



## IN VITRO REGENERATION OF THE BIOFUEL CROP *JATROPHA CURCAS*

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### Abstract

*Jatropha* (*Jatropha curcas*) is a multipurpose plant and gains a special interest as a non-edible biofuel producing plant. The present study aimed to establish an efficient protocol for *in vitro* regeneration of *Jatropha* from cotyledonary leaves. Hundred percent of explants induced green and nodulated regenerative callus when explants were placed on Murashige and Skoog (MS) medium supplemented with 0.45 or 4.54  $\mu\text{M}$  thidiazuron (TDZ). The induced callus gave the highest number of shoots (11.9) on MS medium supplemented with 0.45  $\mu\text{M}$  TDZ. The optimum medium for shoot proliferation and elongation was MS medium supplemented with 8.88  $\mu\text{M}$  6-benzyl adenine (BA) combined with 54.3  $\mu\text{M}$  adenine sulphate (12.7 shoots with a length of 3.72 cm). These shoots were isolated and then rooted in half strength MS medium supplemented with indole-3-butyric acid (IBA) at the concentration of 1.47  $\mu\text{M}$  and gave the highest rooting percentage of 61.66%. This protocol is efficient in mass production of the biofuel producing *Jatropha* plant and could be used in genetic transformation and breeding programs.

**Keywords:** Euphorbiaceae; micropropagation; cotyledonary leaf

### Introduction

*Jatropha* (*Jatropha curcas* L.) is a multipurpose deciduous tree, belongs to Euphorbiaceae family. It is known for its medicinal importance and recently is promising as a standard biodiesel source. The genus *Jatropha* comprises about 70 species, native to America, especially Mexico and Central America, but now it is widely cultivated in tropical and subtropical areas (Om *et al.*, 2013 and Ji *et al.*, 2019). Oil is the major resource obtained from *Jatropha*. Depending on the variety/cultivar, seeds contain 40–60% oil that is utilized for many purposes, such as lighting, lubricating, making soap and most importantly as biodiesel (Kumar *et al.*, 2011). Among all other characteristics of *Jatropha*, it receives a priority attention for production of biodiesel, it complies with European biodiesel standards (Moniruzzaman *et al.*, 2017). Also, *Jatropha* represents an ideal biofuel crop for poor and developing countries, because of its high oil content, rapid growth, pest-tolerance, grows in degraded lands and is adaptable to a wide range of stress conditions; such as drought (Edrisi *et al.*, 2015). *Jatropha* is unpalatable for animals, and as a non-edible plant, it does not compete with food production. Biofuels derived from non-edible oils; such as that of *Jatropha* are more suitable economically, especially in developing countries (Fufa *et al.*, 2019). Moreover, *Jatropha* has medical applications as an anti-tumor, anti-microbial and anti-parasitic (Xu *et al.*, 2019). *Jatropha* contains latex, which is used for medicinal purposes, cosmetics industry and as biopesticides (Laguna *et al.*, 2018). In addition, the seedcake provides organic manure and is rich in protein (60–63%) with a high content of essential amino acids (Maghuly and Laimer, 2013).

In Egypt, since 1997, the Egyptian government started plantation of *Jatropha* as a part of “The National Program for Safe Use of Treated Sewage Water for Afforestation” that aims to cultivate the desert by man-made forests of economically important plants, using treated wastewater, to conserve the fresh water. *Jatropha* gave high

yields in Egypt (Swanberg, 2009), therefore, there is a great opportunity to spread the plantation of *Jatropha* and biodiesel production in Egypt, in addition to increase EU imports of biodiesel for creation of a huge market for the Egyptian biodiesel in the future (Soliman and He, 2015).

The most common method for propagation of *Jatropha* is seed germination, which is severely limited, because of the poor seed viability and low percentage of germination and rooting of seedlings. Moreover, the plants produced from seeds are not true to type and variations occur in seed yield and oil content (Shukla *et al.*, 2013). Oil percentage can vary between 8-54% in plants produced from seeds (Ovando-Medina *et al.*, 2011). On the other hand, plants propagated by cuttings have not deep roots and easily get uprooted when planting in poor top soil lands. Produced plants also have lower longevity and resistance to drought and disease than plants propagated by seeds, therefore the planting materials can be a source of disease transmission (Fufa *et al.*, 2019).

For an efficient large-scale commercial production of disease free true to type *Jatropha* genotypes, *in vitro* propagation is a must. It offers advantages for mass propagation of more vigorous superior quality *Jatropha* in a short period of time and independent of the season (Fufa *et al.*, 2019). However, propagation through tissue culture is difficult (Mweu *et al.*, 2016), especially *in vitro* rooting and acclimatization (Pankaj and Divay, 2011). This species is latex containing, so it is recalcitrant for plant tissue culture (Rajore and Batra, 2007). Also, establishing an *in vitro* regeneration system is the main prerequisite for genetic improvement, transformation technology and breeding strategies. Therefore, developing an efficient *in vitro* regeneration system would be a remarkable progress for the *Jatropha* business and the field of alternative energy technology.

Many studies were carried out to establish a protocol for the *in vitro* propagation of *Jatropha* either by direct or indirect methods. The most recent studies were that of Chiangmai *et al.* (2015), Gangwar *et al.* (2015), Jadon *et al.* (2015), Mishra (2018) and Fufa *et al.* (2019). To the best of our knowledge, despite the available reviews on regeneration in *Jatropha*, there is no reports on its regeneration from plants that are grown in Egypt. It was reported that regeneration of *Jatropha* is highly genotype dependent (Kumar *et al.*, 2011; Mweu *et al.*, 2016 and Fufa *et al.*, 2019). Therefore, the aim of the present study was the development of an *in vitro* regeneration protocol for *Jatropha* from Egypt to be used for mass production of this biofuel crop and a stepping-stone for efficient genetic transformation.

## Materials and Methods

### *In vitro* seed sterilization and germination

Seeds of *Jatropha* were collected from plants grown in Tushka Research Station of Desert Research Center, South Egypt. They were washed under running tap water for 20 min, followed by a detergent for 5 min. Seeds were surface sterilized using 100% commercial bleach solution (containing 5.25% sodium hypochlorite) for 10 min, followed by dipping in 0.1% (w/v) mercuric chloride solution for five min. Finally, seeds were thoroughly rinsed for five times with sterilized distilled water. All surface sterilization steps were carried out in Laminar-air-flow (Holten LaminAir HVR 2448, USA) under complete aseptic conditions. After surface sterilization, seed coats were removed and seeds were germinated on half strength Murashige and Skoog (MS; Duchefa, Haarlem, the Netherland) medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose, 0.01% (w/v) myo-inositol (Fluka, Switzerland). The pH of the medium was adjusted to 5.7-5.8 before gelling with 0.25% (w/v) phytigel (Duchefa, Haarlem, the Netherlands) and autoclaving at a pressure of 1.06 kg cm<sup>-2</sup> and 121°C for 15 min (Wisid, Korea). Seeds were incubated at 25±2°C in the darkness for 10 days, then transferred to normal growth conditions of a 16-h photoperiod under cool white fluorescent tubes (F140t9d/38, Toshiba) and 60-65% relative humidity. Cotyledonary leaves were excised from 21-day-old seedlings, cut into small pieces (1x1 cm) and were used as explants for *in vitro* regeneration.

### Shoot regeneration and proliferation

The induced callus was transferred to MS medium supplemented with 3% (w/v) sucrose, 0.01% (w/v) myo-inositol and TDZ at concentrations of 0.227, 0.454, 1.135, 2.27 and 4.54 µM individually or combined with 0.49 µM IBA. The pH of the medium was adjusted, gelled, autoclaved and cultures were incubated as mentioned above. The percentage of shoot regeneration from callus (%) and mean number of induced shoots were recorded after four weeks.

Shoot proliferation was tested using different concentrations of BA (4.44, 6.66, 8.88 and 13.32 µM) in a preliminary experiment. BA at a concentration of 8.88 µM was the best and used either individually or combined with TDZ at 0.225 and 0.0454 µM for proliferation. Adenine sulphate was tested individually at 54.3 µM and 108.6 µM or

at 54.3 µM in combination with 8.88 µM BA. Abscisic acid (ABA) at 1.89 µM was applied to MS medium supplemented with 8.88 µM BA and 0.49 µM IBA. IBA at 0.49 µM was also added to culture medium supplemented with either 8.88 µM BA individually or in combination with 0.045 µM TDZ. The mean increase in number and length (cm) of shoots were recorded after four weeks of culture.

All media for shoot induction and proliferation were supplemented with 3% w/v sucrose and 0.1% w/v myo-inositol. MS medium without PGRs served as a control. The pH was adjusted to 5.7-5.8 and gelled with 0.25% phytigel before autoclaving. Cultures were incubated at 23±2°C with a 16-h photoperiod.

### Root induction

The elongated shoots were cultured on half strength MS medium supplemented with IBA at 1.47, 2.94 and 4.41 µM. Rooting media were supplemented with 3% w/v sucrose and 0.1% w/v myo-inositol. The pH was adjusted to 5.7-5.8 and gelled with 0.25% phytigel before autoclaving. Half strength MS medium without IBA served as control. Cultures were incubated at 23±2°C with a 16-h photoperiod. The percentage of root induction (%) and number of roots/shoot were recorded after six weeks of culture.

### Experimental design and statistical analysis

Experiments were conducted in a completely randomized design with at least 15 replicates per treatment for each growth stage. Data were subjected to statistical analyses by ANOVA programme using Duncan's multiple range test (Duncan, 1955), as modified by Snedecor and Cochran (1990). The differences among means of the recorded parameters for all treatments were tested for significance at 5% level.

## Results and Discussion

### Callus induction

Callus induction and plant regeneration are one of the key tools in plant biotechnology that exploits the totipotent nature of plant cells (Li *et al.*, 2012). In this experiment, several cytokinins and auxins were used to test their effect on callus induction from cotyledonary leaves of *Jatropha* (Table 1). The results show that the percentage of survived explants ranged between 77 and 100%. Most of the tested PGRs gave 100% of both survived explants and explants forming callus. Callus was different in colour, texture and ability to regenerate shoots according to the used PGRs. In general, the ability of explants to form callus not only depended on the type of PGRs, but also on their concentration. TDZ at 0.454 and 4.54 µM concentrations was the most suitable in producing 100% of regenerative callus, which was green and nodulated (Fig. 1a). This type of callus is suitable for shoot organogenesis as reported by Misra *et al.* (2010) for *Jatropha*. TDZ was proved to be a determinant factor in callus behaviour of *Jatropha*, as mentioned by Gopale *et al.* (2015). In woody plants, callus is induced using TDZ, it involves in modification of cell membrane, energy level, nutrient uptake and nutrient assimilation (Guo *et al.*, 2011). Although, most the other tested PGRs gave

100% callus formation, but callus was not regenerative. Callus obtained on MS medium supplemented with 0.465  $\mu\text{M}$  Kin and 5.74  $\mu\text{M}$  NAA was embryogenic. It was obvious that IBA at 7.35  $\mu\text{M}$  gave significantly the least

callus induction percentage (53.33%). Since, callus induction medium is very critical for shoot regeneration, therefore the media supplemented with TDZ at 0.454 or 4.54  $\mu\text{M}$  were the best for producing regenerative callus of *Jatropha*.

**Table 1:** Effect of PGRs on callus induction of *Jatropha* on MS medium after eight weeks of culture.

Growth regulators concentration ( $\mu\text{M}$ )							Survived explants (%)	Explants forming callus (%)	Colour and texture of callus
BA	Kin	TDZ	IAA	IBA	NAA	2,4-D			
0.00	0.000	0.000	0.00	0.00	0.00	0.000	-	-	-
2.22	0.000	0.000	0.00	0.00	0.00	4.530	100 <sup>a</sup>	100.00 <sup>a</sup>	White & friable
0.00	0.000	0.000	0.00	0.00	0.00	2.265	100 <sup>a</sup>	100.00 <sup>a</sup>	White & compact
2.22	0.000	0.000	5.71	0.00	0.00	4.530	100 <sup>a</sup>	100.00 <sup>a</sup>	Yellow & friable
4.44	0.000	0.000	0.00	0.00	0.00	0.000	77 <sup>c</sup>	93.33 <sup>a</sup>	Pale green & compact
4.44	0.000	0.000	0.00	0.00	0.00	13.59	100 <sup>a</sup>	100.00 <sup>a</sup>	Pale green & friable
6.66	0.000	0.000	0.00	0.49	0.00	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Pale green & compact
4.44	0.000	0.000	0.00	2.45	0.00	0.000	93 <sup>ab</sup>	70.00 <sup>c</sup>	Pale green & compact
6.66	0.000	0.000	0.00	2.45	0.00	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Pale green & compact
0.00	0.465	0.000	0.00	0.00	5.37	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	White & friable, nodulated
22.20	0.000	0.000	0.00	0.00	5.37	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	White & compact
0.00	0.000	0.000	0.00	7.35	0.00	0.000	81 <sup>bc</sup>	53.33 <sup>b</sup>	Yellow & friable
0.00	4.650	0.000	0.00	14.70	0.00	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Yellow & friable
8.88	0.000	0.000	0.00	0.00	10.74	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Pale green & compact
0.00	0.000	0.454	0.00	0.00	0.00	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Green & nodulated
0.00	0.000	4.540	0.00	0.00	0.00	0.000	100 <sup>a</sup>	100.00 <sup>a</sup>	Green & nodulated

Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$ .

### Shoot regeneration and proliferation

The percentage of shoot regeneration from callus of *Jatropha* ranged between 60 and 100% and the mean number of induced shoots ranged between 5.6 and 11.9 (Table 2). The highest percentage of shoot regeneration from callus was 100% and was obtained on MS medium supplemented with 2.27  $\mu\text{M}$  TDZ. TDZ is a potent cytokinin and plays a crucial role in shoot regeneration from callus as compared to other cytokinins. The regeneration efficiency was higher in the medium containing TDZ, compared to other cytokinins as

documented by Kumar *et al.* (2011) for *Jatropha*. The highest number of shoots (11.9) was observed using 0.454  $\mu\text{M}$  TDZ (Fig. 1b). Addition of IBA on the same concentration of TDZ negatively affected the response of callus to produce shoots (73.33%), but the number of shoots remained fair (10.4). This could be attribute to the endogenous auxin, which balances with cytokinin and this is very essential for the growth of plant tissue, since they function in relation to each other (Purkayastha *et al.*, 2010).

**Table 2:** Effect of TDZ and IBA on shoot regeneration from callus of *Jatropha* on MS medium after six weeks of culture.

Growth regulators concentration ( $\mu\text{M}$ )		Shoot regeneration from callus (%)	Mean no. of induced shoots/callus
TDZ	IBA		
0.000	0.00	-	-
0.227	0.00	66.67 <sup>ab</sup>	5.60 <sup>c</sup>
0.454	0.00	82.67 <sup>ab</sup>	11.90 <sup>a</sup>
1.135	0.00	65.33 <sup>ab</sup>	7.00 <sup>bc</sup>
2.270	0.00	100.0 <sup>a</sup>	6.90 <sup>bc</sup>
4.540	0.00	89.33 <sup>ab</sup>	6.80 <sup>bc</sup>
0.227	0.49	62.67 <sup>ab</sup>	9.20 <sup>ab</sup>
0.454	0.49	73.33 <sup>ab</sup>	10.40 <sup>a</sup>
1.135	0.49	73.33 <sup>ab</sup>	9.90 <sup>ab</sup>
2.270	0.49	60.00 <sup>b</sup>	9.80 <sup>ab</sup>
4.540	0.49	60.00 <sup>b</sup>	6.60 <sup>bc</sup>

Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$ .

Regarding shoot proliferation, data presented in Table 3 show that 8.88  $\mu\text{M}$  BA was the best concentration affecting the mean increase in number of shoots either with 54.3  $\mu\text{M}$  adenine sulphate (12.7) or 0.227  $\mu\text{M}$  TDZ (11.8) or even individually (11.1). Also, as recorded in Table 3, ABA in combination with IBA in the culture medium supplemented with 8.88  $\mu\text{M}$  BA, gave a high number of

regenerated shoots, which reached 10.2. The positive effect of ABA is in harmony with the results obtained by Singh *et al.* (2014) on *Jatropha*, ABA enhanced shoot regeneration from callus. They reported that ABA is an auxin inhibitor, therefore it might cause reduction in the auxin level to the required optimum level for shoot regeneration and proliferation.

**Table 3:** Effect of PGRs on shoot proliferation of *Jatropha* on MS medium after four weeks of culture.

Growth regulators concentration ( $\mu\text{M}$ )					Mean increase in no. of shoots/callus	Mean length of shoots (cm)
BA	TDZ	IBA	Adenine sulphate	ABA		
0.00	0.000	0.00	0.0	0.00	4.70 <sup>f</sup>	2.15 <sup>de</sup>
8.88	0.000	0.00	0.0	0.00	11.10 <sup>abcd</sup>	2.21 <sup>d</sup>
8.88	0.227	0.00	0.0	0.00	11.80 <sup>abc</sup>	2.65 <sup>c</sup>
8.88	0.000	0.49	0.0	1.89	10.20 <sup>bcde</sup>	1.99 <sup>e</sup>
8.88	0.045	0.49	0.0	0.00	8.70 <sup>cde</sup>	3.09 <sup>b</sup>
0.00	0.000	0.00	54.3	0.00	7.00 <sup>ef</sup>	2.94 <sup>b</sup>
0.00	0.000	0.00	108.6	0.00	7.90 <sup>def</sup>	2.2 <sup>d</sup>
8.88	0.000	0.00	54.3	0.00	12.70 <sup>ab</sup>	3.72 <sup>a</sup>

Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$ .

Cytokinins have a significant role in shoot regeneration (Thomas and Yoichiro, 2010 and Bala *et al.*, 2015). They are derivatives of adenine and regulate the synthesis of proteins, which are involved in the formation and functioning of mitotic spindle. Cytokinins are also required for adventitious shoot formation and stimulation of cell division (Bala *et al.*, 2015). TDZ is less susceptible to enzymatic degradation than other aminopurine cytokinins (Magyar-Tabori *et al.*, 2010 and Kim and Sivanesan, 2016), so at low concentration, it positively affected the number of multiplied shoots when added to the medium containing other cytokinins. Moreover, a study on *in vitro* regeneration of *Jatropha* was conducted by Li *et al.* (2012) and showed the same effect of using TDZ either individually or combined with other PGRs in increasing the number of shoots (12.7 shoots) from callus. The least response was observed in PGRs free treatment. This is confirmed with Thepsamran *et al.* (2008) and Fufa *et al.* (2019), who reported that the exogenous application of cytokinins is obligatory for *Jatropha* multiple shoot induction.

Regarding length of shoots, it was found that the mean length of shoots ranged between 1.99 to 3.72 cm/shoot. The longest shoots were obtained on MS medium supplemented with 8.88  $\mu\text{M}$  BA and 54.3  $\mu\text{M}$  adenine sulphate, therefore, this medium considered the optimum for *Jatropha* shoot regeneration and proliferation (Fig. 1c). These data are supported by Herrera-Cool *et al.* (2019), who pointed out the effect of the organic additive; adenine sulphate, which has a positive effect on both the induction and elongation of adventitious shoots of *Jatropha*. This may be due to the fact that the interaction of adenine sulphate with cytokinins facilitates the growth and development of shoots in the *in vitro* culture. Adenine sulphate greatly stimulates cell growth and shoot formation. It reinforces the effect of other PGRs in enhancing shoot proliferation (Naaz *et al.*,

2014). This may be due to that adenine sulphate acts as a precursor for cytokinins and consequently increases their biosynthesis. On the other hand, adenine sulphate may act synergistically as a cytokinin (Gatica *et al.*, 2010). It also provides nitrogen to cells, which can be taken up more rapidly than inorganic nitrogen (Naaz *et al.*, 2014).

#### Root induction

The highest root induction percentage (61.66%) and the highest number of roots (8.6) were observed when the elongated shoots were transferred to half strength MS medium supplemented with 1.47  $\mu\text{M}$  IBA (Table 4 and Fig. 1d). Upon preliminary investigation (data not shown), IBA appeared to be superior for *in vitro* root induction as compared to IAA and NAA, individually or in combination or to higher concentrations of IBA (until 14.7  $\mu\text{M}$ ). The same observation was reported by Jadon *et al.* (2015) for *Jatropha*. IBA is an effective auxin for root induction (Tan *et al.*, 2018). The negative effect of NAA in root induction of *Jatropha* shoots could be a reason for the more persistency of NAA than IBA, it remains in the tissue and may block root meristemoids development (Nanda *et al.*, 2004).

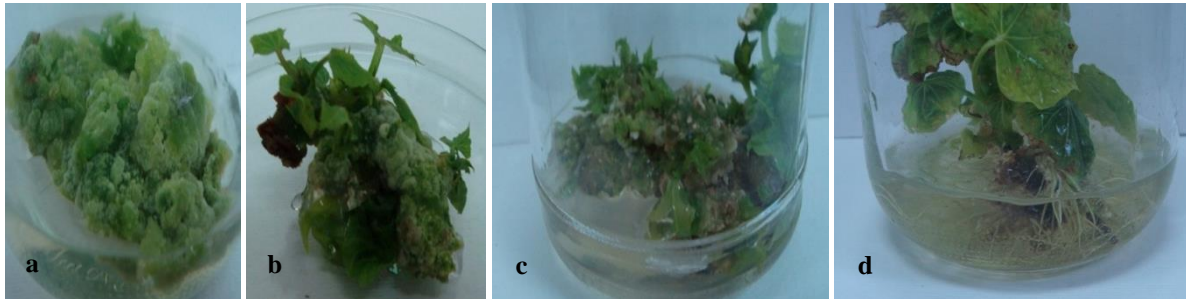
It is clear from data in Table 4 that medium without PGRs was more effective than that with higher IBA concentrations (higher than 1.47  $\mu\text{M}$ ). Medium without PGRs was also applied by Shukla *et al.* (2013) for rooting of *Jatropha* and gave a high response. Moreover, root length was higher in the control medium than high IBA concentration, which confirms the sensitivity of shoot length to auxin concentration, which inhibits the length of roots. This could be contributed to that, these concentrations are supra-optimum and cause the inhibition of root elongation by enhancing the biosynthesis of ethylene, which is a root growth inhibitor (Hartman *et al.*, 2009). Efficiency of IBA at the same range of concentrations on the *in vitro* rooting of

*Jatropha* shoots was previously recorded by Kumar *et al.* (2011), Attaya *et al.* (2012) and Fufa *et al.* (2019).

**Table 4:** Effect of IBA on *in vitro* rooting of *Jatropha* on half strength MS medium after four weeks of culture.

IBA conc. ( $\mu\text{M}$ )	Root induction (%)	Mean no. of roots /shoot
0.0	40.00 <sup>a</sup>	3.5 <sup>b</sup>
1.47	61.66 <sup>a</sup>	8.6 <sup>a</sup>
2.94	30.00 <sup>a</sup>	3.1 <sup>b</sup>
4.41	00.00	0.0

Means followed by the same letter within a column are not significantly different at  $P \leq 0.05$ .



**Fig. 1:** *In vitro* regeneration of *Jatropha*.

- a. Callus on MS medium supplemented with 0.454  $\mu\text{M}$  TDZ.  
 b. Regenerated shoots from callus on MS medium supplemented with 0.454  $\mu\text{M}$  TDZ.  
 c. Proliferated shoots on MS medium supplemented with 8.88  $\mu\text{M}$  BA + 54.3  $\mu\text{M}$  adenine sulphate.  
 d. Rooted shoots on  $\frac{1}{2}$  MS medium supplemented with 1.47  $\mu\text{M}$  IBA.

## Conclusion

The present study represents the establishment of *in vitro* regeneration protocol for *Jatropha* grown in Egypt, using cotyledonary leaves for mass production of this biofuel crop in a short time. The application of this protocol could meet the enormous demand of the plant and ensures easy and continuous supply of this elite species. This method is important for large scale production of the plant and as a step for its genetic improvement by genetic transformation for higher quality of crop and oil yield.

## References

- Agbogidi, O.M.; Mariere, A.E. and Ohwo, O.A. (2013). Metal concentration in plant tissues of *Jatropha curcas* L. grown in crude oil contaminated soil. *J Bio Innov*, 2(3): 137–145.
- Amonum, J.I.; Dachung, G. and Tsa, O.S. (2019). Germination and early growth of *Jatropha curcas* L. from different seed sources. *Forest Res Eng Int J*, 3(1): 1–5.
- Attaya, A.S.; Geelen, D. and Belal, A.E.H. (2012). Progress in *Jatropha curcas* tissue culture. *Am-Eurasian J Sustain Agric*, 6(1): 6-13.
- Bala, R.; Beniwal, V.S. and Laura, J.S. (2015). An efficient and reproducible indirect shoot regeneration from female leaf explants of *Simmondsia chinensis*, a liquid-wax producing shrub. *Physiol Mol Biol Plants*, 21(2): 293–299.
- Chiangmai, P.N.; Pootaeng, O.Y.; Meetum, P.; Kamkajon, K.; Yuiam, W.; Rungphan, N. and Ninkaew, P. (2015). Regeneration of adventitious shoots from callus and leaf explants in *Jatropha curcas* L. 'Phetchaburi'. *Silpakorn U Science Tech J*, 9(1): 28-39.
- Duncan, D.B. (1955). Multiple ranges and multiple "F" test. *Biometrics*, 11: 1-42.
- Edrisi, S.A.; Dubey, R.K.; Tripathi, V.; Bakshi, M.; Srivastava, P. and Jamil, S. (2015). *Jatropha curcas*: a crucified plant waiting for resurgence. *Renew Sust Energ Rev*, 41: 855-62.
- Fufa, H.; Tesema, M. and Daksa, J. (2019). *In vitro* regeneration protocol through direct organogenesis for *Jatropha curcas* L. (Euphorbiaceae) accessions in Ethiopia. *Afr J Biotechnol*, 18(31): 991-1003.
- Gangwar, M.; Sharma, S.; Chauhan, R.S. and Sood, H. (2015). Indirect shoot organogenesis in *Jatropha curcas* (L.) for *in vitro* propagation. *Indian J Res*, 4(12): 56-58.
- Gatica, A.; Valverde, A.M.; Fonseca, P.R. and Melara, M.V. (2010). *In vitro* plant regeneration system for common bean (*Phaseolus vulgaris*): effect of N6 benzylaminopurine and adenine sulphate. *Electron J Biotech*, 13(1):6–7.
- Gopale, K.D.; Zunjarrao, R.S.; Pande, A.K.; Paranjape, S.Y. and Joshi, O.B. (2015). Organogenesis and somatic embryogenesis of *Jatropha curcas* L. *Dnyanamay Journal*, 1(2): 36-42.
- Guo, B.; Abbasi, B.H.; Zeb, A.; Xu, L L, Wei Y H (2011) Thidiazuron: A multi-dimensional plant growth regulator. *Afr J Biotechnol*10(45):8984-9000.
- Hartman, H.D.; Kester, D.Z.; Davies, F.T. and Geneve, R.L. (2009). *Plant propagation: principles and practices*. 7<sup>th</sup> Edition, PHL Learning Private Ltd, New Delhi, India.
- Herrera-Cool, G.J.; Loureiro, J.; Rodríguez-Buenfil, I.M.; Uc-Vázquez, A.; Iglesias-Andreu, L.G.; Góngora-

- Canul, C.C.; Martínez-Sebastian, G.; Aguilera-Cauch, E.A. and López-Puc, G. (2019). Indirect organogenesis and estimation of nuclear DNA content in regenerated clones of a non-toxic variety of *Jatropha curcas*. *Tropi Subtrop Agroecosys*, 22: 451-463.
- Jadon, S.; Singh, V.; Shrivastava, N.; Wahi, N. and Bhadauria, A. (2015). Micropropagation of *Jatropha curcas* L. with different hormonal treatments. *Indian Res J Genet Biotech*, 7(1): 35-40.
- Kim, D.H. and Sivanessan, I. (2016). Influence of benzyladenine and thidiazuron on shoot regeneration from leaf and shoot tip explants of *Sedum sarmentosum* Bunge. *Braz Arch Biol Techn*, 59: 1-6.
- Kumar, N.; Anand, K.G.V. and Reddy, M.P. (2011). Plant regeneration of non-toxic *Jatropha curcas*-impacts of plant growth regulators, source and type of explants. *J Plant Biochem Biotechnol*, 20(1): 125-133.
- Laguna, G.; Molina, M.V.; Padilla, A.; Aviles, Z.; Bonomo, C.; Carrizo, A. and Martinez, R. (2018). Induction of somatic embryogenesis in *Jatropha curcas* L. *Int J Res Agric Sci*, 6(5): 2348-3997.
- Maghuly, F. and Laimer, M. (2013). *Jatropha curcas*, a biofuel crop: Functional genomics for understanding metabolic pathways and genetic improvement. *Biotechnol J*, 8: 1172-1182.
- Magyar-Tabori, K.; Dobranszki, J.; Taeixeira da Silva, J.A.; Bulley, S.N. and Hudak, I. (2010). The role of cytokinins in shoot organogenesis in apple. *Plant Cell Tiss Org Cult*, 101: 251-267.
- Mishra, S. (2018). *In-vitro* direct shoot organogenesis in *Jatropha curcas* L. *J Pharmacogn Phytochem*, 7(2): 1777-1780.
- Misra, P.; Gupta, N.; Dibya, D.; Pandey, T.V.; Mishra, M.K. and Tuli, R. (2010). Establishment of long-term proliferating shoot cultures of elite *Jatropha curcas* L. by controlling endophytic bacterial contamination. *Plant Cell Tiss Org*, 100: 189-197.
- Moniruzzaman, M.; Yaakob, Z. and Khatun, R. (2016). Biotechnology for *Jatropha* improvement: a worthy exploration. *Renew Sust Energ Rev*, 54: 1262-1277.
- Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plantarum*, 15: 473-493.
- Mweu, C.M.; Nyende, A. and Onguso, J. (2016). Efficient somatic embryogenesis of *Jatropha curcas* L. from petiole and leaf discs. *Int J Biotechn Mol Biol Res*, 7(3): 29-35.
- Naaz, A.; Shahzad, A. and Anis, M. (2014). Effect of adenine sulphate interaction on growth and development of shoot regeneration and inhibition of shoot tip necrosis under *in vitro* condition in Adult *Syzygium cumini* L. - a multipurpose Tree. *Appl Biochem Biotechnol*, 173: 90-102.
- Nanda, R.M.; Das, P. and Rout, G.R. (2004). *In vitro* clonal propagation of *Acacia mangium* and its evaluation of genetic stability through RAPD marker. *Ann Forest Sci*, 61(4): 381-386.
- Ovando-Medina, I.; Espinosa-García, J.F.; Nuñez-Farfán, J. and Salvador-Figueroa, M. (2011). Genetic variation in Mexican *Jatropha curcas* estimated with seed oil fatty acids. *J Oleo Sci*, 60(6): 301-311.
- Pankaj, K. and Divay, G. (2011). Plant tissue culture of *Jatropha curcas* L.: a review. *Imperial J Pharmacogn Nat Prod*, 1(1): 2248-9754.
- Purkayastha, J.; Sugla, T.; Paul, A.; Solleti, S.K.; Mazumdar, P.; Basu, A.; Mohommad, A.; Ahmed, Z. and Sahoo, L. (2010). Efficient *in vitro* plant regeneration from shoot apices and gene transfer by particle bombardment in *Jatropha curcas*. *J Plant Biol*, 54(1): 13-20.
- Rajore, S. and Batra, A. (2007). An alternative source for regenerable organogenic callus induction in *Jatropha curcas* L. *Indian J Biotechnol*, 6: 545-548.
- Shukla, P.; Makwana, V.; Bhatt, D. and Robin, A.P. (2013). Efficient method for direct and indirect organogenesis in biofuel crop *Jatropha curcas*. *Int J Pharm Bio Sci*, 4(1): 673-682.
- Singh, A.; Jani, K.; Sagervanshi, A. and Agrawal, P.K. (2014). High-frequency regeneration by abscisic acid (ABA) from petiole callus of *Jatropha curcas*. *In Vitro Cell Dev Biol – Plant*, DOI 10.1007/s11627-014-9628-y
- Snedecor, G.W. and Cochran, W.G. (1990). *Statistical methods*. 8<sup>th</sup> edition. Iowa State University Press, Ames, Iowa, USA.
- Soliman, W. and He, X. (2015). The potentials of *Jatropha* plantations in Egypt: A Review. *Modern Economy*, 6(2): 190-200.
- Swanberg, K. (2009). Economic feasibility of alternative crops with potential for the reuse of treated wastewater in Egypt. International Resources Group, Washington DC, USA.
- Tan, S.N.; Tee, C.S. and Wong, H.L. (2018). Multiple shoot bud induction and plant regeneration studies of *Pongamia pinnata*. *Plant Biotechnol*, 35: 325- 334.
- Thepsamran, N.; Thepsithar, C. and Thongpukdee, A. (2008). *In vitro* induction of shoots and roots from *Jatropha curcas* explants. *J Horticult Sci Biotech*, 83(1): 106-112.
- Thomas, T.D. and Yoichiro, H. (2010). *In vitro* propagation for the conservation of a rare medicinal plant *Justicia gendarussa* Burm. f. by nodal explants and shoot regeneration from callus. *Acta Physiol Plant*, 32: 943-950.
- Xu, G.; Huang, J.; Lei, S.K.; Sun, X.G. and Li, X. (2019). Comparative gene expression profile analysis of ovules provides insights into *Jatropha curcas* L. ovule development. *Sci Rep-UK*, 9: 1.