation of these crops through seeds is plagued with unsynchronized flowering, fruit maturity as well as high degree of heterozygosity which pose problem of genetic fidelity. Moreover, seed viability and rate of germination

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are low (Jepsen *et al*., 2008), thus for commercial scale farming conventional seed propagation alone cannot provide the needed quality planting materials for farmers. Thus, the application of *in vitro* culture for production of planting materials for large-scale propagation of *Ricinus* and *Jatropha* species is inevitable.

There have been reports of successful regeneration of both plant species *in vitro*. *Jatropha* plantlets have been successfully regenerated using hypocotyl, nodal cuttings, peduncle and leaf explants (Sujatha and Reddy, 2000; Rajore and Batra, 2005; Ahn *et al*., 2007; Ahn and Chen, 2008). Similarly, there are reports of successful regeneration of *Ricinus* plantlets from embryogenic axes on an MS Medium modified with benzylaminopurine (BAP), kinetin, thiaduzuron (TDZ) and zeatin at concentration ranging from 0.5 to 10 mg L^{-1}; BAP at a concentration of 2.0 mg L^{-1} resulted in the highest number of shoot production (Sujatha and Reddy, 2000). However, the regeneration rates were low for field application. The present study was therefore, aimed at developing *in vitro* regeneration protocols for *Ricinus communis* and *Jatropha curcas*. Also, the effect of cytokinins or auxins on plant regeneration from shoot tip and meristem explants was investigated.

MATERIALS AND METHODS

Viability of zygotic embryos at different maturity stages and post flask survival: Fruits of *Jatropha curcas* and *Ricinus communis* at different stages of maturity (Fig. 1a-g) were harvested from mature plants and split open to remove the seeds. The seeds were soaked

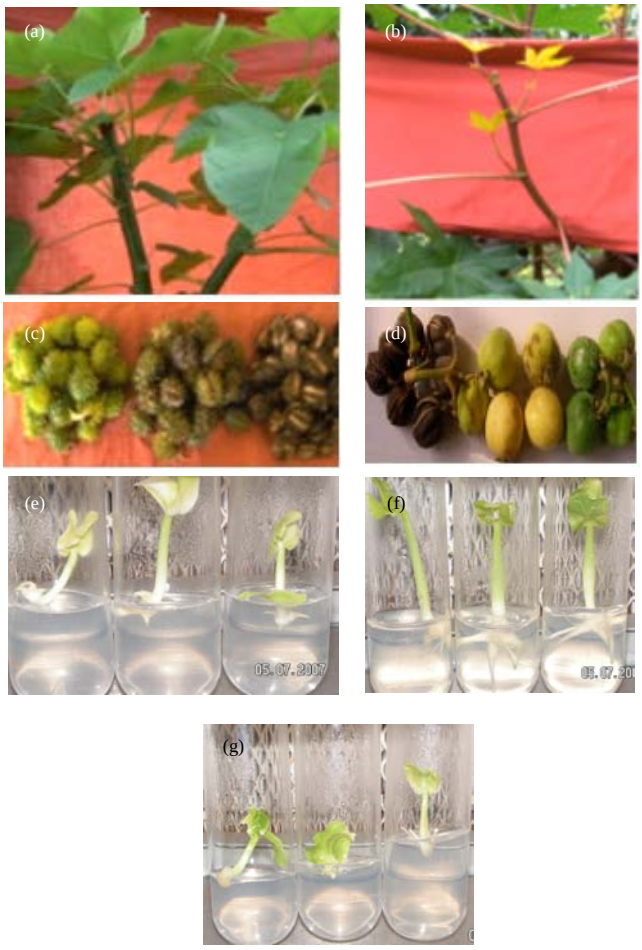

Fig. 1 (a-g): (a) Decapitated tree of *Jatropha curcas*, (b) *Ricinus communis*, (c) Developing juvenile shoots for use as explants. Fruits of *J. curcas*, (d) *R. communis*, (e) at different stages of maturation. Zygotic embryo of *Jatropha* germinating on MS medium supplemented with 1 mg L^{-1} kinetin, (f) BAP and (g) 2iP

overnight to imbibe water, sterilized by immersion in 1% $HgCl_2$ for 10 min and thereafter rinsed in three changes of sterile distilled water. The seeds were then immersed in 70% ethanol, followed by washing with three changes of sterile distilled water. The testa was then removed and the endosperm was split open to remove the zygotic embryos for culture. Dissected zygotic embryo explants were cultured in 30 mL of (Murashige and Skoog, 1962). MS basal salts and vitamins supplemented with 100 mg L^{-1} myoinositol, 30 g L^{-1} sucrose, 3.5 g L^{-1} phytagel and varying concentrations (0.0-1.0 mg L^{-1}) BAP, kinetin and 2iP in test tubes. The pH of the medium was adjusted to 5.8 prior to addition of phytagel and autoclaved at 121°C for 15 min and pressure of 15 psi. The cultures were initially incubated in the dark for three days and thereafter transferred to growth room at temperature of 27°C, 16 h/8 day/dark photoperiod and light intensity of 3,500 lux provided by white fluorescent tubes. The number of embryos that germinated was recorded two weeks after culture. Each experimental treatment was replicated 3 times with twenty explants per treatment. Well-developed plantlets of different ages were weaned in the plant barn prior to field planting.

Moisture and oil content of *Jatropha* and *Ricinus* seeds: The moisture and oil contents of seeds collected from both *Jatropha* and *Ricinus* seeds obtained as above were determined using standard methods described by the Association of Official Analytical Chemist (AOAC) in 1970. Fifty seeds from each plant were ground into fine powder using mortar and pestle. Two grams of the powder was weighed into a Petri dish and then put in an oven at a temperature of 130°C for 1 h without a cover. The dish was again weighed after it has reached room temperature. The moisture content was calculated as:

$$\%MC = \frac{\text{Weight fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Seed oil from *Jatropha* and *Ricinus* was extracted using the Soxlet apparatus. Two grams of the powder was weighed after which petroleum ether was used to extract the oil at a temperature of 40-60°C and a condensation rate of 5 to 6 drops per sec. The extracted oil was dried at 100°C for 30 min in an oven and then cooled at room. The percentage oil content was calculated using the formula below. Each experiment was repeated twice:

$$\%MC = \frac{E_{oil}}{100 - MC} \times 100$$

Where:

MC = Moisture content of seeds and

E_{oil} = Amount of oil extracted

Plant regeneration from shoot tip and meristem explants: Four week-old juvenile shoots harvested from previously decapitated *Jatropha* and *Ricinus* trees (Fig. 1) were washed with running tap water, immersed in 10% commercial bleach (parazone, Jeyes Limited, Norfolk, England) for 10 min and thereafter rinsed with three changes of sterile distilled water. To ensure effective sterilisation, the shoot tips were again immersed in 70% ethanol for 5 min. Half the number of shoot tips was further trimmed and cultured on 40 mL of Murashige and Skoog (1962) basal medium amended with cytokinins (BAP, Kinetin or 2iP) at concentrations ranging from 0.0 (control) to 3.0 mg L^{-1} in 250 mL honey jar bottles. Meristematic tissues (0.4 mm) from the remaining shoot tips were dissected under the microscope and cultured on 15 mL of MS basal medium supplement with various concentrations of cytokinins as described above. A honey jar containing four explants was considered an experimental unit and this was replicated five times.

Statistical analysis: All experiments were set up in a completely randomized design and data collected were subjected to Analysis of Variance (ANOVA) to separate means between treatments using the Minitab Statistical Software (version 12). Means which differed significantly, were separated using the Tukey's pair wise comparison.

RESULTS

Effect of fruit maturity stage on viability of zygotic embryos: Zygotic embryos excised from physic nut (*Jatropha curcas,* L.) and castor bean (*Ricinus communis,* L.) at different fruit maturity stages (Fig. 1a-d) germinated within three days after culture. The developing plantlets were etiolated due to the initial dark incubation, however, after three days of transfer to growth room lighting conditions the plantlets became green (Fig. 1).

The presence of cytokinin in the culture medium generally increased viability of the zygotic embryos over the controls in *Ricinus* with the exception of embryos obtained from the yellow stage cultured on kinetin amended medium. At the green stage, the presence of BAP, kinetin and 2iP significantly increased the viability of *Ricinus* from 18.33% in the controls to 50.0, 56.66 and 65.0%, respectively. A further increase in the

Table 1: Effect of maturity stage on viability of *Jatropha curcas* and *Ricinus communis*

Maturity stage	Growth regulator	Concentration (mg L^{-1})	Germination (%)	
			Ricinus	*Jatropha*
Green	BAP	0.00	18.33±3.22^{a}	90.00±3.16^{a}
		0.50	50.00±0.00^{b}	98.33±1.70^{a}
		1.00	58.33±3.90^{b}	100.00±0.00^{a}
	KIN	0.00	18.33±3.22^{c}	90.00±3.16^{b}
		0.50	56.66±2.76^{d}	93.33±2.76^{b}
		1.00	66.66±3.22^{d}	100.00±2.40^{b}
	2iP	0.00	18.33±3.22^{e}	90.00±3.16^{c}
		0.50	65.00±3.16^{f}	88.33±3.22^{c}
		1.00	58.33±3.39^{f}	98.33±1.70^{c}
Yellow	BAP	0.00	76.66±2.76^{g}	93.33±2.76^{d}
		0.50	83.33±1.69^{g}	98.33±1.70^{d}
		1.00	83.33±1.69^{g}	100.00±0.00^{d}
	KIN	0.00	76.66±2.76^{h}	93.33±2.76^{e}
		0.50	56.66±3.54^{hi}	100.00±0.00^{e}
		1.00	53.33±1.69^{i}	98.33±1.70^{e}
	2iP	0.00	76.66±2.76^{j}	93.33±2.76^{f}
		0.50	81.66±2.76^{j}	88.33±3.54^{f}
		1.00	85.00±2.23^{j}	96.67±2.40^{f}
Black	BAP	0.00	83.33±2.40^{k}	55.00±3.63^{g}
		0.50	96.67±1.70^{m}	78.33±2.76^{gh}
		1.00	96.67±1.70^{m}	88.33±3.54^{h}
	KIN	0.00	83.33±2.40^{n}	55.00±3.63^{i}
		0.50	86.66±1.69^{n}	96.67±2.40^{j}
		1.00	83.33±1.69^{n}	83.33±1.70^{j}
	2iP	0.00	83.33±2.40^{o}	55.00±3.63^{k}
		0.50	91.67±3.22^{p}	51.67±3.39^{k}
		1.00	96.67±1.70^{p}	61.67±3.79^{k}

Percentage means with different letters in a column are significantly different ($p \leq 0.05$) according to the Tukey's test

concentration of these growth regulators to 1.0 mg L^{-1} resulted in 58.33, 66.66 and 58.33%, respectively. At the black stage BAP and 2iP, respectively caused significant ($p \leq 0.05$) increase of 86.66 and 91.67% in viability over the controls while the concentration of kinetin did not have any significant effect. Conversely, in *Jatropha*, only black stage embryos cultured on BAP and kinetin amended medium significantly ($p \leq 0.05$) increase the viability from 55.0% in the controls to 78.33 and 96.67%, respectively; doubling the concentration of BAP and kinetin in the culture medium to 1.0 mg L^{-1} marginally increased the viability to 88.33 in BAP and 83.33% in kinetin (Table 1). Although, green stage embryos cultured on 1.0 mg L BAP or kinetin resulted in 100% viability, they were not significantly ($p \leq 0.05$) different from the control (90.0%). Similarly, at the yellow stage none of concentrations of the growth regulators had significant effect. Generally, percentage germination increased as the fruit matured in *Ricinus communis* while in *Jatropha* percentage germination decreased with fruit maturity.

Moisture and oil contents of *Ricinus* and *Jatropha* seeds: The percentage moisture content in both *Jatropha* and *Ricinus* species decreased as the fruit matured (Fig. 2). In *Ricinus,* the percentage moisture content decreased from 29.4% (green stage) to 10% (black stage) while in *Jatropha* it decreased from 85% (green) to as low as 12% at the black stage. Comparatively, the percentage moisture content of *Jatropha* at the green stage (85%) was more than twice that of *Ricinus* species (29.94%). Conversely, the percentage oil content increased as the fruit matured (green to black) in both plant species. In *Ricinus* it ranged from 35% (green) to 50% (black) while in *Jatropha* it ranged from 19.84 to 33.27%. Unlike the moisture content, the percentage oil content of *Ricinus* was comparatively higher than *Jatropha* species in all the three stages of fruit maturity.

Effect of cytokinins on plantlet regeneration from shoot tip and meristem explants: The effect of cytokinins on shoot regeneration from shoot tip and meristem explants of *Ricinus* and *Jatropha* is presented in Table 2. In *Ricinus* species, shoot tips cultured on 0.5 mg L^{-1} 2iP amended medium significantly ($p \leq 0.05$) increased shoot regeneration from 25.0 to 90.0%. A further increase in the concentration of 2iP to 1.0, 2.0 or 3.0 mg L^{-1} significantly ($p \leq 0.05$) decreased plantlet regeneration to 70.0, 65.0 and 50.0%, respectively, indicating that increasing the concentration of the growth regulator is inhibitory to shoot development. In *Jatropha*, although, the presence of cytokinins comparatively increased shoot regeneration over the controls, the effect was generally not significantly ($p \leq 0.05$) different. The optimal

Table 2: Effect of BAP, Kinetin and 2iP on plantlet regeneration from shoot tip and meristem explants

		Shoot formation (%)			
		Ricinus communis		*Jatropha curcas*	
Cytokinin	Conc. (mg L^{-1})	Shoot tip	Meristem	Shoot tip	Meristem
BAP	0.0	25.00±0.20^{a}	60.00±0.30^{ac}	50.00±0.22^{a}	85.00±0.06^{a}
	0.5	60.00±0.10^{a}	70.00±0.21^{a}	55.00±0.10^{a}	55.00±0.09^{a}
	1.0	75.00±0.40^{a}	70.00±0.21^{ac}	80.00±0.12^{a}	80.00±0.05^{a}
	2.0	70.00±0.15^{a}	60.00±0.31^{ac}	65.00±0.15^{a}	65.00±0.13^{a}
	3.0	40.00±0.13^{a}	40.00±0.31^{bc}	50.00±0.11^{a}	65.00±0.10^{a}
Kinetin	0.0	25.00±0.20^{b}	60.00±0.30^{de}	50.00±0.00^{b}	85.00±0.06^{d}
	0.5	75.00±0.30^{b}	60.00±0.30^{de}	75.00±0.00^{b}	85.00±0.06^{d}
	1.0	70.00±0.20^{b}	65.00±0.22^{d}	50.00±0.00^{b}	30.00±0.14^{b}
	2.0	60.00±0.20^{b}	45.00±0.40^{de}	70.00±0.67^{b}	40.00±0.12^{bc}
	3.0	25.00±0.10^{b}	35.00±0.22^{fe}	40.00±0.74^{b}	40.00±0.12^{bc}
2iP	0.0	25.00±0.20^{c}	60.00±0.30^{cd}	50.00±0.22^{c}	85.00±0.06^{e}
	0.5	90.00±0.06^{e}	40.00±0.13^{c}	75.00±0.19^{e}	90.00±0.06^{e}
	1.0	70.00±0.12^{cd}	90.00±0.06^{e}	60.00±0.24^{c}	80.00±0.09^{e}
	2.0	65.00±0.12^{cd}	55.00±0.14^{cd}	85.00±0.10^{c}	80.00±0.14^{e}
	3.0	50.00±0.14^{cd}	45.00±0.10^{cd}	45.00±1.14^{c}	80.00±0.09^{e}

Percentage means with different letters in a column are significantly different ($p \leq 0.05$) according to the Tukey's test

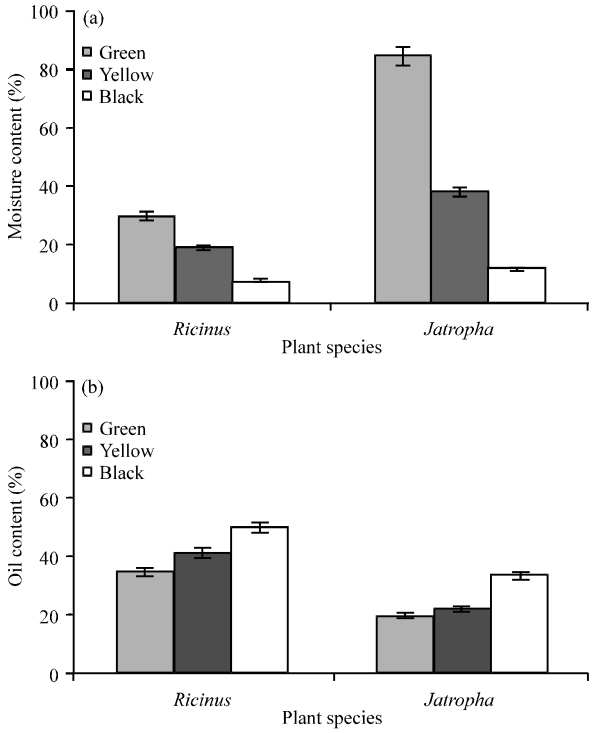

Fig. 2(a-b): (a) Moisture and (b) Oil content of zygotic embryos of *Jatropha curcas* and *Ricinus communis* at different stages of fruit maturity

concentration of cytokinin needed for shoot formation in *Jatropha* shoot tips varied; for kinetin 0.5 mg L^{-1} resulted in 75.0% shoot formation while in BAP and 2iP the optimal concentration was 1.0 mg L^{-1} (80.0% shoot formation) and 2.0 mg L^{-1} (85% shoot formation).

Similarly, meristem explants cultured on BAP, kinetin or 2iP also showed varied response in both *Ricinus* and *Jatropha* species. In *Ricinus*, shoot formation generally increased from the control to an optimal concentration before declining to the lowest. Meristem explants of *Ricinus* cultured on 1.0 mg L^{-1} 2iP significantly ($p \leq 0.05$) produced the highest shoots (90.0%) compared to 60.0% in the controls while a further increase to 2.0 or 3.0 mg L^{-1} significantly reduced shoot production to 55.0 and 45.0%, respectively. Similarly, in BAP amended medium, a concentration of 0.5 or 1.0 mg L^{-1} resulted in 70% shoot

Fig. 3 (a-d): Plant regeneration from shoot tip and meristem explants: (a) Shoot tip explants of *Ricinus communis* showing profuse callus formation on a medium amended with 3.0 mg L^{-1} BAP, (b) Shoot tip explants of *Jatropha curcas* cultured on a medium amended with 2 mg L^{-1} 2iP while, (c) and (d) are plant regenration from *Jatropha* and *Ricinus* meristematic explants

formation while at 3.0 mg L^{-1} shoot formation significantly reduced to 40%. Also, for kinetin, 1.0 mg L^{-1} significantly produced 65% shoots and a further increase in kinetin concentration to 3.0 mg L^{-1} reduced shoot formation to 35.0% (Table 2).

Although, the response of meristem explants of *Jatropha* to BAP and 2iP varied according to the concentration in the culture medium, the differences were not significantly ($p \leq 0.05$) different. However, for kinetin higher concentrations (1.0-3.0 mg L^{-1} significantly ($p \leq 0.05$) reduced shoot formation from 85% in the controls to 30 or 40% indicating that higher concentration of kinetin was inhibitory to shoot development (Table 2). Shoot development from both shoot tip and meristem explants of *Ricinus* was preceded by callus formation (Fig. 3a, c) which suppressed further plantlet development. Contrarily, in *Jatropha* shoot development occurred without prior callus formation (Fig. 3b, d) thereby resulting in well developed shoot with leaves.

DISCUSSION

The germination of zygotic embryos of *Ricinus communis* and *Jatropha curcas* was influenced by the stage of fruit maturity, presence of cytokinins in the culture medium as well as the oil content of the seeds. The addition of cytokinins in the culture medium significantly increased the viability of zygotic embryos of *Ricinus* depending on the stage at which the fruit were collected. However, in *Jatropha* although, the viability of zygotic embryos was increased by the presence of the cytokinins the increase was not generally significant over the controls compared to *Ricinus*. These contrasting results indicate that although, the two plant species (*Ricinus* and *Jatropha*) belong to the same family they respond differently to cytokinins in the culture medium.

The stage of fruit maturity had influence on the viability, moisture as well as the oil content of the seeds. In *Ricinus*, embryo viability increased as the fruit matured while in *Jatropha* it generally decreased. Kaushik (2003) and Feike *et al*. (2007) have demonstrated that seeds from yellow fruits of *Jatropha* are ideal for propagation, thus confirming the observation made in this study. Although, the exact cause of low viability or germination in matured *Jatropha* seeds is not known it may be due to some kind of fruit degradation while hanging on the tree (Feike *et al*., 2007). It may also be attributed to high oil contents which increased as the fruits matured and thus had inhibitory effect on germination.

In both plant species, the oil contents of the seeds increased with fruit maturity. The increase in oil content

may probably be due to the decrease in moisture content at fruit maturity. Our study showed that the percentage moisture content was comparatively higher in *Jatropha* than *Ricinus* while the oil content of *Ricinus* (35-50%) was conversely higher than *Jatropha* (22-33%). Johnston (2006) and Jongschaap *et al.* (2007) have independently reported that *Ricinus* seeds contain high oil content than *Jatropha*. According to Kandpal and Madan (1995) and Ginwal *et al.* (2004), the oil content of *Jatropha* seeds range from 28.4 to 42.3%. In this study, the oil content of *Jatropha* seeds ranged from 22% in green fruits to 33% in matured seeds (black fruit). The oil obtained from both *Ricinus* and *Jatropha* seeds may be commercially exploited in the biofuel industry as they have good physicochemical properties (Lele, 2006). Thus, alternative mode of propagation via *in vitro* culture which lends itself to genetic modification to improve on the oil content is relevant to the biofuel industry.

Although, the need for specific growth regulators for germination of zygotic embryos is not clear (Raghavan, 2003), there are various reports on the successful use of cytokinins for regeneration of *Jatropha* (Li *et al.*, 2007; Kalimuthu *et al.*, 2007) and *Ricinus* species from vegetative explants (Malathi *et al.*, 2006; Sailaja *et al.*, 2008). For example, Sujatha and Mukta (1996) successfully regenerated *Jatropha curcas* from cultured hypocotyl, petiole and leaf explants on an MS medium supplemented with varying concentrations of zeatin, kinetin and Benzyladenine (BA). We also observed in the present study that varying concentrations of BAP, kinetin or 2iP in the MS culture medium improved plant regeneration from shoot tip and meristem explants depending on the species. In both plant species the presence of cytokinins improved shoot regeneration from the explants but in *Jatropha* the increase from shoot tip explants is not significant over the controls.

Among the cytokinins tested, 2iP had the highest effect on shoot regeneration. Benzylaminopurine (BAP) has been shown to improve shoot bud induction compared to kinetin in *Jatropha* using shoot tip explants (Rajore and Batra, 2005). In their report BAP at 2 mg L^{-1} was most effective in shoot regeneration from *Jatropha* shoot tips while in the present study, BAP at 1 mg L^{-1} was most effective. The difference in the response of shoot tip explants to BAP may be attributed to the specific age and physiological condition of the donor plant from which shoot tip explants were excised. The use of 2iP and kinetin in the culture medium to enhance shoot induction in *Jatropha* has also been reported (Datta *et al.*, 2005). In their study 2iP enhanced comparatively higher shoot regeneration than kinetin, an observation similar to the present report.

Comparatively, the percentage shoot regeneration in *Jatropha* from meristem explants was higher than that of *Ricinus* species. The differences observed may probably be due to genotypic differences as well as the excessive exudation of phenolics by *Ricinus* explants. Phenols are secondary metabolites synthesized by plants which are often excreted into the culture medium and when oxidized leads to the darkening of the explant (Laukkanen *et al.*, 1999) as well as the culture medium thereby negatively affecting *in vitro* regeneration (Ozyigit, 2008). According to Vimala *et al.* (2005), *Ricinus* contains twice as much phenols compared to *Jatropha* and this may explain the excessive browning and subsequent deaths observed in our studies on *Ricinus* culture.

However, regeneration of plantlets via meristematic explants drastically reduced browning compared to the use of shoot tip explants. Consequently, comparatively more plantlets were regenerated from meristematic explants than shoot tips. The reduction in browning may be explained by the absence of polyphenolic compounds in the relatively smaller meristem explants.

CONCLUSION

This study demonstrates an attempt to regenerate *in vitro* two important oil producing plant species (*Ricinus communis* and *Jatropha curcas*) for biofuel production. Both plant species showed decrease in moisture content and an increase in oil content as the fruit matured from green to black stage. The oil can be exploited commercially to supplement fossil fuel. Although, the culture of shoot tips and meristems resulted in regeneration of plantlets, further investigations are needed to improve the production of sub-culturable plantlets to supplement conventional seed production. Such successful regeneration may augur well for future genetic transformation of these crops for increased biofuel production.

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