

1 Regulation of Organogenesis via PGRs and LEDs Light Technology for *Jatropha curcas* L. Plants

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Received: 29/11/2016

Accepted: 13/2/2017

BECAUSE of the increased interest in the potential of *Jatropha* as a biofuel plant, more attention is given to methods that allow the mass production of elite material. A simple and efficient protocol has been developed for high frequency regeneration using nodal explants of Madagascar and Ecuador *Jatropha curcas* lines. The optimum shoot growth with high number and without callus formation was observed using MS medium supplemented with 1.0 mg/l BA in both lines within a period of 30-40 days which shows 3.8 and 3.4 shoot buds in Madagascar and Ecuador lines, respectively. Healthy elongated shoots were harvested and cultured on ½ strength MS basal salts without vitamins medium containing different concentrations of IBA individual or in combination with IAA or 2,4-D. Moreover, the optimum rooting medium with good root formation (46.6 and 40%) in both *Jatropha* lines and (3.0 – 2.4) roots per shoot containing half-strength MS supplemented with 0.5 mg/l IBA. In the present study, different light sources like cool white fluorescent, warm white fluorescent and different high-brightness LEDs (red – blue – white) were used to monitor the root initiation of *in vitro* *Jatropha* shoots. Red LED light provided the most favorable growth conditions inducing a rooting response in 66.6% of the shoots in Madagascar *Jatropha* and 56.5% in Ecuador *Jatropha* which produced on average 4.2 and 3.5 roots per shoot, respectively in both lines. Thus, white LED light was more effective on root elongation than all studied light treatments that recorded (5.6 and 4.4 cm) root length in both lines. Therefore, well developed healthy *in vitro* rooted plantlets after 6 weeks were successfully acclimatized and the survival rate of *in vitro* propagated plantlets after transplanting was 40-60% and they grew as normal plants in the greenhouse.

Keywords *Jatropha curcas*, Organogenesis, Plant growth regulators (PGRs), *in vitro* Rooting, Light-Emitting-Diodes (LEDs)

INTRODUCTION

Jatropha curcas is a species of flowering plant in the spurge family (Euphorbiaceae). *Jatropha* genus is large comprising more than 170 species succulent plants. Most of shrubs and trees are ornamental except *J. curcas* and *J. glandulifera* that are oil-yielding species (Swarup, 2004). It has rapid growth, easy propagation, low seeds cost, high oil content (up 60%) which can be transformed into fuel through esterification (Li *et al.*, 2007). Easy, wide adaptation and root architecture involves a pen root that grows to great depths highly suitable for fixing the soil and the prevention of erosion so it can be used for reclamation of waste lands (Achten *et al.*, 2007). The optimum plant size makes the seed collection convenient (Jones & Miller, 1991; Francis *et al.*, 2005 and Kumar & Sharma, 2008).

Energy experts claim that *Jatropha* oil is an environmentally safe, cost-effective and a

promising substitute for diesel, kerosene and other fuels (Abdulla *et al.*, 2011). *Jatropha* oil is high in cetane value and can be used directly in diesel engines added to diesel fuel as an extender or transesterized to a biodiesel fuel but also it is not edible due to the presence of several toxic substances such as "curcasine" or curcin, phorbol esters, saponins, protease inhibitors and phytates (Menezes *et al.*, 2006 and Datta *et al.*, 2007). Conventionally, propagation through seeds is a widely used but it is not favorable because of loss of genotype homogeneity due to out-crossing and it is beset with problems of poor seed viability, low germination, scanty and delayed rooting of seedlings (Daud *et al.*, 2013). On the other hand, mass propagation through stem cuttings are seasonal, seed yield are low, were reported to generate plants with a lower longevity, a lower drought and disease resistance therefore the established plants are not deep rooted, hence are easily uprooted as they do not form a taproot

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DOI :10.21608/agro.2017.419.1043

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system (Sujatha *et al.*, 2005 and Datta *et al.*, 2007). As Attaya *et al.* (2012) reported *in vitro* techniques are being increasingly applied for clonal propagation of selected tree species to supplement conventional methods which have their limitations especially when a large number of genetically uniform propagules are required. Moreover, these technologies would help in producing the active compounds *in vitro* with better productivities without cutting down the natural resources. Recently, many studies have been reported on *Jatropha in vitro* propagation through organogenesis from different explants (Datta *et al.*, 2007; Deore & Johanson, 2008 and Misra *et al.*, 2010) but few are through somatic embryogenesis (Sardana *et al.*, 2000 and Jha *et al.*, 2007). Although shoot multiplication is good but it records low growth quality, show callus formation, low rooting percentage and difficult acclimatization which restricts clonal propagation of quality planting material.

This study aimed to investigate the influence of different plant growth regulators and focusing on the process of adventitious root induction to improve rooting of regenerated shoots, to develop an efficient and reproducible protocol for *Jatropha* micro propagation that shows no or limited callus induction using shoot tips on different *Jatropha curcas* lines for mass propagation and genetic improvement.

MATERIALS AND METHODS

Plant material

Jatropha curcas seeds were collected from Madagascar and Ecuador by Prof. Dr. Patrick Van Damme, Laboratory of Tropical and Subtropical Agronomy and Ethnobotany, Bioscience Engineering Faculty, Ghent University. The seeds were germinated in soil and maintained in laboratory greenhouse. During the period from 2015 to 2016 at *in vitro* biology and horticulture unit, Plant Science Department, McGill University, Canada, shoot tips of (1.5 cm long) and (0.5 cm wide) from six-month-old donor plants were cut. The shoot tips were washed and then submerged in tap water with a drop of liquid soap (Dreff™ detergent; Procter and Gamble, Surrey, UK) in a flask and shaken by hand for 5 min then rinsed in tap water to remove the soap then explants were sterilized with 70 % (v/v) ethanol for 30 sec and subsequently surface sterilized with 70% (v/v) solution of a commercial disinfection product (Haz-taps; Guest Medical, Kent, UK) followed by repeated washing (3 times) with sterile distilled water under aseptic condition in

laminar air-flow hood.

Culture media and conditions

The sterilized explants were trimmed (0.5-1.0 cm) at the base and cultured with the cut surface in contact with MS basal salt mixtures including vitamins medium (Murashige & Skoog, 1962) supplemented with 30 g/l sucrose and included different concentrations of BA, meta-methoxytopolin-riboside (Mem-Tr) and Zeatin in addition to 8 g/l of agar. pH of the medium was adjusted to 5.6 - 5.8 after gelling with agar and autoclaved at 121°C and 1.1kg / cm² for 20 min. The cultures were maintained in an air conditioned incubation room at 25 ± 2°C under 16 h/ day photoperiod which provided by cool white fluorescent lamps (light intensity 2000 Lux.).

In vitro rooting and hardening

Shoots (2-4 cm) in length regenerated from *in vitro* explants were rooted on ½ strength MS medium supplemented with 30 g/l sucrose and 8 g/l agar with different concentrations of auxins like (IBA – IAA – 2,4-D). To monitor the initiation and quality of adventitious roots on the regenerated shoots, the cultures were maintained by cool white fluorescent lamps as a control treatment provided by Philips, warm white fluorescent lamps provided by Osram (München, Germany) and three Light-emitting-diodes (LEDs) treatments which were blue (450 nm), red (660 nm) and white. In the present investigation, healthy *in vitro* plantlets with well-developed shoots and roots were produced from nodes of two *Jatropha curcas* lines (Madagascar and Ecuador) through direct adventitious shoot regeneration. Then, well rooted shoots after 6 weeks of culture on root induction medium were carefully taken out of the medium and washed thoroughly in sterilized distilled water to remove medium attached to the roots for *ex vitro*. The plantlets were then planted into pots containing a mixture of organic soil and sand in the ratio of 1:1 then placed in a plastic tunnel and wetted with tap water followed by covering with transparent plastic bags to maintain humidity. After 3-4 weeks, the established plants were transplanted to poly ethylene bags containing garden soil and farmyard manure for further growth

Statistical analysis

Statistical difference among the means was analyzed by Duncan's multiple range test (DMRT) at 0.05 level using the SPSS (version 17) and the results were expressed as the mean ± SE. Data were also subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Shoot multiplication and elongation

Effect of PGRs on multiple shoot formation

The experiments conducted to optimize the medium for multiplication of *Jatropha curcas* lines are summarized in Table 1. The effect of different plant growth regulators on multiple shoot formation was clearly dependent on the used lines. Explants were cultured on full strength MS basal medium including vitamins supplemented with 30 g/l sucrose and 8 g/l of agar in addition to various cytokinins like (BA, Mem-Tr, Zeatin) individually with different concentrations (0.1, 0.5, 1.0 mg/l). The optimum shoot growth with high number was observed using MS medium supplemented with 1.0 mg/l BA in both lines within a period of 30-40 days (Table 1; Fig 1) which shows 3.86 and 3.4 shoot buds in Madagascar and Ecuador lines, respectively. followed by the concentration of 1.0 mg/l mem-tr that gave the longest shoot length which shows 4.66 and 4.36 cm, respectively but proved to be second best concentration recording 3.66 and 3.13 number of shoots in Madagascar and Ecuador *Jatropha*, respectively. According to Shrivastava & Banerjee (2008), there are several reasons for preferred use of BA as cytokinins, slow degradation and it can be autoclaved without losing its activity. On the other hand, by increasing the concentration of BA, mem-tr or zeatin the shoot formation increased in both lines. Moreover, using 1.0 mg/l zeatin proved to be second best concentration recording 4.33 and 3.63 cm shoot length in Madagascar and Ecuador lines, respectively.

At the same concentration of 1.0 mg/l BA but here in combination with 1.0 mg/l IBA (Table 2), a maximum initiation of healthy shoot buds (1.43 and 1.60) were obtained in both lines after 6 weeks of culture. The obtained shoots were lesser in its number than the number in the 1st experiment. However, using 0.5 mg/l BA in the presence of 0.5 mg/l IBA recorded the second best shoot formation (1.3 and 1.4 shoots /explant) in both lines. The results are in harmony with that found by Datta *et al.* (2007). In this experiment, the best response of 1.0 mg/l Mem-Tr (1.0 and 1.43 shoots per explant) was obtained in the presence of 1.0 mg/l IBA which recorded the best shoot length (1.9 and 2.1 cm) in both lines, respectively. Moreover, presence of NAA in combination with BA or Mem-Tr did not form any shoots in both *Jatropha* lines.

Also, addition of IBA along with BA has been reported to regenerate shoot buds from the nodal explants of *Jatropha curcas* (Shrivastava & Banerjee, 2008). Moreover, Maharana *et al.* (2012) mentioned that cytokinins at its high concentration stimulated development of meristems and its optimal concentration promotes shoot proliferations, and the inclusion of low concentration of auxins along with cytokinins triggers the rate of shoot proliferations. This differential response may be attributed to the specific age and physiological condition of the donor plant from which the nodal explants were excised.

Callus formation is a significant problem for *Jatropha* micropropagation and needs to be minimized. It was observed in the presence of different NAA concentrations. However, adding mem-Tr to the MS medium showed free callus and produced elongated stems and well-developed leaves in comparison to BA and Zeatin (data not shown) thus, in the presence of different concentrations of IBA except using it with NAA (Table 2).

In vitro root induction

Effect of PGRs on root formation

Adventitious rooting is a complex process and a key step in the vegetative propagation of economically important woody, horticultural and agricultural plant species and is a critical factor for successful production of elite clones (Davis & Haissig, 1994). In this study, Generated shoots (1-1.5 cm long) were cultured on half MS basal salt without vitamins medium containing IBA concentrations (0.0 – 0.1 – 0.5 – 1.0 – 2.5 – 12.5) mg/l with five folds rate. IBA significantly increased the root formation percentage and the number of roots per shoot compared to the control. The optimum rooting medium with good root formation (46.6 and 40%) in both *Jatropha* lines and (3.03 – 2.43) roots per shoot containing half-strength MS supplemented with 0.5 mg/l IBA (Table 3). However, a higher concentration of IBA (12.5 mg/l) in MS medium inhibited the root formation and formed small callus growth less than 5 mm diameter at the basal end of shoots. similar findings have also been reported by Bhatt & Tomar (2010) and Toppo *et al.* (2012) that indicate low IBA concentration (0.5 mg/l) were found to be more effective for root primordia initiation. Rajore & Batra (2005) cleared that IBA from 0.5 to 5 mg/l incorporated singly in MS found suitable for root induction. In other papers,

TABLE1. Influence of plant growth regulator (PGRs) on multiple shoot formation from nodal explants of different *Jatropha curcas* lines.

Plant growth regulator	Amount of PGR (mg/l)	Shoot formation (%)		No. of shoots/explant		Average shoot length (cm)	
		Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.
BA	0.0	43.3	50.0	0 ^g	0 ^g	1.26±0.14 ^f	0.73±0.12 ^h
	0.1	73.3	76.6	2.96±0.08 ^c	2.66±0.12 ^c	1.7±0.11 ^c	1.3±0.05 ^g
	0.5	96.6	93.3	3.46±0.14 ^b	3.03±0.08 ^b	2.66±0.08 ^c	2.2±0.10 ^e
	1.0	100	100	3.86±0.14 ^a	3.4±0.11 ^a	3.4±0.11 ^b	3.16±0.12 ^c
Mem-Tr	0.1	66.6	73.3	1.23±0.14 ^f	0.9±0.15 ^f	2.26±0.14 ^d	1.7±0.15 ^f
	0.5	93.3	96.6	2.33±0.17 ^d	1.93±0.08 ^d	3.2±0.11 ^b	2.6±0.15 ^d
	1.0	96.6	100	3.66±0.08 ^{ab}	3.13±0.08 ^{ab}	4.66±0.08 ^a	4.36±0.03 ^a
Zeatin	0.1	40.0	53.3	1.26±0.08 ^f	1.06±0.12 ^{ef}	2.26±0.08 ^d	1.73±0.12 ^f
	0.5	43.3	56.6	1.83±0.12 ^e	1.26±0.12 ^e	3.16±0.12 ^b	2.56±0.13 ^d
	1.0	66.6	70.0	1.46±0.08 ^f	1.1±0.05 ^{ef}	4.33±0.08 ^a	3.63±0.16 ^b

Means ± SE (standard error) in each column followed by same letters are not significantly different according to Dunchan's multiple range test (DMRT) at $\alpha = 0.05$

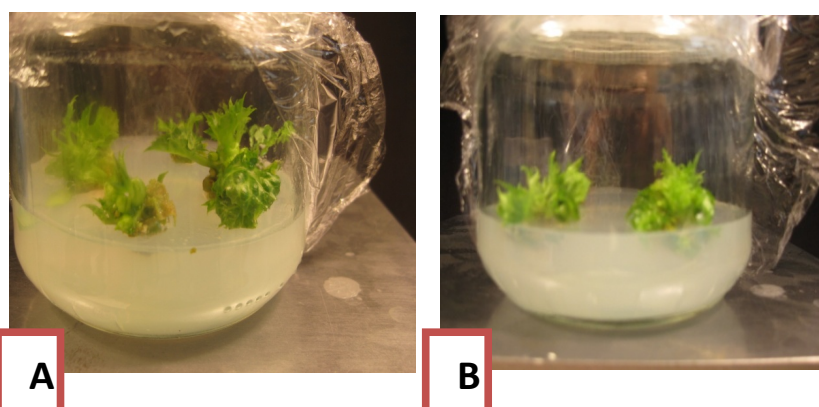


Fig.1. Shoot multiplication on *Jatropha curcas* explants using 1.0 mg/l BA: A, Madagascar line; B, Ecuador line

TABLE 2 . Effect of different concentrations and combination of PGRs on multiple shoot formation of different *jatropha curcas* lines.

Plant growth regulators (mg/l)				No. of shoots/explant		Shoot length (cm)		Degree of callus formation	
BA	Mem-TR	IBA	NAA	Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.
0.1	-	0.1	-	1.0±0.00 ^c	1.26±0.08 ^b	0.7±0.05 ^d	0.83±0.03 ^d	-	-
0.5	-	0.5	-	1.3±0.11 ^b	1.4±0.05 ^b	1.03±0.03 ^c	1.5±0.05 ^b	+	+
1.0	-	1.0	-	1.43±0.06 ^a	1.6±0.11 ^a	0.73±0.06 ^d	1.03±0.06 ^c	+	+
0.1	-	-	0.1	0 ^e	0 ^e	0 ^e	0 ^f	+	+
0.5	-	-	0.5	0 ^e	0 ^e	0 ^e	0 ^f	++	++
1.0	-	-	1.0	0 ^e	0 ^e	0 ^e	0 ^f	+++	+++
-	0.1	0.1	-	0 ^e	0.63±0.06 ^d	0 ^e	0.53±0.03 ^e	-	-
-	0.5	0.5	-	0.8±0.05 ^d	1.06±0.06 ^c	1.23±0.12 ^b	1.56±0.03 ^b	-	-
-	1.0	1.0	-	1.1±0.05 ^c	1.43±0.08 ^{ab}	1.96±0.12 ^a	2.13±0.08 ^a	-	-
-	0.1	-	0.1	0 ^e	0 ^e	0 ^e	0 ^f	+	+
-	0.5	-	0.5	0 ^e	0 ^e	0 ^e	0 ^f	+	+
-	1.0	-	1.0	0 ^e	0 ^e	0 ^e	0 ^f	+	+

Data (Mean ± SE) recorded after 6 weeks. callus formation (-, no callus ; +, small callus less than 5 mm diameter ; .(++, moderate callus 5-10 mm diameter ; +++, large callus more than 10 mm diameter

TABLE 3 . Influence of different concentrations of IBA on adventitious root formation of different *Jatropha*

IBA concentration (mg)	Average growth							
	Root formation (%)		No. of roots/explant		Root length (cm)		Callus formation	
	Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.
0	0	0	0 ^d	0 ^d	0 ^d	0 ^d	-	-
0.1	36.6	33.3	2.16±0.08 ^b	1.76±0.08 ^b	1.8±0.05 ^b	1.33±0.12 ^b	-	-
0.5	46.6	40.0	3.03±0.12 ^a	2.43±0.21 ^a	2.7±0.25 ^a	2.13±0.08 ^a	-	-
2.5	13.3	23.3	1.1±0.11 ^c	0.66±0.08 ^c	0.7±0.11 ^c	0.7±0.05 ^c	-	-
12.5	0	0	0 ^d	0 ^d	0 ^d	0 ^d	+	+

Means ± SE (standard error) in each column followed by same letters are not significantly different according to Duncan's multiple range test (DMRT) at $\alpha = 0.05$

IBA was reported as most effective auxin for in vitro rhizogenesis in *Jatropha curcas*. Also, these results are in agreement with those of Datta et al. (2007), Rajore & Batra (2007), Shrivastava & Banerjee (2008) and Kaewpoo & Te-chato (2009). In contrast, Kalimuthu et al. (2007) and Sujatha & Mukta (1996) found in their studies that IAA was found to be more suitable hormone than IBA for root induction.

In this investigation, 0.5 mg/l IBA individual and in combination with three concentrations of IAA and 2,4-D (0.1 – 0.5 – 1.0 mg/l) were used to study its effect on adventitious root formation. The maximum number of roots (3.1 and 2.7) were observed in half strength MS basal salts supplemented with 0.5 mg/l with longest root length (2.7 and 2.1 cm) (Table 4). However, increasing the concentration of IBA from 0.5 to 2.0 mg/l decreased root formation and number of roots per shoot of different *Jatropha* lines. Also, it was found that addition of auxins like IAA or 2,4-D with different concentrations to the basal MS medium including 0.5 mg/l IBA inhibited the root formation and observed small to large callus growth. The induction of excessive callus formation was avoided only on IBA containing medium individually but in the presence of IAA and 2,4-D, excessive callus growth was formed at the basal end of shoots.

Influence of LED plant light technology on root formation

In the present study, different light sources like cool white fluorescent (CWF), warm white fluorescent (WWF) and different high- brightness LEDs (red – blue – white) were used to monitor the root initiation of *in vitro* *Jatropha* shoots. Red light provided the most favorable growth conditions inducing a rooting response in 66.6%

of the shoots in Madagascar *Jatropha* and 56.5% in Ecuador *Jatropha* (Table 5 and Fig. 2) which produced on average 4.2 and 3.5 roots per shoot, respectively in both lines. Followed by incubation under LED blue light that recorded the second best number of roots (3.5 and 2.9) in both lines, respectively. Then, cool white fluorescent lamps that produced 3.0 roots per shoot in Madagascar line and 2.5 roots per shoot in Ecuador line, these findings are the same according to Shin *et al.* (2008) and Nhut & Nam (2010) who found that root growth was optimum and higher in plants grown under blue light than that under PGF. Furthermore, white LED light was more effective on root elongation than all studied light treatments that recorded (5.6 and 4.4 cm) root length in both lines, followed by LED blue light which resulted (3.8 and 3.1 cm) root length. On the other side, shoots incubated under warm white fluorescent lamps did not form any root initiation.

The results are in agreement with Daud *et al.* (2013) who mentioned that *Jatropha curcas* plantlets regenerated from shoots incubated under constant red light performed slightly better than plantlets regenerated from shoots incubated under fluorescent light because the led red light increases the percentage of root formation, number of roots and root length of *Jatropha curcas* with limited or no necrosis. Also, the results in agreement with Nhut *et al.* (2003) who cultured strawberry plantlets under different blue to red LED ratios as well as irradiation levels and compare its growth, they noticed that the number of roots and root length of strawberry under red LEDs was higher than that of blue LEDs. On the contrary, the data disagree with those observed by (Iacona and Muleo 2010) who found that blue light was more effective for the induction of adventitious rooting of cherry rootstock.

TABLE 4 . Effects of different concentrations and combination of auxins on adventitious root formation from *in vitro* grown shoots cultured on ½ MS medium.

Plant growth regulators	Amount of PGR (mg/l)	No. of roots/shoot		Average root length (cm)		Degree of callus formation	
		Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.
IBA	0	0 ^e	0 ^d	0 ^d	0 ^e	-	-
	0.5	3.1±0.11 ^a	2.7±0.05 ^a	2.73±0.12 ^a	2.13±0.08 ^a	-	-
	1.0	2.1±0.05 ^b	1.73±0.17 ^b	1.43±0.06 ^b	1±0.03 ^b	-	-
	2.0	1.26±0.08 ^c	0.93±0.06 ^c	0.8±0.05 ^c	0.63±0.03 ^d	-	-
IBA 0.5 + IAA	0.1	1±0.00 ^d	0.76±0.03 ^c	1.23±0.08 ^b	0.76±0.03 ^c	+	+
	0.5	1±0.00 ^d	0.73±0.03 ^c	0.66±0.12 ^c	0.56±0.03 ^d	+	++
	1.0	0 ^e	0 ^d	0 ^d	0 ^e	+++	+++
IBA 0.5 + 2,4-D	0.1	1±0.00 ^d	0.83±0.08 ^c	0.76±0.08 ^c	0.63±0.03 ^d	+	+
	0.5	0 ^e	0 ^d	0 ^d	0 ^e	+	+
	1.0	0 ^e	0 ^d	0 ^d	0 ^e	++	+++

Data (Means ± SE) in each column followed by same letters are not significantly different according to Dunchan's multiple range test (DMRT) at $\alpha = 0.05$

TABLE 5. Influence of different light treatments on *Jatropha curcas* root formation.

Light treatment	Root formation (%)		No. of roots/explant		Root length (cm)	
	Mad.	Ecu.	Mad.	Ecu.	Mad.	Ecu.
Cool light fluorescent	53.3	56.6	3±0.05 ^c	2.56±0.12 ^c	2.53±0.14 ^d	1.93±0.08 ^c
Warm light fluorescent	0	0	0 ^e	0 ^e	0 ^e	0 ^d
LED red	66.6	56.6	4.23±0.14 ^a	3.5±0.05 ^a	3.2±0.11 ^c	2.8±0.15 ^b
LED blue	43.3	46.6	3.5±0.15 ^b	2.93±0.08 ^b	3.83±0.14 ^b	3.16±0.03 ^b
LED white	26.6	33.0	1.36±0.12 ^d	1±0.00 ^d	5.6±0.11 ^a	4.43±0.21 ^a

Data collected after 45 days, 30 explants per condition were used, Means ± SE (standard error) in each column followed by same letters are not significantly different according to Duncan's multiple range test (DMRT) at $\alpha = 0.05$

Acclimatization and field transfer

Well-developed healthy *in vitro* rooted plantlets after 6 weeks were washed thoroughly in running tap water and hardened for *ex vitro*. The plantlets were planted into pots containing a mixture of organic soil and sand in the ratio of 1:1 then placed in a plastic tunnel and wetted with tap water followed by covering with transparent plastic bags to maintain humidity. After 3-4 weeks, the established plants were transplanted to poly ethylene bags containing garden soil and farmyard manure for further growth. The survival rate of *in vitro* propagated plantlets after transplanting was 40-60% and they grew as normal plants.

CONCLUSIONS

An efficient and reproducible protocol was developed for plant regeneration of elite genotypes (Madagascar and Ecuador) of *Jatropha curcas* plants. A reliable micropropagation method of *Jatropha* plants from nodal explants with much

higher rate of multiplication was described . Using new LED light technology, is a promising method for elite propagation, especially red for roots number and blue for root length.

ACKNOWLEDGMENTS

Deep gratitude to Prof. Dr. ir. Patrick Van Damme, Laboratory of Tropical, Subtropical Agronomy & Ethnobotany for using *Jatropha* plant material from laboratory greenhouse. Also, my gratitude is further extended to employees of *In vitro* culture and horticulture Unit, for encouragement and for all facilities supported during the present study.

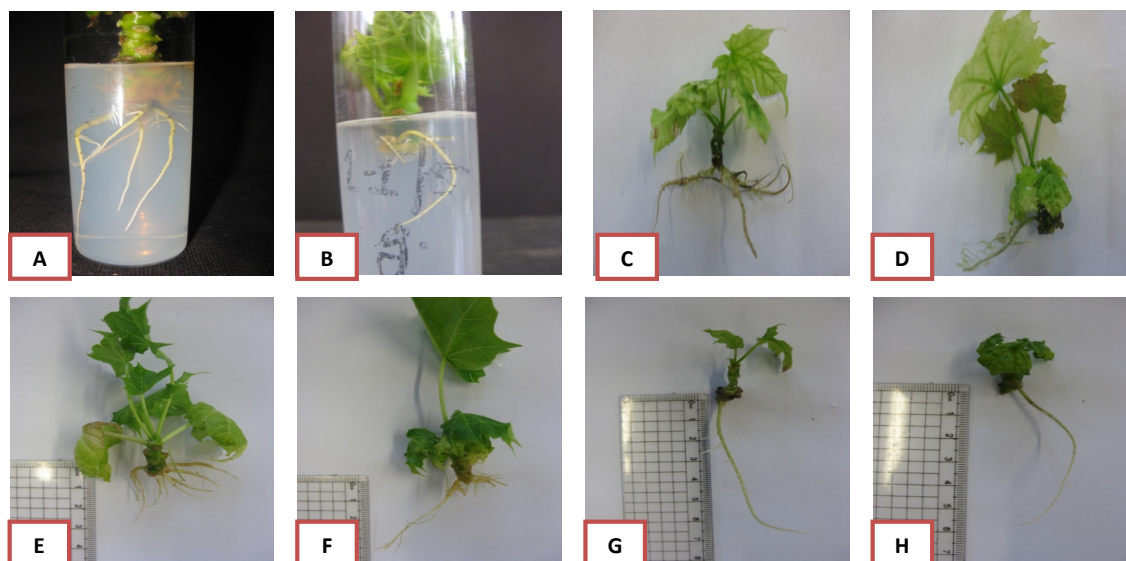


Fig 2. Adventitious rooting of Madagascar and Ecuador *J. curcas* lines respectively using 0.5 mg/l IBA under different light treatments: A,B, cool white fluorescent; C,D, LED red light; E,F, LED blue light; G,H, LED white light

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تنظيم التكشف العضوي باستخدام منظمات النمو النباتية وتكنولوجيا الإضاءة الحديثة (الليد) لنباتات الجاتروفا

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نظراً للاهتمام المتزايد لإمكانات الجاتروفا كمصدر للوقود الحيوي تم إعطاء المزيد من الاهتمام إلى الأساليب التي تسمح لإنتاج كميات كبيرة من المواد النخبة. ولذلك تم وضع بروتوكول بسيط وفعال للحصول على معدل تضاعف وتوالد عالي باستخدام أجزاء نباتية برعمية من أصناف الجاتروفا التي منشأها مدغشقر والاكوادور حيث تم الحصول على معدل نمو خضري مثالي وبدون تكوين الكالس باستخدام بيئة موراشيجي وسكوج المضاف إليها 1 ملليجرام/لتر من مركب البنزويل ادنينين في خلال فترة 30 إلى 40 يوم من زراعة المنفصل النباتي والتي أعطت معدلات تضاعف 3.4 و 3.8 برعم خضري ناتج من زراعة منفصل نباتي واحد لكل الصنفين على التوالي

كذلك فإن الأجزاء الخضرية الصحية التي تمت استطالها تم زراعتها على نصف قوة بيئة موراشيجي وسكوج المحتوية على الاملاح القاعدية وبدون فيتامينات والتي أيضاً تحتوي على تركيزات مختلفة من اندول حامض البيوتريك منفرداً او في توليفة مع اندول حامض الخليك او مركب 2, 4 داي كلوروفينوكسي اسينك اسيد. بالإضافة إلى أن بيئة التجذير المثالية ذات نسبة التجذير (46.6% ، 40%) لصنفي الجاتروفا ومعدل (3 - 2.4) جذور للبرعم الخضري الواحد هي بيئة موراشيجي وسكوج بنصف قوتها مضافاً إليها 0.5 ملليجرام/لتر من اندول حامض البيوتريك. كذلك في هذه الدراسة تم استخدام مصادر مختلفة من الإضاءة مثل لمبات الفلورسنت البيضاء التي تعطي برودة ، لمبات الفلورسنت البيضاء التي تعطي دفء ، ثلاث مصادر للمبات الإضاءة القوية الليد ذات اللون (احمر - ازرقي - ابيض) وذلك لدراسة تأثيرها على تكشف الجذور من البراعم الخضرية للجاتروفا. واتضح من الدراسة أن لمبات الليد الحمراء وفرت ظروف النمو الأكثر ملائمة لتشجيع تجذير البراعم بنسبة 66.6% للسنف مدغشقر ، 56.5% للسنف الجاتروفا الكوادور والتي أعطت في المتوسط عدد 4.2 ، 3.5 جذور لكل برعم خضري على التوالي لكل الصنفين. إضافة إلى أن لمبات الليد البيضاء كانت الأكثر تأثيراً على معدل استطالة الجذور بخلاف مصادر الإضاءة الأخرى والتي سجلت (5.6 سم ، 4.4 سم) لصفة طول الجذر لكل الصنفين. وفي النهاية الشتلات الصحية والمتطورة المتحصل عليها معملياً تمت اقلمتها بنجاح بعد 6 أسابيع ومعدل البقاء لهذه الشتلات بعد نقلها وزراعتها في الاصص يتراوح من 40-60% لكل الصنفين واخذت تنمو طبيعياً في الصوبة الزجاجية.