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MICROPROPAGATION POSSIBILITY OF TWO *Jatropha curcas* LINES AS A VALUABLE BIOFUEL CROP

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ABSTRACT

There is a great opportunity to create a wide demand and expand to Jatropha plantation for produce Egyptian biodiesel in the future, so more focus is now being imposed on methods that permits commercial production of Jatropha elite material. A protocol for micropropagation has been developed by using different types of explants, medium, growth regulators with various concentrations along the two lines of Jatropha Ecuador and Madagascar. Results showed that, both lines achieved optimal number of shoots and no callus formation by culture shoot tip explants on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l 6-Benzyladenine (BA) for the two lines during establishment stage. Moreover, used shoot tip with MS medium fortified with 1.0 mg/l BA gave the highest number for each of shoots and leaves than the other treatments with the two lines. Also, in the presence of 1.0 mg/l BA+ 0.5 Indole butyric acid (IBA), multiple shoot formation and elongation were observed, with 5.0, 5.4 shoot buds/explant, 3.46, and 3.70 cm shoot length in both lines, respectively. However, harvested elongated shoots were transplanted on a half-strength MS medium with various concentrations of IBA, Indole-3-acetic acid (IAA), and α-Naphthaleneacetic acid (NAA). Furthermore, the optimal concentration of 0.5 mg/l IBA on half-MS medium for Jatropha root formation was found to be 2.70 and 3.10 roots per shoot with 2.13 and 2.73 cm root length in both Ecuador and Madagascar Jatropha. Furthermore, well-developed plantlets were successfully rooted, acclimatized and produced a 40-60 percent survival rate after 6 weeks.

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INTRODUCTION

Jatropha curcas Linn., commonly known as physic nut, belongs to the Euphorbiaceae family and is currently being considered as an alternative substitute for fossil fuels. In addition, the seed yield of Jatropha varies from 0.5 to 12 ton/hectare yearly, depending on soil, nutrient and rainfall conditions. The tree has a productive life of over 30 years. Both the kernel and the hull have a large rate of oil amount up to 60%, which can be extracted to diesel *via* esterification (**Attaya and El-Sarag, 2017**). **Abdulla *et al.* (2011)** reported that, according to energy experts, Jatropha oil is cost-effective, environmentally stable, and promising alternative to fuels such as kerosene, gasoline, and others. Jatropha oil contains many poisonous substances such as curcasine, phytates, and saponins. In addition, the oil has a high cetane content, as an extender in diesel fuel, or transesterified into biodiesel fuel (**Sobrinho *et al.*, 2022**). There is a great opportunity to expand Jatropha plantations and biodiesel production in Egypt, to create a wide demand for Egyptian biodiesel in the future (**Soliman and He, 2015**). It is valued for its rapid growth, ability of propagation, low seed costs, high oil value, short gestation period, large adaptability, production on both good and poor soils, and the ideal plant size

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for agricultural practices (**El-Sayed *et al.*, 2020**). On the other hand, Jatropha can sustain drought short periods, making it suitable for use in arid areas where other oil crops such as palm cannot survive. Furthermore, some semi-arid land areas are vulnerable to erosion, and Jatropha root architecture has a pen root, which can reach lower depths, making it perfect for soil stabilisation, and preventing erosion (**Chacuttayapong *et al.*, 2021**). Traditional seed propagation is commonly used, but it is insufficient due to out-crossing that has resulted in a lack of genotype homogenization. It is plagued by issues such as low germination, poor seed viability, and sparse and seedlings delayed rooting (**Daud *et al.*, 2013**).

Many plant species have been propagated using plant cell and tissue culture techniques, which provide a constant supply of plantation substrate of plants that are hard to cultivate, take a long time to cultivate and low yield (**Maharana *et al.*, 2012**). According to **Toppo *et al.* (2012)** *J. curcas* plants propagated *in vitro* have maximum yield traits than the other plants which propagated by seeds. However, because of its versatility in reacting to market demand and the ability to produce pathogen-free products, this method may be favoured over cuttings. Besides, tissue culture techniques are increasingly used to complement traditional methods for mass-propagation of tree species that have limitations. Also, tissue culture technologies will also allow the development of active natural compounds with optimum amounts. Many studies were done using various explants and have recently been published (**Misra *et al.*, 2010; Attaya *et al.*, 2012**). **Hegazi *et al.* (2020)** established a new Jatropha regeneration protocol for cotyledonary leaves. When the explants were placed on Murashige and Skoog (MS) medium supplemented with either 0.45 or 4.54 µM thidiazuron (TDZ), they discovered that 100% of the explants induced green and nodulated regenerative callus. The maximum number of shoots (11.9) was obtained when the induced callus was placed on MS medium supplemented with 0.45 µM Thidiazuron (TDZ). For shoot proliferation and elongation, the optimal medium was found to be MS medium supplemented with 8.88 µM 6-benzyl adenine (BA) in combination with 54.3 µM adenine sulphate, where it resulted to 12.7 shoots with average length 3.72 cm. Furthermore, these shoots were then isolated and rooted in half strength MS medium supplemented with indole-3-butyric acid (IBA) at a concentration of 1.47 µM, which resulted in the highest rooting percentage of 61.66%. **Qasim *et al.* (2021)** demonstrated that adding various phytohormones to MS media significantly increased Jatropha micropropagation. Adding 1.0 mg/l Benzyl-amino-purine (BAP) to MS media provided the optimum number of shoots (80 shoot/explant) in the shortest period (60 days). MS containing 2, 4-D plus 0.5 mg/l BAP provided the most callus initiation from leaves (100 percent).

The present study conducted to develop an effective and repeatable protocol for Jatropha mass propagation using shoot tips from seeds of two *J. curcas* lines obtained from Ecuador and Madagascar to be used for mass production of this biofuel crop and a stepping-stone for efficient genetic transformation.

MATERIALS AND METHODS

Plant Materials Source and Sterilization

Jatropha curcas seeds were obtained friendly from Ecuador and Madagascar by Prof. Dr. Patrick Van Damme, who works at the Laboratory of Tropical and Subtropical Agronomy and Ethnobotany in the Bioscience Engineering Faculty at Ghent University. During the period from 2019-2021, the seeds were germinated in soil and kept in greenhouse at the experimental farm, Fac. of Environ. Agric. Sci., Arish Univ. Axillary

nodes and shoot tips from 1 - 5 cm in length and 1.5 cm in diameter were cut from six-month-old donor plants then transferred to Prof. Dr. Abd El-Fatah Helmy Belal Plant Tissue Culture Laboratory, Fac. Environ. Agric. Sci., Arish Univ. These plant parts were washed, and then placed in tap water with a drop of liquid soap in a flask. The flask was shaken by hand for 5 minutes and then rinsed with tap water to remove the soap. Then the explants were sterilized with 70% (*V/V*) ethanol for 30 seconds and subsequently surface sterilized with Clorox solution (NaOCl, 5.25% free chlorine) at concentration of 20% for 10 min followed by three washes with sterile distilled water under aseptic conditions in a laminar air-flow hood.

Establishment stage

Shoot tip explants that had been sterilized were trimmed at the base by 0.5 cm and then cultured with the cut surface in contact with MS basal salt mixtures including vitamins medium (**Murashige and Skoog, 1962**), The medium was supplemented with 30 g/l sucrose and 8 g/l agar, various concentrations of cytokinins for shoot induction (0, 1, 2 or 3 mg/l) of four cytokinin compounds (BA, TDZ, kin, and zeatin) were individually tested.

Proliferation stage

For the multiplication stage another experiment was conducted by using different media *i.e.*, MS medium, B5 medium (**Gamborg *et al.*, 1968**) and WPM woody plant medium (**Lioyed and McCowen, 1980**) fortified with BA, TDZ at 1.0 mg/l using two explant types (shoot tip or Axillary node). Additionally, 1.0 mg/l of BA or TDZ in combination with 0.5, 1.0, and 2.0 mg/l of IBA or IAA were added to the MS medium with shoot tip to investigate which combination of plant growth regulators would yield the best results for the shoot multiplication in different Jatropha lines.

Culture conditions

After being gelled with agar and autoclaved at 121°C and 1.1 Kg/cm^2 for 20 min, the pH of all media was adjusted to 5.6 - 5.8. The cultures were then incubated in an air-conditioned room at 25 ± 2°C under a 16 hour/day photoperiod, with a light intensity of 2000 Lux provided by cool white, fluorescent lamps.

Root Formation and Acclimatization Stage

In vitro shoots measuring 2-4 cm in length were placed on a root induction medium consisting of ½ strength MS, 8 g/l agar and 30 g/l sucrose. Different doses (0.0, 0.5, 1.0, and 2.0 mg/l) of different auxins such as IBA, IAA, and NAA were added to the medium. After 42 days, the shoots with roots were carefully removed from the medium and washed with sterilized distilled water to remove any remaining medium. The plantlets were then transferred to plastic bags filled with a mixture of sterilized sand and soil in a 1:1 ratio. Tap water was used to moisten the mixture, and transparent plastic bags were placed over the plantlets to maintain humidity. After 3-4 weeks, the established plants were transplanted to polyethylene bags filled with garden soil and farmyard manure then transfer to a greenhouse.

Recorded Data

After 6 weeks the next data were recorded

Number of shoots, shoot length (cm), number of leaves, root number and length as well as callus performed.

Analytical Statistics

To assess the statistical difference among the means at the 0.05 level, Duncan's multiple range test (DMRT) was conducted using SPSS (version 17). The results were presented as mean ± STDEV standard deviation. Additionally, analysis of variance (ANOVA) was performed on the data.

RESULTS AND DISCUSSION

Establishment Stage

Results illustrated in Table 1 and Fig. 1 (A, B) indicate that the effect of different both cytokinins and concentrations on shoot growth/ number of Ecuador *Jatropha curcas* after 6 weeks of culture. The highest number of shoots was belonged to MS supplemented with 1.0 mg/l BA (3.40) followed by using 1.0 mg/l TDZ (3.23) and 1.0 mg/l kin (3.13) with significant difference among them. While the lowest number of shoots (0.66) was obtained by 3.0 mg/l kin. The results are in agreement with the outcomes of **Hegazi *et al.* (2020)** and **Qasim *et al.* (2021)** who indicated that MS medium with 2.22-4.44 µM BA was effective for apical shoot culture, while 4.44 µM BA was suitable for node culture.

For shoot length, the tallest shoot was obtained using 1.0 mg/l TDZ (4.40 cm) then 1.0 mg/l kin (4.36 cm) without significant differences between them. Then 1.0 mg/l Zeatin gave (3.63 cm) shoot length. In contrast, the shortest shoots (0.73 cm) were belonged to free MS medium. Also, the maximum leaf number/shoot was showed by 1.0 mg/l kin (9.16) followed by (7.13) that recorded with 2.0 mg/l kin and 1.0 mg/l Zeatin, then (6.56) leaf/shoot which observed by 3.0 mg/l kin.

On the contrary, the least leaf number/ shoot (1.03 and 1.26, respectively) was belonged to using 2.0 or 3.0 mg/l TDZ without significant differences between them. As for callus formation, (1.0 or 2.0 mg/l) BA, (1.0 or 2.0 mg/l) kin and Zeatin at different doses (1.0, 2.0 and 3.0 mg/l) did not form any callus after 6 weeks. Furthermore, 3.0 mg/l of BA or Kin and 1.0 mg/l TDZ gave callusing less than 5 mm in diameter on MS medium, while 2.0 mg/l TDZ resulted callusing 5-10 mm diameter but 3.0 mg/l TDZ gave callusing more than 10 mm in diameter.

Table 2 and Fig. 1 (C, D) stated that the effect of different cytokinins and its concentrations on shoot growth/number of Madagascar *Jatropha curcas* after 6 weeks of culture. The optimum number of shoots/ explant was belonged to MS supplemented with 1.0 mg/l BA (3.86) followed by using 1.0 mg/l TDZ which gave (3.76) without significant differences between them, then (3.63) shoots/explant was recorded using 1.0 mg/l kin. while the least number of shoots (1.00) was belonged to using 3.0 mg/l kin. The findings are in agreement with **Qasim *et al.* (2021)** they found that MS medium containing 2.22 - 4.44 µM BA was effective for growing apical shoot cultures, while 4.44 µM BA was suitable for node culture. These results are in agreement with **Attaya and El-Sarag (2017) and Hegazi *et al.* (2020)**.

The tallest shoot was obtained by using 1.0 mg/l kin (4.66 cm) followed by 1.0 mg/l Zeatin (4.33 cm) without significant differences between them. Then 1.0 mg/l BA that gave (3.40 cm) shoot length. In contrast, the shortest shoot (1.46 cm) was belonged to 3.0 mg/l BA. The maximum leaf number/shoot was obtained by 1.0 mg/l kin (9.33) followed by (7.26) that recorded with 2.0 mg/l kin and 1.0 mg/l Zeatin, then (6.76) leaf/shoot which observed by 3.0 mg/l kin. On the other hand, the least leaf number/shoot (1.13 and 1.43, respectively) was belonged to using 3 or 2 mg/l TDZ without significant differences between them. As for callus formation, (1.0 or 2.0 mg/l) BA, (1.0 or 2.0 mg/l) kin and Zeatin at different doses (1.0, 2.0 and 3.0 mg/l) did not form any callus after 6 weeks. Furthermore, 3.0 mg/l of BA or kin and 1.0 mg/l TDZ gave callusing less than 5 mm diameter on MS medium, while 2.0 mg/l TDZ produced callusing 5-10 mm diameter and 3.0 mg/l TDZ resulted callusing more than 10 mm diameter.

Table 1. Effect of different both cytokinins and concentrations on shoot growth of *Jatropha curcas* (Ecuador line) after 6 weeks during establishment stage

Cytokinin	Concentration (mg/l)	Shoot No./ explant	Shoot length (cm)	Leaf No./shoot	Callus performed
BA	0.0	0.00 ± 0.00^{i}	0.73 ± 0.20^{j}	1.66 ± 0.15^{g}	No
	1.0	3.40 ± 0.20^{a}	3.16 ± 0.20^{c}	3.96 ± 0.15^{e}	No
	2.0	2.80 ± 0.10^{bc}	1.96 ± 0.15^{f}	2.56 ± 0.05^{f}	No
	3.0	2.43 ± 0.15^{c}	1.06 ± 0.5^{i}	2.20 ± 0.26^{f}	small callus
Kin	1.0	3.13 ± 0.15^{bc}	4.36 ± 0.05^{a}	9.16 ± 0.15^{a}	No
	2.0	1.70 ± 0.10^{e}	2.36 ± 0.23^{de}	7.13 ± 0.11^{b}	No
	3.0	0.66 ± 0.20^{h}	1.46 ± 0.23^{gh}	6.56 ± 0.23^{c}	small callus
TDZ	1.0	3.23 ± 0.05^{b}	4.40 ± 0.10^{a}	1.73 ± 0.20^{g}	small callus
	2.0	2.06 ± 0.20^{d}	2.56 ± 0.23^{d}	1.26 ± 0.05^{h}	moderate callus
	3.0	0.90 ± 0.10^{g}	1.56 ± 0.15^{g}	1.03 ± 0.05^{h}	large callus
Zeatin	1.0	1.10 ± 0.10^{f}	3.63 ± 0.28^{b}	7.13 ± 0.20^{b}	No
	2.0	1.03 ± 0.15^{f}	2.33 ± 0.20^{de}	5.56 ± 0.15^{d}	No
	3.0	0.83 ± 0.15^{g}	1.50 ± 0.17^{gh}	5.16 ± 0.15^{d}	No

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

Table 2. Effect of different both cytokinins and concentrations on shoot growth of *Jatropha curcas* (Madagascar line) after 6 weeks during establishment stage

Cytokinin	Concentration (mg/l)	Shoot No./explant	Shoot length (cm)	Leaf No./shoot	Callus performed
BA	0.0	0.00 ± 0.00^{h}	1.70 ± 0.20^{g}	1.80 ± 0.20^{g}	No
	1.0	3.86 ± 0.25^{a}	3.40 ± 0.20^{b}	4.13 ± 0.15^{e}	No
	2.0	3.23 ± 0.20^{c}	2.43 ± 0.11^{d}	2.66 ± 0.20^{f}	No
	3.0	2.80 ± 0.20^{d}	1.46 ± 0.15^{h}	2.36 ± 0.25^{f}	small callus
Kin	1.0	3.63 ± 0.15^{b}	4.66 ± 0.15^{a}	9.33 ± 0.15^{a}	No
	2.0	2.10 ± 0.26^{e}	2.96 ± 0.15^{bc}	7.26 ± 0.20^{b}	No
	3.0	1.00 ± 0.20^{g}	2.03 ± 0.20^{e}	6.76 ± 0.25^{c}	small callus
TDZ	1.0	3.76 ± 0.05^{a}	3.16 ± 0.20^{b}	1.90 ± 0.20^{g}	small callus
	2.0	2.36 ± 0.11^{e}	2.83 ± 0.15^{bc}	1.43 ± 0.15^{h}	moderate callus
	3.0	1.50 ± 0.10^{f}	2.53 ± 0.15^{d}	1.13 ± 0.15^{h}	large callus
Zeatin	1.0	1.46 ± 0.15^{f}	4.33 ± 0.15^{a}	7.26 ± 0.20^{b}	No
	2.0	1.23 ± 0.15^{fg}	2.93 ± 0.15^{bc}	5.76 ± 0.20^{d}	No
	3.0	1.03 ± 0.11^{g}	1.93 ± 0.15^{f}	5.33 ± 0.15^{d}	No

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

Multiplication Stage

Results presented in Table 3 show that the impact of medium kinds, explant types with BA and TDZ at 1 mg/L on shoot growth of Ecuador *Jatropha curcas* during multiplication stage after 6 weeks. The optimal number of shoots was belonged to MS media fortified with BA (3.60) or with TDZ (3.30) using shoot tip explants without significant difference between them, followed by using axillary nodes on the same medium which supplemented with BA or TDZ (3.23 and 3.16, respectively) while the least number of shoots (0.93 and 0.76) was belonged to using either shoot tips or axillary nodes with TDZ in WPM medium, respectively. The tallest shoot was recorded when subjected shoot tip (4.5 cm) and axillary node (4.26 cm) to TDZ in MS medium. On the other side, the shortest shoot tended to exhibit WPM medium, especially for axillary node treated with TDZ (0.76 cm). The highest leaf number/ shoot was observed for MS medium supplemented with BA using either shoot tip or axillary nodes (4.03 and 3.80, respectively), followed by TDZ for both explants (1.86 and 1.60, respectively). On the other side, the least leaf number/shoot (0.76) was belonged to B5 medium with TDZ using axillary node as explant. As for callus formation, results in Table 3 demonstrated that both types of explants (shoot tip and axillary node) on MS medium supplemented with BA did not form any callus after 6 weeks in Ecuador line. Otherwise B5 and WPM media form callus depending on the cytokinin used where BA gave callusing less than 5 mm in diameter and TDZ gave callusing 5-10 mm in diameter.

Moreover, Table 4 shows that the impact of medium, explant and cytokinin types on shoots formation and growth of Madagascar *Jatropha curcas* after 42 days of culture. It can be observed that Madagascar *J. curcas* line behaved in similar manner to Ecuador *J. curcas* line in their effects with medium, explant and cytokinin types. The highest value for each of shoot number, shoot length and leaf number exhibited MS medium explants. Whereas, the relatively low values of these growth parameters were observed for axillary node supplemented with TDZ in WPM medium. Regarding the above-mentioned results, the two cytokinins used in this study, BA was more suitable than TDZ for shoot-bud induction. Moreover, shoot tip explants found to be more effective for shoot induction than axillary nodes. Furthermore, MS medium found to be the best medium for shoot bud induction. These results are in agreement with the findings of **Purkayastha *et al.* (2010) and Qasim *et al.* (2021).**

In this respect, using shoot tip with the same concentration of 1.0 mg/l of BA or TDZ but in combination with three different doses (0.5, 1.0 and 2.0 mg/l) of IBA or IAA as shown in Table 5 and Fig. 1 (C, D), the maximum multiplication of healthy shoot number (5.06 and 5.46) with the greatest number of leaves (4.16 and 4.53) were achieved in Ecuador and Madagascar Jatropha lines after 6 weeks of culture by using 1.0 mg/l BA combined with 0.5 mg/l IBA. The findings align with **Bhatt and Tomar (2010**) as well as **Murthy *et al.* (2010)** who reported that the combination of BA and IBA has been shown to regenerate shoot buds from nodal segments of *Jatropha curcas.* **Attaya and El-Sarag (2017)** noticed that 1.0 mg/l BA + 0.5 or 1.0 IBA in combination was more suitable and effective of Jatropha multiplication. Furthermore, **Maharana *et al.* (2012)** stated that high levels of cytokinins stimulate the production of meristems, while an optimal amount promotes shoot proliferation. Additionally, the addition of low concentrations of auxins in combination with cytokinins accelerates the rate of shoot proliferation. This may be related to the physiological condition of the donor plant of nodal explants.

Table 3. Impact of medium, explant types combined with BA and TDZ at 1 mg/L on shoot growth of Ecuador *Jatropha curcas* after 6 weeks during multiplication stage

Media type	Explant type	Cytokinin (1 mg/L)	Shoot No./explant	Shoot length (cm)	Leaf No./shoot	Callus performed
			After 6 weeks			
MS	Shoot tip	BA	3.60±0.30^{a}	3.23±0.11^{b}	4.03±0.05^{a}	no
		TDZ	3.30±0.10^{a}	4.50±0.10^{a}	1.86±0.05^{c}	small callus
	Axillary node	BA	3.23±0.15^{b}	3.06±0.05^{b}	3.80±0.10^{b}	no
		TDZ	3.16±0.11^{b}	4.26±0.05^{a}	1.60±0.10^{c}	small callus
B5	Shoot tip	BA	1.26±0.15^{c}	1.20±0.10^{c}	1.10±.010de	small callus
		TDZ	0.96±0.11^{d}	0.83±0.15^{e}	0.83±0.15^{ef}	moderate callus
	Axillary node	BA	1.16±0.05^{cd}	1.20±0.10^{c}	0.90±0.10^{e}	small callus
		TDZ	0.96±0.05^{d}	0.93±0.15^{d}	0.76±0.05^{f}	moderate callus
WPM	Shoot tip	BA	1.23±0.11^{c}	0.93±0.20^{d}	1.30±0.10^{d}	small callus
		TDZ	0.93±0.15^{d}	0.90±0.10^{de}	0.86±0.05^{e}	moderate callus
	Axillary node	BA	1.33±0.05^{c}	0.90±0.10^{de}	1.13±0.25^{de}	small callus
		TDZ	0.76±0.05^{e}	0.76±0.05^{e}	0.83±0.05^{ef}	moderate callus

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

Table 4. Impact of medium, explant types combined with BA and TDZ at 1 mg/L on shoot growth of Madagascar *Jatropha curcas* after 6 weeks during multiplication stage

Media type	Explant type	Cytokinin (1 mg/l)	Shoot No./explant	Shoot length (cm)	Leaf No./shoot	Callus performed
			After 6 weeks			
MS	Shoot tip	BA	4.00±0.10^{a}	3.63±0.05^{a}	4.20±0.26^{a}	no
		TDZ	3.90±0.10^{a}	3.33±0.05^{ab}	2.00±0.00^{c}	small callus
	Axillary node	BA	3.60±0.10^{b}	3.26±0.05^{b}	3.93±0.05^{b}	no
		TDZ	3.53±0.05^{b}	2.90±0.10^{c}	1.80±0.10^{d}	small callus
B5	Shoot tip	BA	1.36±0.15^{c}	1.20±0.10^{d}	1.10±0.10^{e}	small callus
		TDZ	0.86±0.11^{f}	0.93±0.15^{f}	0.93±0.15^{f}	moderate callus
	Axillary node	BA	1.33±0.05^{c}	1.16±0.15^{d}	0.93±0.05^{f}	small callus
		TDZ	0.90±0.00^{f}	0.86±0.11^{g}	0.76±0.11^{h}	moderate callus
WPM	Shoot tip	BA	1.16±0.15^{d}	1.03±0.20^{e}	1.30±0.10^{e}	small callus
		TDZ	1.00±0.17^{e}	0.96±0.11^{f}	0.86±0.05^{g}	moderate callus
	Axillary node	BA	1.30±0.10^{c}	0.83±0.15^{g}	1.13±0.25^{e}	small callus
		TDZ	0.76±0.05^{g}	0.80±0.10^{h}	0.83±0.05^{g}	moderate callus

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

Table 5. Influence of both different concentrations and combinations of PGRs on the formation of multiple shoots in different *jatropha curcas* lines during multiplication stage

Plant growth regulator (mg/l)				No. of shoots/explant		Shoot length (cm)		No. of leaves/shoot	
				Ecuador	Madagascar	Ecuador	Madagascar	Ecuador	Madagascar
BA	TDZ	IBA	IAA	After 6 weeks					
1.0	-	0.5	-	5.06±0.25^{a}	5.46±0.15^{a}	3.46±0.15^{d}	3.70±0.10^{c}	4.16±0.15^{a}	4.53±0.25^{a}
		1.0	-	4.26±0.05^{b}	4.33±0.05^{b}	2.36±0.05^{f}	2.56±0.15^{d}	3.83±0.05^{b}	4.03±0.15^{b}
		2.0	-	3.16±0.05^{de}	3.40±0.20^{cd}	1.46±0.20^{h}	1.80±0.10^{e}	2.73±0.15^{cd}	3.03±0.15^{d}
		-	0.5	3.30±0.17^{d}	3.56±0.15^{c}	3.03±0.15^{e}	3.33±0.15^{c}	3.86±0.05^{b}	4.03±0.15^{b}
		-	1.0	2.50±0.20^{f}	2.80±0.10^{e}	2.23±0.15^{fg}	2.56±0.15^{d}	2.50±0.20^{d}	2.83±0.11^{e}
		-	2.0	1.83±0.15^{h}	2.06±0.05^{g}	1.33±0.15^{h}	1.53±0.05^{f}	1.63±0.05^{e}	1.73±0.05^{gh}
-	1.0	0.5	-	2.26±0.15^{g}	2.56±0.15^{f}	0.53±0.05^{i}	0.83±0.25^{g}	1.36±0.15^{f}	1.53±0.05^{h}
		1.0	-	1.63±0.11^{h}	1.73±0.05^{h}	1.56±0.05^{h}	1.70±0.10^{e}	1.13±0.15^{g}	1.16±0.11^{i}
		2.0	-	1.10±0.10^{i}	1.33±0.11^{i}	2.13±0.15^{g}	2.50±0.20^{d}	0.96±0.15^{h}	1.23±0.15^{i}
		-	0.5	3.80±0.20^{c}	4.20±0.20^{b}	4.36±0.20^{a}	4.76±0.15^{a}	2.93±0.15^{c}	3.33±0.20^{c}
		-	1.0	2.93±0.15^{e}	3.26±0.15^{d}	4.03±0.11^{b}	4.23±0.11^{b}	2.30±0.10^{d}	2.63±0.20^{e}
		-	2.0	1.86±0.05^{h}	2.06±0.15^{g}	3.66±0.15^{c}	4.10±0.10^{b}	1.70±0.10^{e}	2.03±0.25^{f}

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

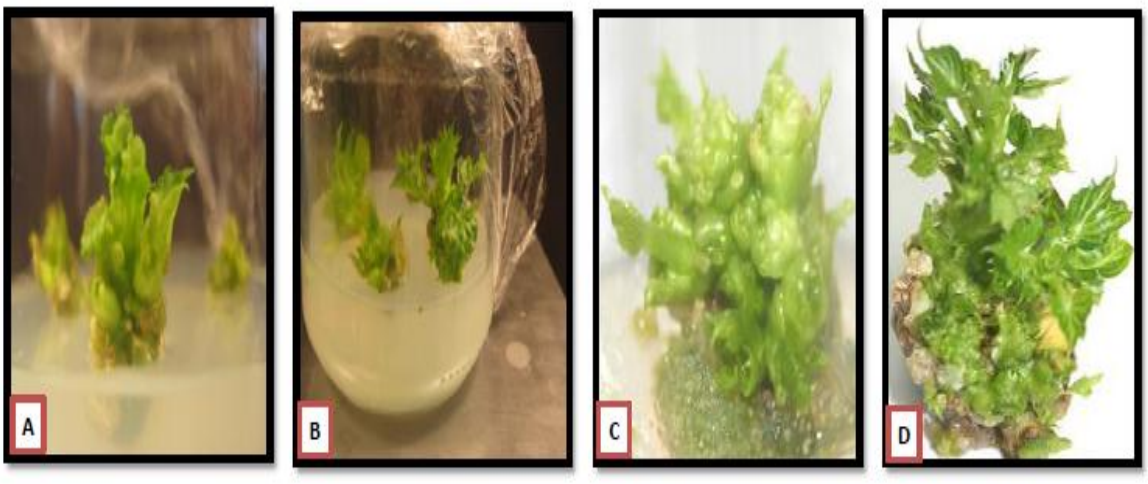

Fig. 1. (A, B) *in vitro* shoot initiation using 1.0 mg/l BA of Ecuador and Madagascar *Jatropha curcas* (C, D) Shoot multiplication and elongation of Ecuador and Madagascar using 1.0 mg/l BA with 0.5 mg/l IBA

Generally, in this experiment, the best influence of 1.0 mg/l TDZ in combination with 0.5 mg/l IAA gave the best shoot length (4.36 and 4.76 cm, respectively) in Ecuador and Madagascar. Followed by 1.0 mg/l TDZ along with 1.0 IAA that obtained (4.03 and 4.23 cm), then the combination of 1.0 mg/l TDZ+ 2.0 IAA that observed (3.66 and 4.10 cm) in both lines.

In vitro Root Induction

Table 6 indicates that the highest number of roots/shoot after 6 weeks was resulted with 0.5 mg/l IBA (2.70 and 3.10) in Ecuador and Madagascar Jatropha lines respectively, followed by using 0.5 mg/l IAA which gave (1.80 and 2.66) roots/shoot in both lines, respectively. Then (1.73 and 2.10) roots/shoot that obtained using 1.0 mg/l IBA with significant difference between all of them. On the otherwise, adding NAA at different concentrations (0.5, 1.0 or 2.0 mg/l) to half-MS medium gave the least number of roots (from 0.3 to 1.1) in Ecuador and Madagascar Jatropha lines. These results were agreed with those of **Murthy *et al.* (2010), Maharana *et al.* (2012) and El-Sayed *et al.* (2020)**. Also, the tallest root after 6 weeks was observed by using 0.5 mg/l IBA (2.13 and 2.73 cm, respectively) in both lines, followed by 1.0 mg/l IBA (1.10 and 1.43 cm). In contrast, adding NAA at different concentrations (0.5, 1.0 or 2.0 mg/l) to half-MS medium gave the shortest roots (from 0.23 to 0.73 cm). As for callus formation, IBA at different concentrations did not form any callus after 6 weeks. However, IAA at different concentrations of 1.0 or 2.0 mg/l produced small callus formation up to 5 mm in diameter. Furthermore, the concentration of NAA is increased from 0.5 to 2.0 mg/l on half-MS containing medium increased callus formation degree from small callus (0-5 mm in diameter) to large callus formation. These results were obtained for the two lines.

Increasing the amounts of IBA, IAA and NAA from 0.5 to 2.0 mg/l on half-strength MS medium decreased the values of roots number/shoot and average root length in different Ecuador and Madagascar *Jatropha curcas* lines. IBA on half-strength MS found to be more suitable on Jatropha root formation than IAA or NAA without forming any callus formation during this stage. According to previous studies (**Toppo *et al.*, 2012; Attaya and El-Sarag, 2017; El-Sayed *et al.*, 2020**) they found that a low concentration of IBA (0.5 mg/l) is more effective and suitable for initiating root primordia and *in vitro* rhizogenesis in *Jatropha curcas*. **Hegazi *et al.* (2020)** also found that IBA concentrations ranging from 0.5 to 5 mg/l in MS were effective for inducing root growth. These findings are consistent with other research papers (**Daud *et al.*, 2013; Chacuttayapong *et al.*, 2021**). However, **Sobrinho *et al.* (2022)** discovered in their studies that IAA was a more efficient phytohormone than IBA for promoting root formation.

Furthermore, well-developed plantlets were successfully acclimatized (as reported in materials and methods section) and produced a 40-60 percent survival rate after 6 weeks.

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Conclusions

A successful and reproducible protocol was developed for the plant regeneration of elite genotypes of *Jatropha curcas* plants from Ecuador and Madagascar. A reliable method for propagating Jatropha plants from shoot tip explants was identified, resulting in a significantly higher multiplication rate.

Table 6. Influence of both auxin types and concentrations on the formation of Jatropha roots from shoots grown *in vitro* on ½ MS

Auxin	Concentration (mg/l)	No. of roots/shoot		Average root length (cm)		Callus formation	
		Ecuador	Madagascar	Ecuador	Madagascar	Ecuador	Madagascar
IBA	0	0.00±0.00^{j}	0.00±0.00^{j}	0.00±0.00^{h}	0.00±0.00^{h}	no	no
	0.5	2.70±0.10^{a}	3.10±0.20^{a}	2.13±0.15^{a}	2.73±0.20^{a}	no	no
	1.0	1.73±0.30^{c}	2.10±0.10^{c}	1.10±0.10^{b}	1.43±0.11^{b}	no	no
	2.0	0.93±0.11^{e}	1.26±0.15^{e}	0.63±0.05^{d}	0.80±0.10^{c}	no	no
IAA	0.5	1.80±0.10^{b}	2.66±0.15^{b}	0.76±0.05^{c}	1.40±0.10^{b}	no	no
	1.0	1.50±0.10^{d}	1.90±0.10^{d}	0.60±0.10^{d}	0.76±0.15^{d}	small ca.	small ca.
	2.0	0.70±0.10^{g}	0.96±0.15^{g}	0.43±0.05^{e}	0.60±0.10^{e}	small ca.	small ca.
NAA	0.5	0.83±0.15^{f}	1.10±0.10^{f}	0.63±0.05^{d}	0.73±0.15^{d}	small ca.	small ca.
	1.0	0.53±0.05^{h}	0.63±0.05^{h}	0.33±0.05^{f}	0.40±0.10^{f}	moderate ca.	moderate ca.
	2.0	0.33±0.05^{i}	0.33±0.05^{i}	0.23±0.05^{g}	0.26±0.05^{g}	large ca.	large ca.

Based on the Dunchans multiple range test (DMRT) at a significance level of 0.5, the means ± STDEV (standard deviation) in each column that share the same letters are not significantly different.

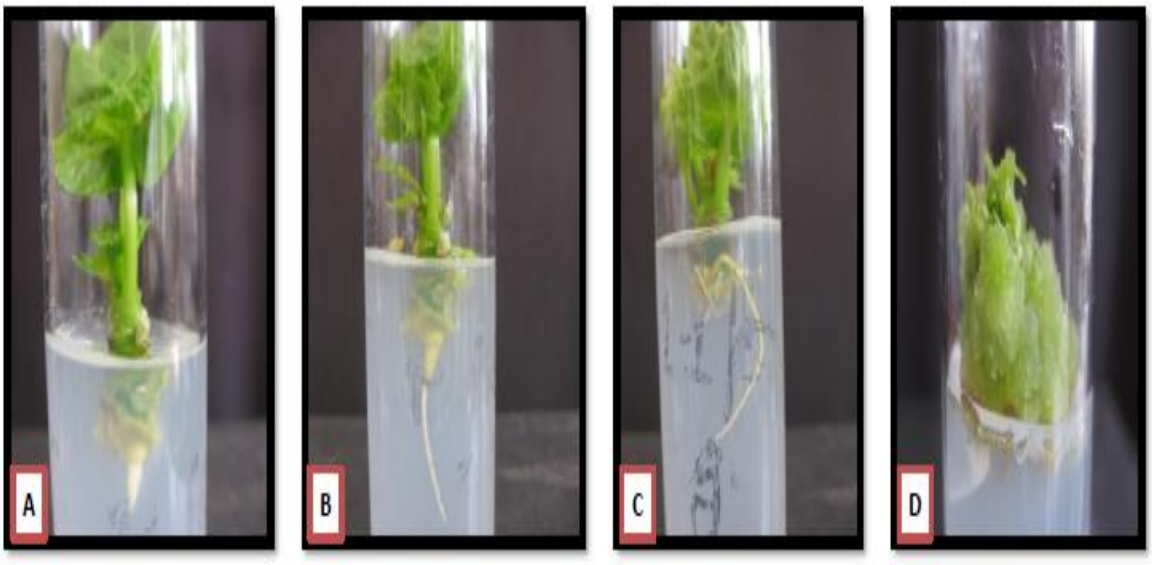

Fig. 2. (A) *In vitro* root induction of *Jatropha curcas* using 1.0 mg/l IBA (B) 0.5 mg/l IBA on Ecuador line. (C) 0.5 mg/l IBA on Madagascar line. (D) Large callus formation using 2.0 mg/l NAA

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الملخص العربي

إمكانية الإكثار المعملى الدقيق لنوعين من نبات الجاتروفا كمحصول وقود حيوي ذو قيمة عالية

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هناك فرصة كبيرة لخلق طلب واسع والتوسع في زراعة الجاتروفا لإنتاج وقود الديزل الحيوي المصري في المستقبل، لذلك يتم الان فرض المزيد من التركيز على الأساليب التي تسمح بالإنتاج التجاري للجاتروفا المتميزة. تم تطوير بروتوكول للتكاثر الدقيق باستخدام انواع مختلفة من المنفصلات النباتية والبيئات ومنظمات النمو النباتية بتركيزات مختلفة لنبات الجاتروفا من الاكوادور ومدغشقر، وأظهرت النتائج المتحصل عليها أن كلا نوعي الجاتروفا حققا العدد الامثل من الافرع مع عدم تكوين الكالس بواسطة استخدام القمم النامية على بيئة موراشيج وسكوج المضاف إليها 1 ملجم/ لتر بنزيل أدينين لكلا النوعين خلال مرحلة التأسيس. علاوه على ذلك، اعطت القمم النامية المستخدمة على بيئة موراشيج وسكوج المضاف إليها 1 ملجم/ لتر بنزيل أدينين اعلى عدد من البراعم اوالافرع والاوراق مقارنة بالمعاملات الاخرى لذات النوعين. كذلك فان التوليفة المكونة من 1 ملجم/ لتر بنزيل أدينين مضاف اليها 0.5 ملجم/ لتر اندول حامض البيوتريك كانت الأفضل للحصول على معدل تضاعف واستطالة عالي للبراعم الناتجة حيث اعطي (5)، (5.4) براعم للمنفصل النباتي الواحد وبطول (3.46 سم)، (3.70 سم) لكلا من نوعي الجاتروفا ذات المنشأ الاكوادور ومدغشقر على التوالي. كذلك فان الأجزاء الخضرية الصحية التي تمت استطالتها في المرحلة السابقة تم زراعتها على بيئة موراشيجي وسكوج بنصف قوتها والمحتوية على تركيزات مختلفة من أندول حامض البيوتريك أو أندول حامض الخليك أو نفثالين حامض الخليك. بالإضافة إلى أن 0.5 ملجم/ لتر اندول حامض البيوتريك كان أفضل تركيز من الاوكسينات والذي اعطى أعلى معدل للجذور (2.70)، (3.10) وبطول جذر (2.13 سم)، (2.73 سم) لكلا من نوعي الجاتروفا ذات المنشأ الاكوادور ومدغشقر على التوالي. كذلك فإن النبتات المتحصل عليها معمليا وبعد 6 أسابيع تمت أقلمتها وبقائها بمعدل 40-60 بالمائة.

الكلمات الإسترشادية: الجاتروفا، الاكثار الدقيق، التجذير، منظمات النمو النباتية.

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