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Leaf Culture Technique For High-frequency Regeneration of Non-toxic Variants of *Jatropha curcas* Plantlets

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ABSTRACT

Recognizing the economic value of biofuels and due to the current growing interest in the non-toxic Jatropha plant's potential as a biofuel plant for the Egyptians future, Leaf culture of non-toxic *Jatropha curcas* Ecuador and Madagascar lines has been conducted at Fac. of Environ. Agric. Scie., Arish University during the period from 2019-2021 to identify an effective, simple, and rapid protocol for improvement the frequency regeneration of *Jatropha curcas* plantlets, depending on modification of various media, including MS, B5, and WPM. MS modified with 0.5 mgl-1 N-phenyl-N'-l,2,3-thidiazol-5-yl urea (TDZ) + 0.5 mgl-1 BA + 0.1 mgl-1 IBA resulted in the highest induction of adventitious shoot buds (5.3) in Ecuador line and (4.7) in Madagascar line from leaf culture. In in vitro rooting stage of different Jatropha, stable elongated shoots were then planted on half-strength MS, B5, and WPM medium, all of which contained varying concentrations of IBA. The MS medium modified with 0.5 mgl-1 IBA provided the largest frequency of root induction. The highest number of roots/shoot (6.06) with (2.93 cm) root length for Ecuador line, therefore, the largest number of roots/shoot (5.76) with (2.76 cm) root length for Madagascar line, after 6 weeks of culture. In the greenhouse, the in vitro Jatropha plantlets were successfully acclimatized and survived to other study in the future and it could be concluded that, the modified MS medium with TDZ or IBA and Ecuador line were more effective for leaf culture technique..

KEYWORDS: *Jatropha curcas*, leaf explant, plant growth regulators (PGRs), plant regeneration, in vitro rooting.

1. INTRODUCTION

Jatropha curcas (L.) is called Purgative nut in the Euphorbiaceae family. *J. Curcas* and *J. glandulifera* are the only species that produce oil; otherwise, several members of the family have some ornamental value. The oil has a high cetane content and can be used directly in diesel engines, as an extender in diesel fuel, or transesterified into biofuel. The seed or oil cannot be consumed by humans or animals because of the toxins phytates, protease inhibitors, saponins, phorbol esters, and curcin are present (Sobrinho *et al.*, 2022). Some Jatropha accessions from Mexico and Ecuador are edible (Chacuttayapong *et al.*, 2021). The oil

is also utilised in production of medicine, lubricants, paints, candles, and soaps (Attaya and El-Sarag, 2017). Recently, *J. curcas* is considering a fundamental production source of biofuel in a wide range of West-Africa especially in Senegal and South-East Africa (Namibia, Tanzania, Zambia), furthermore in Asia (India and Philippines). Jatropha oil, according to energy experts, is cost-effective, environmentally stable, and promising alternative to fuels such as kerosene, gasoline, and others (Abdulla *et al.*, 2011). There is a great opportunity to expand Jatropha plantations in Egyptian lands, as well as increase the imports of European biodiesel, to create in the future a wide demand for Egyptian biofuel (Soliman and He, 2015).

Besides that, according to Attaya *et al.*, (2012) plant tissue culture techniques are increasingly being used to complement traditional methods for mass propagation of tree species that have limitations, particularly when a sizable quantity of genetically identical are needed. Leaf explants have a higher multiplication potential than other forms of explants. Based on nodal proliferation is restricted to the amount of leaf explants, axillary buds, on the other hand, can induce a large number of fresh shoots, based on the multiplication potential. The disadvantage of using explants from leaf is that the start of a shoot is delayed by a brief or prolonged period of callus development. As a result, some colonel variations derived from calluses can appear in propagated material. Leaf discs react well to (2 mgl^{-1}) BA and (0.5 mgl^{-1}) IBA, with 80-90 percentage producing adventitious shoots Daud *et al.* (2013). The largest value of regenerated shoots was 5.4 produced on the second node of stems from leaves petiole pieces, and the highest percent was 70% of explants growing shoots, Toppo *et al.* (2012). Older leaf explants can regenerate, according to Maharana *et al.*, (2012), but they need various mixtures of hormones. In these cases, a high cytokinin amount and TDZ are particularly important. Kumar and Reddy (2010) used MS medium in the presence of 0.5 mgl^{-1} TDZ to establish repeatable and effective method from petiole explants for Jatropha regeneration without forming any callus. They recorded a 58.3 percent of shoot initiation rate with ten shoots/explant. Leafy explants cultivated on MS medium enhanced with TDZ (0.2 mgl^{-1}) and IBA (0.2 mgl^{-1}) specifically generated (18.8) adventitious buds with no callus. Thus, when shoots were transplanted to PM2 medium with $CuSO_4$ amounts, their multiplication improved. The maximum multiplication rate (22.8 shoot) was attained in the presence of $CuSO_4$ at (0.15 mgl^{-1}) concentration, Khurana-Kaul *et al.* (2010).

Liu *et al.* (2010) demonstrated that impact of various amounts of IBA and BA given to MS medium on adventitious shoots regeneration were studied using leaves from seedlings planted in a glasshouse. When the dosage of gibberellic acid (GA3) was 0.05 mg/l, the rate of leaf-disc regeneration was 90.9 percent with 4.6 shoots. Moreover, (Ying *et al.*, 2016) the treatment of TDZ solution from 10-60 mgl^{-1} for (10-60 min) on cotyledon explants, significantly improved the frequency of regeneration and improved the consistency of shoot-bud proliferation. The optimum initiation (87.4 percent) with (11.23 shoots) were observed when equipped the segments with 20 mgl^{-1} TDZ solvent for 40 minutes upon being immunized into free MS medium in one month. Furthermore, (Hegazi *et al.*, 2020) created a successful multiplication Jatropha protocol from cotyledonary leaves. Explants treated with 0.45 or 4.54 μM (TDZ) on MS produced green and modulate regenerative callus in 100% of cases. however, the induced callus produced the greatest number of shoots (11.9) at 0.45 μM TDZ. It was discovered that the optimal medium for shoot initiation and elongation was MS medium supplemented with 8.88 μM (BA) and 54.3 μM adenine sulphate (12.7 shoots with a length of 3.72 cm). After being separated and rooted in half-strength MS with 1.47 μM IBA, the acquired shoots produced the greatest rooting percentage 61.66 percent.

In their study, El-Sayed *et al.*, (2020) MS medium with 2.5mgl^{-1} BA + 1.0 mgl^{-1} NAA resulted in the highest percentage of callus formation from leaf explant. The callus derived from the leaf was cultivated on MS in presence of 2.0 mg/l BA + 0.2 mg/l IBA to promote regenerated shoots. Qasim *et al.*, (2021) found that the inclusion of different phytohormones in MS media had a significant positive impact on micropropagation of

Jatropha. Specifically, the addition of BAP at the concentration of (1.0 mgl^{-1}) to MS media established the optimum number of shoots (80) within 60 days. Moreover, the combination of MS media with 0.5 mgl^{-1} BAP and 2,4-D led to forming callus initiation from leaves (100 percent).

There is a great opportunity in Egypt to expand Jatropha plantations and its biofuel production, to create a wide demand for Egyptian biodiesel in the future. In this research, the main objective is, identify an effective, simple, and rapid protocol for improvement the frequency regeneration of *Jatropha curcas* plantlets and the better response to leaf culture of various Ecuador and Madagascar Jatropha lines.

2. MATERIALS AND METHODS

2.1. Culture conditions and plant materials:

The seeds were friendly collected from Ecuador and Madagascar by Prof. Patrick Van Damme, Faculty of Bioscience Engineering at Ghent University, Belgium. At plant tissue culture laboratory, Environ. Agric. Sci. Faculty, Arish Univ., and during the period from 2019-2021. At the experimental farm, the seeds were planted in soil and kept alive in our lab greenhouse. Six-month-old donor plants were chopped into 1 to 1.5-cm-long shoot tips, cleaned, and then immersed in tap water with a drop of liquid soap in a flask. The soap was then removed by hand shredding the tips for five minutes, followed by rinsing in tap water. After sterilizing the explants for 30 seconds with 70% (v/v) ethanol, they were surface sterilized for 10 minutes with a 20% Clorox solution (NaOCl, 5.25% free chlorine). After that, they were cleaned three times in a laminar air-flow hood using aseptic conditions and sterile distilled water. The sterilized explants from the shoot tips were cut at the base (0.5–1.0 cm), and the cut surface was cultivated in MS baseline salt mixes containing vitamins medium (Murashige and Skoog 1962), with 30 g/l sucrose and 8 g/l agar added as supplements. The media's pH was adjusted to 5.6–5.8 following agar gelling and autoclaving at 121°C and 1.1 kg/cm2 for 20 minutes. For six weeks, the cultures were incubated at 25 ± 2°C in an air-conditioned room with a 16-hour photoperiod and 2000 Lux of light intensity from cool white fluorescent lights.

2.2. Shoot bud initiation from leaf cultures

After that, *in vitro* leaf discs (1.0-1.5 cm diameter) from Ecuador and Madagascar *Jatropha curcas* seedlings were cultured in contact with various media, MS, B5 (Gamborg, 1968), and WPM woody plant medium (Lioyed and Mccown, 1980) in the presence of $30gl^{-1}$ sucrose with 8 gl^{-1} of agar in addition of PGRs in various amounts as noted in tables 1, 2, and 3. The media's pH was adjusted to 5.6–5.8 and autoclaved at 121°C and 1.1 kg/cm2 for 20 minutes. For six weeks, the cultures were incubated at 25 ± 2°C in an air-conditioned room with a 16-hour photoperiod and 2000 Lux of light intensity from cool white fluorescent lights.

2.3. Root formation and acclimatization

In vitro regenerated shoots in length (2-4 cm) that previously established on leaf segments were planted on half strength of different media types MS, B5 and WPM woody plant medium complemented by 8 gl^{-1} agar and 30 gl^{-1} sucrose with different concentrations of IBA as noted in table 3 and 4. Furthermore, after 42 days of culture, in order to remove the medium that was adhered to the roots, the rooted shoots were gently removed from the medium and properly cleaned in distilled water that had been sterilized. After that, the plantlets were placed in plastic bags with sand and soil that had been sterilized in a 1:1 ratio. The bags were wetted with tap water and then covered with clear plastic bags to preserve humidity. The established plants were moved to polyethylene bags filled with garden soil and farmyard manure after three to four weeks, and they were then placed in a greenhouse.

Data were recorded on shoot induction %, No. of shoots/leaf disc, shoot length, response of calls formation %, adventitious shoots/leaf disc, degree of callus formation, rooting %, No. of roots/explants and root length as recommended by Murashige and Skoog, (1962).

2.4. Statistics for Analysis

The data statistically analyzed according to the Randomized Complete Design with three replications. Using SPSS (version 17), the

statistical difference between the means was assessed at the 0.05 level using Duncan's multiple range test (DMRT), Duncan (1955). The results were reported as mean ± STDEV standard deviation. On the data, analysis of variance (ANOVA) was also carried out.

3. RESULTS AND DISCUSSION

3.1. Impact of cytokinin and medium types on shoot initiation of different Jatropha lines (Ecuador and Madagascar) after 6 weeks of culture.

Results in table (1) revealed that, the largest shoot induction percentage (93.33%) in Ecuador line were scored with TDZ (0.5 mg/l) on MS followed by (90.00%) recorded with BA on the same medium, then using TDZ and BA on WPM obtained (60.33 and 50.00%, respectively. While, using different cytokinins on B5 did not give any percentage of shoot induction. In Madagascar line, the data indicate that the highest shoot induction (90.00%) were scored with TDZ on MS followed by (86.66%) recorded with BA on the same medium, then using TDZ and BA on WPM obtained (60 and 50.33%, respectively.

The largest number of shoots/leaf disc of Ecuador line (2.90) after 6 weeks was belonged to MS complemented by TDZ (0.5 mg/l) followed by BA on the same medium that record (2.70) shoots/leaf disc. then (1.40 and 1.10) shoots/leaf disc was recorded on WPM with TDZ and BA, respectively without significant difference between them. While, using different cytokinins on B5 did not give any shoots/leaf disc, indicated to the MS media was more effective for leaf culture technique. The similar results were obtained by Kumar and Reddy (2010) used MS medium supplemented with 0.5 mg/l TDZ to establish an effective and repeatable protocol for the regeneration of *J. curcas* from petiole explants without the formation of an intervening callus. They recorded a 58.3 percent shoot bud initiation rate and 10 shoots per explants.

Table 1. Effect of medium type and cytokinins on all the studied traits of different Jatropha lines.

Medium type	Cytokinin (0.5mg/l)	Shoot induction %	No. of shoots/leaf disc	Shoot length	Response of calls formation
Ecuador line					
MS	**TDZ**	93.33	2.90±0.26^{a}	2.30±0.10^{a}	no
	BA	90.00	2.70±0.20^{b}	2.15±0.15^{b}	no
B5	**TDZ**	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
	BA	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
WPM	**TDZ**	60.33	1.40±0.10^{c}	0.96±0.15^{c}	no
	BA	50.00	1.10±0.10^{c}	0.83±0.10^{c}	no
Madagascar line					
MS	**TDZ**	90.00	2.73±0.20^{a}	2.30±0.26^{a}	no
	BA	86.66	2.30±0.10^{b}	2.10±0.20^{b}	no
B5	**TDZ**	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
	BA	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
WPM	**TDZ**	60.00	1.32±0.10^{c}	0.83±0.15^{c}	no
	BA	50.33	0.96±0.15^{c}	0.76±0.20^{c}	no

The means ± STDEV (standard deviation) followed by the same letter in each column are not significantly different according to the Dunchan multiple range test (DMRT) at the 0.5 level.

Leaf explants cultivated on MS medium enhanced with 0.2 mg/l TDZ and 0.2 mg/l IBA specifically generated adventitious shoot buds (18.8) with no callus. In Madagascar line, the data indicate that the highest number of shoots/leaf disc (2.73) after 6 weeks was belonged to MS with TDZ followed by BA on the same medium that record (2.30) shoot/leaf disc. Then (1.32 and 0.96) shoots/leaf disc was recorded on WPM with TDZ and BA, respectively without significancy between them. The findings are aligned with Purkayastha *et al.*

(2010) and Qasim *et al.,* (2021) found that, the inclusion of different phytohormones in MS media had a significant positive impact on micropropagation of Jatropha.

The tallest shoot after 6 weeks in Ecuador line was recorded on MS with TDZ (2.30 cm) followed by BA on MS which gave (2.15 cm), then WPM with TDZ and BA gave (0.96 and 0.83 cm, respectively) without significant differences. On the other hand, using different cytokinins on B5 did not give any shoot length as well. However, in Madagascar line the data state that the tallest shoot after 6 weeks was recorded on MS with TDZ (2.30 cm) followed by BA on MS which gave (2.10 cm). Then WPM with TDZ and BA gave (0.83 and 0.76 cm, respectively) without significant differences between them.

On the other hand, using different cytokinins on B5 did not give any shoots/leaf disc and shoot length as well. All media supplemented with TDZ or BA in both lines (Ecuador or Madagascar) did not form any callus after 6 weeks. The findings are in harmony with Kumar and Reddy (2010), used MS medium supplemented with 0.5 mg/l TDZ to establish an effective and repeatable protocol for the regeneration of *J. curcas* from petiole explants without the formation of an intervening callus.

3.2. Influence of various PGRs on adventitious bud proliferation of Ecuador Jatropha leaf segments.

According to the results showed in (Table 2 and Fig. 1) which recorded after 6 weeks, the TDZ at 0.5 mgl^{-1} concentration of containing medium established the most shoot induction from leaf culture (93.33 percent). followed by (90 %) with 0.5 TDZ + 1.0 BA + 0.1 NAA (in mgl^{-1}), then (86.66%) that recorded with 0.5 TDZ + 0.5 BA + 0.1 NAA. Using 0.5 TDZ + 1.0 BA + 0.1 IBA, on the contrary, established the least amount of shoot bud induction (50.00%). The obtained data are in line with Daud *et al.* (2013), findings.

Table 2. Influence of various PGRs on adventitious bud proliferation of Ecuador Jatropha leaf segments.

(PGRs) (mg/l)				% Response	Adventitious shoots/leaf disc	Degree of callus formation
TDZ	BA	IBA	NAA			
0.5	-	-	-	93.33	2.90 ± 0.26^{c}	no
0.5	0.5	0.1	-	76.66	5.30 ± 0.20^{a}	small
0.5	1.0	0.1	-	50.00	3.70 ± 0.36^{b}	moderate
0.5	0.5	-	0.1	86.66	2.00 ± 0.20^{d}	large
0.5	1.0	-	0.1	90.00	1.63 ± 0.30^{e}	large
1.0	-	0.1	-	63.33	2.53 ± 0.35^{c}	small
1.0	-	-	0.1	60.00	1.63 ± 0.15^{e}	moderate

The means ± STDEV (standard deviation) followed by the same letter in each column are not significantly different according to the Dunchan multiple range test (DMRT) at the 0.5 level.

0.5 TDZ + 0.5 BA + 0.1 IBA had the largest number of adventitious shoots/explants (5.30), followed by 0.5 TDZ + 1.0 BA + 0.1 IBA with (3.70) adventitious shoots/explants. Then, using 0.5 TDZ and 1.0 TDZ + 0.1 IBA, (2.90) and (2.53) adventitious shoots/explant were reported, without significant difference between them. Using 1.0 TDZ + 0.1 NAA and 0.5 TDZ + 1.0 BA + 0.1 NAA, on the contrary, resulted in the least number of adventitious shoots (1.63). The findings are consistent with those of Kumar and Reddy (2010), and Daud *et al.* (2013), who found that using BA and TDZ supplemented with IBA was the most effective form to induce adventitious shoot buds. 0.5 TDZ did not form any callus formation but it was formed a small callus (less than 5 mm in diameter) on MS by 0.5 TDZ + 0.5 BA + 0.1 IBA, and 1.0 TDZ + 0.1 IBA. Furthermore, 0.5 TDZ +1.0 BA + 0.1 IBA or 1.0 TDZ + 0.1 NAA resulted in moderate callus formation of 5-10 mm diameter, while 0.5 TDZ + 0.5 or 1.0 BA + 0.1 NAA resulted in large callus of greater than 10 mm diameter.

3.3. Impact of various PGRs on adventitious bud regeneration of Madagascar Jatropha leaf segments.

Data in (Table 3 and Fig. 1) cleared the largest shoot bud induction from leaf culture (90.00%) was recorded with TDZ at 0.5 mgl^{-1} containing medium, followed by (86.66 %) with 0.5 TDZ + 0.5 BA + 0.1 NAA (in mgl^{-1}), then (76.66%) that recorded with 0.5 TDZ + 1.0 BA + 0.1 NAA. On the contrary, the least shoot bud induction (53.33%) was belonged to using 0.5 TDZ + 1.0 BA + 0.1 IBA. The obtaining data are in line with the findings of Hegazi *et al.*, (2020).

The optimum adventitious bud number/explant after 6 weeks (4.70) was belonged to 0.5 TDZ + 0.5 BA + 0.1 IBA followed by using 0.5 TDZ + 1.0 BA + 0.1 IBA which recorded (3.50) adventitious shoots/explants. Then (2.73) and (2.40) adventitious shoots/explant was recorded using 0.5 TDZ and 1.0 TDZ + 0.1 IBA, respectively without significant difference between them. On other hand, the least number of adventitious shoots (1.10) was belonged to using 1.0 TDZ + 0.1 NAA. The findings are in line with Kumar and Reddy (2010), and Qasim *et al.*, (2021) that indicated the best combination for optimal adventitious shoot bud initiation was TDZ and BA in combination with IBA.

As for callus formation, 0.5 TDZ did not form any callus formation but 0.5 TDZ + 0.5 BA + 0.1 IBA and 1.0 TDZ + 0.1 IBA (all in mg/l) established small callus after 6 weeks. However, 0.5 TDZ + 1.0 BA + 0.1 IBA or 1.0 TDZ + 0.1 NAA gave moderate callus formation (5-10 mm diameter), while 0.5 TDZ + 0.5 or 1.0 BA + 0.1 NAA obtained more than 10 mm diameter (large callus), indicated to the important role for NAA to callus induction of Jatropha leaf segments.

Table 3. Influence of various PGRs on adventitious bud proliferation of Madagascar Jatropha leaf segments.

(PGRs) (mg/l)				% Response	Adventitious shoots/leaf disc	Degree of callus formation
TDZ	BA	IBA	NAA			
0.5	-	-	-	90.00	2.73 ± 0.20^{c}	no
0.5	0.5	0.1	-	60.00	4.70 ± 0.36^{a}	small
0.5	1.0	0.1	-	53.33	3.50 ± 0.20^{b}	moderate
0.5	0.5	-	0.1	86.66	1.86 ± 0.15^{d}	large
0.5	1.0	-	0.1	76.66	1.43 ± 0.15^{e}	large
1.0	-	0.1	-	73.33	2.40 ± 0.30^{c}	small
1.0	-	-	0.1	66.66	1.10 ± 0.17^{e}	moderate

The means ± STDEV (standard deviation) followed by the same letter in each column are not significantly different according to the Dunchan multiple range test (DMRT) at the 0.5 level.

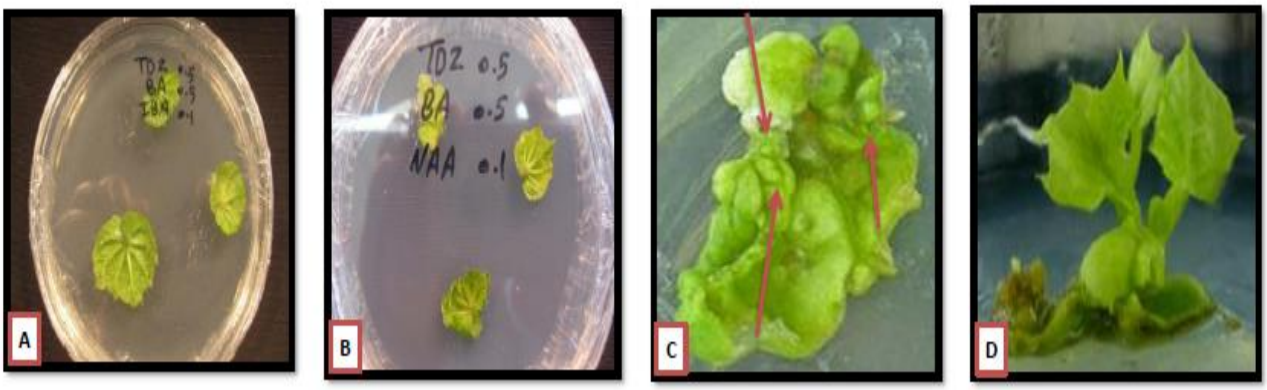

Fig. 1. (A, B) Petri dishes contain MS medium and leaf discs of Ecuador and Madagascar Jatropha
(C) Initiation of shoot buds on Jatropha leaf explant
(D) Direct organogenesis from Jatropha leaf culture

Root induction

3.4. Effect of media strength and two amounts of IBA on root formation of different Jatropha lines (Ecuador and Madagascar) after 6 weeks of culture.

Results in table (4) revealed that, the largest rooting percentage (96.66%) in Ecuador line were scored with 1.0 IBA (mg/l) on half solidity MS followed by (93.33%) recorded with 0.5 IBA on the same medium, then using 0.5 and 1.0 IBA on half solidity WPM obtained (36.66 and 20.00%, respectively. While, using 0.5 or 1.0 IBA on half solidity B5 did not give any percentage of *in vitro* rooting. In Madagascar line the data indicate that the highest rooting percentage (86.66%) were scored with 1.0 IBA on half solidity MS followed by (80.00%) recorded with 0.5 IBA on the same medium, then using 0.5 and 1.0 IBA on half solidity WPM obtained (40 and 23.33%, respectively. While, using 0.5 or 1.0 IBA on half solidity B5 did not give any percentage of *in vitro* rooting. The findings are consistent with those of Maharana *et al.,* (2012); Sobrinho *et al.,* (2022) that 0.5 IBA (mg/l) on MS produced a high frequency of root induction after 4-6 weeks.

Table 4. Effect of media strength and two amounts of IBA on root formation of different lines.

Media strength	IBA (mg/l)	Rooting %	No. of roots/explant	Root length	Response of calls
Ecuador line					
½ MS	**0.5**	93.33	5.30±0.20^{a}	2.50±0.26^{a}	no
	1.0	96.66	3.73±0.15^{b}	2.03±0.20^{b}	no
½ B5	**0.5**	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
	1.0	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
½ WPM	**0.5**	36.66	1.26±0.15^{c}	0.76±0.05^{c}	no
	1.0	20.00	1.16±0.11^{c}	0.80±0.10^{c}	no
Madagascar line					
½ MS	**0.5**	80.00	5.40±0.30^{a}	2.60±0.10^{a}	no
	1.0	86.66	3.36±0.15^{b}	2.10±0.10^{b}	no
½ B5	**0.5**	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
	1.0	00.00	0.00±0.00^{d}	0.00±0.00^{d}	no
½ WPM	**0.5**	40.00	1.10±0.00^{c}	0.73±0.11^{c}	no
	1.0	23.33	1.06±0.20^{c}	0.80±0.17^{c}	no

The means ± STDEV (standard deviation) followed by the same letter in each column are not significantly different according to the Dunchan multiple range test (DMRT) at the 0.5 level.

The largest amount of roots/shoot of Ecuador line (5.30) after 6 weeks was belonged to half solidity MS complemented by 0.5 IBA (mgl^{-1}) followed by 1.0 IBA on the same medium that record (3.73) root/explant. then (1.26 and 1.16) roots/explant was recorded on half solidity WPM with 0.5 and 1.0 IBA, respectively without significant difference between them. While, using 0.5 or 1.0 IBA on half solidity B5 did not give any roots/explant. In Madagascar line, the data indicate that the highest number of roots/explant (5.40) after 6 weeks was belonged to half solidity MS with 0.5 IBA followed by 1.0 IBA on the same medium that record (3.36) root/explant. Then (1.10 and 1.06) roots/explant was recorded on half solidity WPM with 0.5 and 1.0 mgl^{-1} IBA, respectively without significant difference between them. While, using 0.5 or 1.0 IBA on half solidity B5 did not give any roots/explant.

The tallest root after 6 weeks in Ecuador line was recorded on half solidity MS with 0.5 IBA (2.50 cm) followed by 1.0 IBA on half solidity MS which gave (2.03 cm), then half solidity WPM with 1.0 and 0.5 IBA gave (0.80 and 0.76 cm, respectively) without significant differences. On the other hand, using 0.5 or 1.0 IBA on half solidity B5 did not give any roots/explants and root length as well. However, in Madagascar line the data state that the tallest root after 6 weeks was recorded on half solidity MS with 0.5 IBA (2.60 cm) followed by 1.0 IBA on half solidity MS which gave (2.10 cm). Then half solidity WPM with 1.0 and 0.5 IBA gave (0.80 and 0.73 cm, respectively) without significant differences between them. On the

other hand, using 0.5 or 1.0 IBA on half solidity B5 did not give any roots/explants and root length as well, indicated to increase the concentration of IBA decrease the root length, that meaning the IBA enhancement producing adventitious shoots than the root length for that it could be recommended to use 0.5 mg/l of IBA.

All media complemented by 0.5 or 1.0 IBA (mgl^{-1}) in both lines (Ecuador or Madagascar) did not form any callus after 6 weeks.

3.5. Impact of different amounts of IBA on in vitro rooting

The results in (Table 5 and Fig. 2) indicate the largest number of roots/shoot after 6 weeks for Ecuador line was resulted with 0.5 IBA (6.06) followed by using 1.0 IBA which gave (4.03) then (3.30) roots/shoot was recorded using 2.0 IBA with significant difference between them. Moreover, in Madagascar line, the largest number of roots/shoot after 6 weeks was resulted with 0.5 IBA (5.76) followed by using 1.0 IBA which gave (3.53) then (3.16) roots/shoot was recorded using 2.0 IBA with significant difference between them. This result was agreed with that of Murthy *et al.*, (2010); Toppo *et al.*, (2012); Attaya and El-Sarag (2017); El-Sayed *et al.*, (2020), who found that using 0.5 mg/l IBA was the most effective and suitable for root induction.

The tallest root after 6 weeks in Ecuador line was obtained using 0.5 IBA (2.93 cm), then 1.0 IBA (2.33 cm), then 2.0 IBA which gave (2.06 cm) root length as shown in Table (5). However, the tallest root in Madagascar line after 6 weeks was obtained using 0.5 IBA (2.76 cm) then 1.0 IBA (2.26 cm), then 2.0 IBA which gave (2.03 cm) root length, indicated to increase the concentration of IBA decrease the root length, that meaning the IBA enhancement producing adventitious shoots than the root length. As for callus formation, IBA at different concentrations did not form any callus in both lines after 6 weeks. 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.

Table 5. Influences of various amounts of IBA on root formation of different lines.

IBA	Roots no./shoot		Root length (cm)		Response of callus	
(mg/l)	Ecuador	Madagascar	Ecuador	Madagascar	Ecuador	Madagascar
0	-	-	-	-	no	no
0.5	6.06 ± 0.11^{a}	5.76 ± 0.25^{a}	2.93 ± 0.05^{a}	2.76 ± 0.11^{a}	no	no
1.0	4.03 ± 0.25^{b}	3.53 ± 0.11^{b}	2.33 ± 0.11^{b}	2.26 ± 0.05^{b}	no	no
2.0	3.30 ± 0.10^{c}	3.16 ± 0.15^{c}	2.06 ± 0.11^{c}	2.03 ± 0.15^{c}	no	no
4.0	2.36 ± 0.11^{d}	2.43 ± 0.25^{d}	1.46 ± 0.11^{d}	1.43 ± 0.05^{d}	no	no

The means ± STDEV (standard deviation) followed by the same letter in each column are not significantly different according to the Dunchan multiple range test (DMRT) at the 0.5 level.

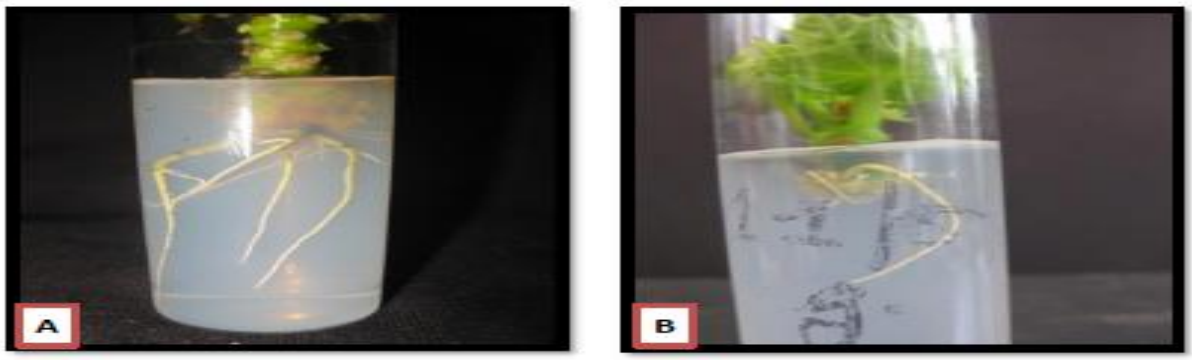

Fig. 2. *In vitro* root induction of *Jatropha curcas*
(A) IBA at the concentration of 0.5 mgl^{-1} with Ecuador Jatropha
(B) IBA at the concentration of 1.0 mgl^{-1} with Madagascar Jatropha

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5. CONCLUSIONS

Elite genotypes plant regeneration of *Jatropha curcas* lines (Ecuador and Madagascar) was developed using an effective and repeatable protocol. A reliable method for Jatropha plant propagation from leaf explants was identified to have a significantly better rate of multiplication and could be concluded that, the modified MS media with TDZ or IBA and Ecuador line were more effective for leaf culture technique.

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الملخص العربي

تقنية زراعة الأوراق للتكشف المتعدد للبراعم الخضرية لنباتات الجاتروفا غير السامة

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إدراكًا للقيمة الاقتصادية للوقود الحيوي ونظرًا للاهتمام المتزايد حاليًا بإمكانيات نبات الجاتروفا غير السام باعتباره مصدر للوقود الحيوي النباتي لمستقبل افضل للمصريين، فقد تم إجراء زراعة أوراق نبات الجاتروفا من الاكوادور ومدغشقر المنشأ بمعمل زراعة الانسجة بكلية العلوم الزراعية البيئية جامعة العريش خلال الفترة من ٢٠١٩ حتى ٢٠٢١ والتي تهدف إلى إنتاج بروتوكول فعال وبسيط وسريع للتكشف المتعدد لنباتات الجاتروفا اعتماداً على تعديل البيئات المختلفة من مثل بيئة موراشيج وسكوج وبيئة جامبورج وكذلك بيئة مكاون. بيئة موراشيج وسكوج المضاف إليها ٠,٥ ملجم/ لتر تي– ديازورون + ٠,٥ ملجم/ لتر بنزيل أدينين + ٠,١ ملجم/ لتر أندول حامض البيوتريك اعطت أعلى معدل براعم خضرية نامية من زراعة الأوراق. حيث أعطت هذه التوليفة (٥,٣) برعم للمنفصل النباتي الورقي للجاتروفا ذات اكوادور المنشأ وكذلك أعطت (٤,٧) برعم للمنفصل النباتي الورقي للجاتروفا ذات مدغشقر المنشأ. اما خلال مرحلة التجذير المعملي لنوعي الجاتروفا محل الدراسة فتمت زراعة البراعم الخضرية الصحية والتي تمت استطالتها خلال المرحلة السابقة على أنواع مختلفة من البيئات ولكن بنصف قوتها والمحتوية على تركيزات مختلفة من حامض أندول البيوتريك. وجد أن أعلى إنتاج للجذور تم الحصول عليه باستخدام بيئة موراشيج وسكوج المضاف إليها ٠,٥ ملجم/ لتر من أندول حامض البيوتريك حيث أعطت (٦,٠٦) جذر للبرعم الخضري بطول (٢,٩٣ سم) في الجاتروفا ذات الاكوادور المنشأ أما في الجاتروفا ذات مدغشقر المنشأ فأعطت (٥,٧٦) جذر للبرعم الخضري بطول (٢,٧٦ سم). بالإضافة الى ان النبتات المتحصل عليها معمليا تمت أقلمتها وبقائها بنجاح في الصوبة الزجاجية لدراسات اخرى في المستقبل ويمكن استنتاج ان بيئة موراشيج وسكوج المضاف اليها تي– ديازورون أو أندول حامض البيوتريك لصنف الجاتروفا الاكوادورية المنشأ كانت أكثر فاعلية لتقنية زراعة الاوراق.

الكلمات المفتاحية: الجاتروفا، المنفصل النباتي الورقي، منظمات النمو النباتية، الاكثار النباتي، التجذير المعملي.