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Adventitious rooting of *Jatropha curcas* L. is stimulated by phloroglucinol and by red LED light

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Abstract An efficient root induction system has been established for *in vitro*-regenerated *Jatropha curcas* L. shoots. Callus formation on shoots transferred to auxin containing medium was found to be a prominent and recurrent problem for rooting of *in vitro*-cultivated *J. curcas*. In particular, the type of auxins and cytokinins applied in the culture media were shown to strongly influence the severity of callus formation. Shoots cultivated on meta-methoxytopolin riboside (MemTR) were free of callus and produced elongated stems and well-developed leaves in comparison to the cytokinins benzyl adenine, zeatin, and thidiazuron. Subsequent root induction experiments were performed with shoots precultured on MemTR-containing medium. Shoots were excised and transferred to Murashige and Skoog (MS) medium supplemented with different concentrations of indole-3-butyric acid (IBA), indole-3-acetic acid (IAA), and α-naphtaleneacetic acid (NAA). The induction of excessive callus formation was avoided only on IBA-containing medium. The optimum rooting medium with good root induction (35%) and 1.2 roots per shoot contained half-strength MS salts supplemented with 2.5 μM IBA. The same medium supplemented with 0.25% (*w*/*v*) activated charcoal produced 46% rooted shoots. Further improvement of rooting was obtained by transferring *in vitro* grown shoots to woody plant medium containing phloroglucinol (PG). In the presence of 2.5 μM IBA and 238 μM PG, 83% of the shoots rooted with on average 3.1 roots per shoot. We also analyzed the impact of light quality on the rooting capacity of *Jatropha in vitro* grown shoots. In general, light-emitting diodes (LEDs) light sources were less efficient for root induction. Red LED light provided the most favorable growth conditions, inducing a rooting response in 65% of the shoots, which produced on average 5.5 roots per shoot. These results indicate that adventitious rooting in *J. curcas* is under control of photoreceptors and that optimal rooting requires fine-tuning of the salt concentration, auxin, and cytokinin balance and application of synergistic compounds.

Keywords *In vitro* root induction · *Jatropha curcas* · Light-emitting diodes (LEDs) · Phloroglucinol

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Introduction

Jatropha curcas L. belongs to the Euphorbiaceae family. It is a multipurpose plant: an emerging energy crop, but certain varieties are also valued for their nutritional and medicinal properties (Openshaw 2000). *J. curcas* seed contains high amounts of oil that have been evaluated prospectively as a substitute for diesel engine fuel (Reddy and Pamidimarri 2010). Furthermore, studies on the chemical and biological active constituents of *J. curcas*, revealed the presence of saponins, steroids, tannin, glycosides, alkaloids, and flavonoids in stem barks extracts from this plant (Igbinosa et al. 2009) and presence of phenolics, flavonoids, and saponins in seed kernel extracts (Oskoueian et al. 2011). Among the terpenes, diterpenoid compounds have dominated the research area in *Jatropha* species with respect to their novel chemical structures and potential medicinal value (Devappa et al. 2011).

In order to meet the demand of biofuel in the future, the development of an appropriate technology for the large-scale production of elite varieties will become critical. Until now, conventional propagation of *J. curcas* is produced in two ways, either through seed or stem cuttings.

Propagation through seeds is not favorable because of loss of genotype homogeneity due to out-crossing and the limited seed production capacity of plants. Propagation through stem cuttings is satisfactory for small-scale reproduction, but the multiplication rate is too low for the establishment of large plantations. Moreover, the root system from regenerated stem cuttings is not the same as that of plants grown from seed. Seedlings produce a single taproot with four lateral roots (Reubens et al. 2011), whereas stem cuttings produce a very different root system with mainly superficial and thin roots (Severino et al. 2011). The *in vitro* cultivation of *J. curcas* provides an alternative to multiplication from stem cuttings with a much higher propagation potential (Deore and Johnson 2008). However, one of the significant problems with *in vitro* cultivation of *J. curcas* remains to be difficulty in forming high-quality roots.

Adventitious rooting is a complex process and a key step in the vegetative propagation of economically important woody, horticultural, and agricultural species and is a critical factor for successful production of elite clones (Davis and Haissig 1994). The quality of roots affects the survival rate and acclimatization of young plantlets. There is a significant variation in the rooting potential of different plant species, and systematic trials are often needed to define the conditions required for root induction (Rout et al. 2000).

The aim of this work was to improve rooting of regenerated shoots of *J. curcas*. So far, several studies have been reported on the micropropagation of *J. curcas* (Rajore and Batra 2005; Sujatha et al. 2005; Datta et al. 2007; Kalimuthu et al. 2007; Li et al. 2008; Shrivastava and Banerjee 2008; Sujatha et al. 2008; Kumar et al. 2010; Mazumdar et al. 2010; Kumar et al. 2011). These studies have contributed to understanding the effects of a variety of medium components and various explants leading to enhanced frequency of *in vitro* regeneration. In our laboratory, we are focusing on the process of adventitious root induction. Preliminary experiments had shown that the *in vitro* induction of roots on *J. curcas* shoots was difficult and usually result in poor root quality, indicating that there was a need for optimization. The initial experiments showed that callus formation is a major problem for *J. curcas* root induction. Here we present a protocol for the *in vitro* rooting of *J. curcas* that shows no or limited induction of callus and involves using IBA as root-inducing auxin, phloroglucinol, and constant red light as enhancers of adventitious root induction.

Materials and Methods

Plant materials and source of explants. The seeds of *J. curcas* were collected in Kiambere, an Eastern Province of Kenya. A single seed was germinated in soil and maintained in greenhouse conditions. The shoot tips from the 6-mo-old plant were cut, washed with tap water, and surface-sterilized for 30 min with 10% (*v*/*v*) of Haz-tab solution (Guest Medical, Kent, UK) with a drop of Dreft™ detergent (Procter and Gamble, Surrey, UK), and rinsed three times with sterile distilled water. Surface-sterilized explants were propagated on Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with 0.8% (*w*/*v*) agar (Lab M plant tissue culture agar MC29, Amersham, London, UK), 3% (*w*/*v*) sucrose, and 4.4 μM benzyl adenine (BA). The pH was adjusted to 5.6. Shoots were subcultured every 4 wk in the same medium. After six subcultures, several hundred shoots were obtained. Then, shoots were tested for propagation on MS medium containing the cytokinins BA, meta-methoxytopolin riboside (MemTR), thidiazuron (TDZ), or zeatin. In subsequent rooting experiments, shoots propagated on 4.1 μM MemTR containing medium were used. Three shoots were grown in screw cap jars (300 mL) containing 50 mL media and maintained at 26°C in 16/8-h light/dark photoperiod (32.7 ± 3.23 $\mu mol\, m^{-2} s^{-1}$) provided by warm white fluorescent light Osram (München, Germany). For root initiation, shoots were separated individually and grown on medium with various auxins and supplements. The data obtained for micropropagation and root induction are the average of at least three biological repeats with each at least 15 shoots per experiment.

Hormonal composition of medium. Shoots (1.0–1.5 cm in length) were cultured on either full- or half-strength MS medium containing different concentrations of α-naphthaleneacetic acid (NAA; 2.9, 5.7, or 8.6 μM), indole-3-butyric acid (IBA; 2.5, 4.9, 7.4, 9.8, or 14.7 μM), or indole-3-acetic acid (IAA; 2.9, 5.7, or 8.6 μM), plus sucrose 3.0% (*w*/*v*), and 0.8% (*w*/*v*) agar. The pH was adjusted to 5.6. To test whether the toxic effect of metabolites, which are presumed to be produced and leak into the culturing medium upon IBA application, could be prevented we included activated charcoal (AC; 0.25%, *w*/*v*) in the medium.

Phloroglucinol treatment. Shoots were separated individually and transferred to rooting medium comprising half- or full-strength MS or woody plant medium (WPM) including vitamins (Lloyd and McCown 1981). The basic salts were supplemented with 0.17% (*w*/*v*) gelrite, 3.0% (*w*/*v*) sucrose, 2.5 μM IBA, and phloroglucinol (PG). Freshly prepared PG (anhydrous; MW= $126.1\ g\, mol^{-1}$) stock solution was filter sterilized (0.22 μm; Millipore, Billerica, MA) and diluted as required. PG was added to the rooting medium to a final concentration of 119 or 238 μM. Shoots were cultured on nutrient medium without PG as the control.

Light-quality effect. The cultures were maintained at 23±2°C under a 16-h light photoperiod. Control treatment used cool fluorescent light (FL), provided by PHILIPS master TLD 36 W 830 Reflex ECO (45 μmol$m^{-2}s^{-1}$ PAR). Light-emitting diode (LED) treatments were blue (450 nm), red (660 nm), or red+ blue (50:50 photon flux density) light. For each treatment, 20 PHILIPS GreenPower LED strings were mounted 15 mm apart in a rack constructed of white walls and a white door. Total photon flux density was adjusted to 45 μmol photons $m^{-2}s^{-1}$. For the red and blue combination, red and blue strips were alternated.

Acclimatization of rooted plantlets. *In vitro*-rooted plantlets of *J. curcas* were randomly selected for acclimatization. Plantlets were taken from the solid medium and agar was removed from the roots under running tap water. Each plantlet was then planted into pots containing a mixture of organic soil and sand (1:1), then placed in a plastic tunnel in the greenhouse. After 2 wk, the relative humidity was slowly reduced by gradually removing the cover. Plant survival was recorded 4 wk after cultivation in a greenhouse under natural daylight conditions.

Statistical analysis. Data were collected as the frequency of shoots with root formation (in percent) and number of roots per shoot. The statistical analysis of data, one-way ANOVA followed by Duncan's multiple range tests, was performed at the level of *P* value less than 0.05 using SPSS 17.0 (SPSS Inc., Chicago, IL).

Results and Discussion

Shoot multiplication experiments. Initially, we used a previously reported *J. curcas* micropropagation protocol that included 4.4 μM BA in full-strength MS medium (Sujatha et al. 2005). Under these conditions, we frequently observed the formation of callus, which prevented the induction of high-quality adventitious roots. We therefore tested different cytokinins, MemTR, zeatin, and TDZ (Table 1) to reduce the callus growth. The optimal conditions for callus-free *in vitro* shoot growth was obtained using MS medium supplemented with 4.1 μM MemTR (Table 1). This medium gave acceptable multiplication rates and, in addition, generated high-quality shoots with elongated stems and well-developed leaves without necrosis (Fig. 1).

Effect of growth regulators on root induction. We initially investigated whether auxins would promote adventitious root formation of *J. curcas in vitro* grown shoots. To do this, 2-mo-old *in vitro* shoots (Fig. 1) were cultured on MS medium comprising full- or half-strength MS containing IBA, IAA, or NAA at different concentrations. In the presence of NAA and IAA, excessive callus growth was induced at the basal end of the *Jatropha* shoots and these hormones were therefore omitted from further testing. The results for adventitious root induction on half-strength MS containing IBA are presented in Table 2. Full-strength MS medium caused frequent necrosis, which started at the shoot tips. The leaves turned yellow and plants died ~4 wk after culturing, preventing the collection of root induction data. The symptoms were most prevalent on shoots that had been treated with the highest IAA dose. However, on half-strength MS supplemented with 2.5 μM IBA, we observed that, at 6 wk after transfer to hormone containing medium, 35% of shoots had formed on average 1.2 roots per shoot (Table 2; Fig. 2*a*). In contrast, IAA, at any of the applied concentrations (2.9, 5.7, or 8.6 μM) induced profuse callus growth and no roots (data not shown). Our data show that high doses of auxin do not favor rooting, a result that deviates from the report by Kumar et al. (2011) who showed that high auxin concentrations (5 μM and above) or combinations of IAA, IBA, and NAA promoted rooting. This apparent contradiction may result from the different application technique. Here, auxin was applied continuously via the root induction medium and roots were measured at the end of the 6 wk incubation period, whereas Kumar et al. applied a pulse treatment of 4 d and scored rooting after 4 wk incubation on medium without hormones. It is of interest though that our method leads to a higher rooting frequency (35% compared with roughly 20% by pulse treatment as reported by Kumar et al. (2011)). These results suggest that continuous exposure to auxin enhances the frequency of root formation. Further support for this comes from several reports showing that IBA often performs better than IAA, as is the case for *Jatropha* shoots shown here. Indeed, IBA is readily converted to IAA (George 2008), causing a slow release of IAA and thereby providing a continuous supply of the most common active auxin at concentrations that may be more adequate for rooting.

Callus formation is a significant problem for commercial micropropagation and needs to be minimized at all times. At low doses, IBA rarely stimulated callus formation in *J. curcas*. Also, the addition of AC to the culture medium stimulated callus formation probably because it caused a shift in the auxin/cytokinin balance (Table 2). Ozel et al. (2006) reported that higher levels of IBA applied to plants inhibited the formation of shoot buds and this might further prevent the production of roots. In our experiments, at 4.9 μM IBA and higher concentrations, root induction was inhibited.

According to Bhatt and Tomar (2010), low concentrations of IBA were found to be more effective for root primordia initiation. However, the increase in IBA above the optimum level showed an inhibitory effect on rooting.

Table 1. Effect of four plant growth regulators on shoot regeneration from shoot tips explants of *J. curcas*

Plant growth regulators (μM)				Response of shoot (%)	No. shoots/explant (±SE)[z]	Callus formation[y]
BA	MemTR	TDZ	Zeatin			
–	–	–	–	42	0.0 f	–
2.2	–	–	–	100	3.2±0.08 b	+
4.4	–	–	–	100	3.6±0.10 a	+
6.7	–	–	–	83	2.2±0.09 c	++
–	2.1	–	–	100	2.0±0.00 c	–
–	4.1	–	–	100	3.5±0.10 a	–
–	6.2	–	–	92	3.0±0.09 b	–
–	–	2.3	–	42	1.2±0.13 e	+
–	–	4.6	–	50	1.0±0.00 e	++
–	–	6.8	–	50	1.5±0.15 d, e	++
–	–	–	2.3	42	1.6±0.16 d	++
–	–	–	4.5	58	1.3±0.13 e	++
–	–	–	6.8	58	1.0±0.00 e	+++

Shoot induction was evaluated and scored after 6 wk of culture on MS medium

[z] Different *letters* indicate significant differences within each treatment according to Duncan's test ($P<0.05$)

[y] Surface of explants covered with callus was scored as "–"=no callus, "+"=0.1–0.5 cm^2, "++"=0.5–1.0 cm^2, and "+++"=>1.0 cm^2

Higher concentrations of IBA can induce higher levels of secondary metabolites, which may lead to the inhibition of the root formation process (Baker and Wetzstein 1994). Moreover, high levels of IBA can result in ethylene accumulation in the tissue culture vessel, which also inhibits the induction of root primordia (De Klerk 2002).

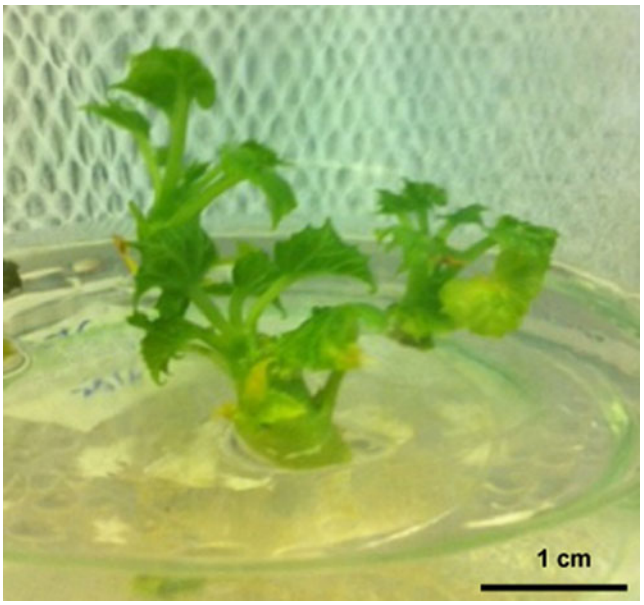

Figure 1. Shoots of *J. curcas* used for rooting experiments. Individual axillary shoots were cut and propagated on MS medium containing 4.1 μM MemTR.

Effect of activated charcoal on root induction. In the presence of AC, we observed a 10% increase in the frequency of shoots that produced roots, with on average 1.4 roots per shoot (Table 2; Fig. 2*b*). On full- and half-strength MS medium supplemented with IAA and AC, none of the conditions resulted in the induction of adventitious roots and only callus formed (data not shown). The addition of AC indeed seemed to promote callus formation as we also observed more frequent callus at the shoot base in medium with high levels of IBA (Table 2*b*). It is well known that AC adsorbs cytokinin, which may have accumulated during the shoot propagation process. By removing excess cytokinin, the balance of auxin/cytokinin shifts to a ratio that promotes callus formation at the cut end of the *Jatropha* shoots.

Comparison of woody plant medium and MS medium. To further optimize the root induction protocol, we tested the influence of the nutrient composition of the medium. The root induction capacity was measured for shoots grown on half-strength MS and half-strength WPM, both containing 2.5 μM IBA (Table 3). In this series of experiments, 44% of the shoots incubated on half-strength MS+2.5 μM IBA produced roots, with on average one root per shoot. The percentage of shoots producing roots was very similar to the results obtained in the first series of experiments. IBA-stimulated root induction was as effective in half-strength WPM as in half-strength MS, despite earlier reports that woody species are sensitive to the relatively high ion strength of MS, which may inhibit adventitious root induction and growth (Bairu et al. 2009). Similar to MS medium,

Table 2. Effect of IBA and activated charcoal (0.25%) on root formation in *J. curcas* shoots after 6 wk of *in vitro* culture

Medium	IBA (μM)	Root formation (%)	No. of roots/shoot (±SE)[z]	Callus formation[y]	Acclimatization	
					No. of plantlets	Survival (%)
½ MS	–	0	0.0 b	–	–	–
	2.5	35	1.2±0.13 a	–	5	0
	4.9	0	0.0 b	–	–	–
	7.4	0	0.0 b	–	–	–
	9.8	0	0.0 b	–	–	–
	14.7	0	0.0 b	–	–	–
½ MS+activated charcoal	–	0	0.0 b	–	–	–
	2.5	46	1.4±0.15 a	–	5	0
	4.9	0	0.0 b	–	–	–
	7.4	0	0.0 b	–	–	–
	9.8	0	0.0 b	+	–	–
	14.7	0	0.0 b	++	–	–

[z] Different *letters* indicate significant differences within each treatment according to Duncan's test ($P<0.05$)

[y] Surface of explants covered with callus was scored as "–"=no callus, "+"=0.1–0.5 cm^2, and "++"=0.5–1.0 cm^2

WPM medium resulted in a satisfactory rooting frequency (48%) and one root per shoot, although both treatments had a 0% survival rate following acclimatization (Table 3).

Phloroglucinol strongly promotes adventitious root induction in WPM medium. Rooting efficiency is a critical parameter for the success of commercial micropropagation technology. We, therefore, tested various additives that may promote rooting (unpublished results). One of these components was phloroglucinol, a benzenetriol that has been used previously for *in vitro* rooting of apple cultivars (Dubranszki and da Silva 2010) and *Prunus avium* (Hammatt and Grant 1997). The response varied depending on the applied PG concentration (Table 3). The highest percentage (83%) of root formation with 3 roots per shoot was observed on half-strength WPM medium supplemented with 238 μM PG (Table 3; Fig. 2*c*). Compared with the control experiment, roots emerged 5 d earlier, and the number of roots per shoot was increased up to threefold. Interestingly, both the enhanced root inductions as well as the increased growth vigor were not observed when PG was applied in MS medium. In fact, at the highest concentration

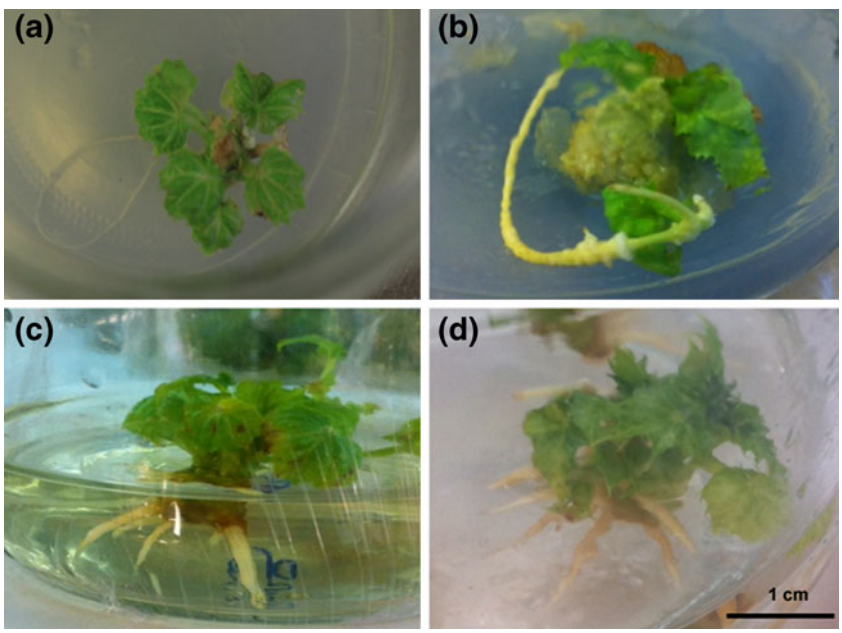

Figure 2. Adventitious rooting of *J. curcas in vitro* grown shoots under various conditions: *a*, ½ MS medium supplemented with 2.5 μM IBA; *b*, ½ MS medium supplemented with 2.5 μM IBA+0.25% (*w*/*v*) AC; *c*, ½ WPM medium supplemented with 2.5 μM IBA+238 μM PG; *d*, ½ WPM supplemented with 2.5 μM IBA+238 μM PG placed under constant red light.

Table 3. Effect of culture medium, phloroglucinol, and IBA on rooting of shoots and plantlet survival of *J. curcas* after 6 wk of culture

Medium	Phloroglucinol (μM)	IBA (μM)	Root formation (%)	No. of root/shoot (±SE)[z]	Acclimatization	
					No. of plantlet	Survival (%)
	0	0	0	0.0 d	0	0
½ MS	0	2.5	44	1.0±0.00 c	5	0
	119	2.5	46	1.0±0.00 c	5	0
	238	2.5	36	1.0±0.00 c	5	0
	0	0	0	0.0 d	0	0
½ WPM	0	2.5	48	1.0±0.00 c	5	0
	119	2.5	73	2.8±0.09 b	10	60
	238	2.5	83	3.1±0.07 a	10	60

[z] Different *letters* indicate significant differences within each treatment according to Duncan's test ($P<0.05$)

of PG (238 μM), we observed a 10% reduction in the root induction frequency, suggesting an inhibitory effect.

Several reports indicate that PG acts as an auxin synergist during the auxin sensitive phase of root initiation (Jones 1976; Jones and Hatfield 1976; Hammatt 1994; James and Thurbon 1981; Dubranszki and da Silva 2010). De Klerk et al. (2011) reported that phenolic compounds, such as PG protects the auxin IAA from decarboxylation at wound sites in apple slices and *in vitro* shoots. Here, we found a synergistic effect of PG with IBA, an auxin analog that also may be degraded by decarboxylation similar to that reported for IAA (De Klerk et al. 2011). In this regard, the observation that PG is effective in WPM medium and not in MS suggests that decarboxylation would be more pronounced in WPM medium. Currently, there is no evidence for such correlation, but the difference between MS and WPM is likely to be attributable to the high nitrogen content present in MS (60 μM) as compared with WPM (14.7 μM) or the ten times higher copper content in WPM compared with MS. Copper-containing enzymes catalyze the oxidation and decarboxylation of IAA (Wagenknecht and Burris 1950) and may be responsible for the presumed acceleration of IBA breakdown in WPM medium. By adding PG, these enzymes would be inhibited and allow a more sustained presence of IBA in WPM medium, which is favorable for the cultivation of woody plant species due to a reduced nitrogen content.

Table 4. Effect of different light treatments on root formation on *J. curcas* shoots

Light treatment	Root formation (%)	No. of root/shoot (mean±SE)[z]	Acclimatization	
			No. of plantlet	Survival (%)
Fluorescent light	56	3.1±0.10 c	10	40
LED Blue+ Red	0	0.0±0.00 e	0	0
LED red	65	5.5±0.17 a	10	80
LED blue	54	4.2±0.10 b	10	60
LED white	32	2.1±0.14 d	5	20

Shoots were cultured in ½ WPM medium supplemented with 2.5 μM IBA, 238 μM PG, 3% sucrose, and 0.17% gelrite. Roots were assessed after 6 wk of culture

[z] Different *letters* indicate significant differences within each treatment according to Duncan's test ($P<0.05$)

Effect of light quality on Jatropha root induction. The spectrum of light during *in vitro* culture is important not only for healthy morphological characteristics but also for the efficiency of adventitious root formation (Iacona and Muleo 2010). LED allow illumination of relatively narrow wavelengths of light within the photosynthetic spectrum (Tennessen et al. 1994). We used different LED light sources to monitor the impact on rooting of *in vitro Jatropha* shoots. Shoots were rooted after 2 wk of cultures in half-

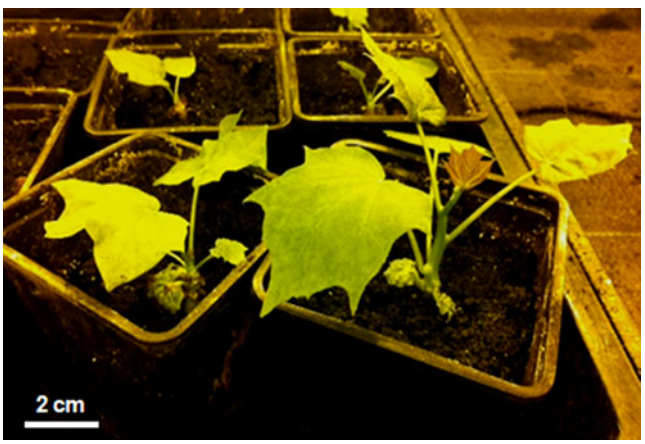

Figure 3. Potted *J. curcas* plantlets after 6 wk on rooting medium ½ WPM+2.5 μM IBA+238 μM PG followed by 6 wk of acclimatization in the glasshouse.

strength WPM medium supplemented with 2.5 μM IBA, 238 μM PG, sucrose 3% (*w*/*v*), and 0.17% (*w*/*v*) gelrite under white, blue, red, and mixed blue and red LED. Exposure to white, red and blue LEDs were suitable for *Jatropha* root induction, whereas the combined red plus blue LED completely prevented rooting (Table 4). Red light provided a better root formation response than did blue and white light. Moreover, shoots grown under the red LED formed more roots (5.5 roots per shoot; Fig. 2*d*) than those placed under white or blue LED (Table 4). Within the same growth room, the comparison of LED with FL showed a slight advantage when red LED was applied.

Similar red light stimulated adventitious root induction has been reported for grape (Poudel et al. 2008), *Ficus benjamina* (Gabryszewska and Rudnicki 1997), and *Morinda citrifolia* (Baque et al. 2010). In these species, the combination of red and blue light did not favor adventitious root induction. Different results were reported for cherry rootstock, whereby blue light was more effective for the induction of adventitious rooting (Iacona and Muleo 2010). It is clear that the impact of light quality varies for different species. For *Jatropha*, we found that red light increases the rate of rooting as well as the number of roots per shoot. Moreover, the *in vitro* grown plants were of high quality showing little or no necrosis (Fig. 2*d*).

*Acclimatization efficiency. Jatroph*a shoots carrying well-developed roots were transferred to soil and acclimatized in a plastic tunnel for adaptation to greenhouse conditions. The acclimatization process tested rooted shoots from the different experiments. The survival rate of plantlets is presented in Tables 2, 3, and 4. In general, pre-incubation conditions that favored rooting also favored successful acclimatization. Those conditions that produced the highest percentage of rooted shoots allowed the highest recovery after transfer to soil. Interestingly, phloroglucinol pretreatment appeared to be required for survival (Tables 2, 3, and 4), suggesting that it may have had other beneficial effects, possibly by promoting shoot growth (Jones 1976). In addition, we found that shoots with more than one root were more likely to regenerate (data not shown). Finally, the plantlets regenerated from shoots incubated under constant red light performed slightly better than plants regenerated from shoots incubated under FL (Fig. 3).

Conclusions

In this study, we optimized the rooting process of *in vitro* cultivated *J. curcas* shoots. Callus formation is a prominent problem for *in vitro* cultivation of *Jatropha* shoots, and under those conditions, the root quality is often inadequate. Half-strength WPM supplemented with 2.5 μM IBA and 238 μM PG was the best performing medium composition, whereby callus formation was absent or minimal. The rooting ability of *J. curcas* was strongly improved by adding PG to the rooting medium. The effect of PG was dependent on the basic salt composition as stimulation of rooting was observed in WPM medium but not in MS medium. A second stimulating effect was the incubation of shoots under constant red LED light. Red LED light had additional beneficiary effects on shoot growth and improved the regeneration capacity of the plants. Collectively, our results contribute to defining optimal conditions for *in vitro* culture of *J. curcas*, which will become increasingly relevant with the rising demands for the clonal propagation of elite varieties.

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