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EFFECT OF JATROPHA LEAVES EXTRACT ON *IN VITRO* DATE PALM EMBRYOS GERMINATION AND SHOOT REGENERATION

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ABSTRACT

The aim of this study was to enhance the shoot production of date palm, Hayani cv. by investigating the effect of adding jatropha leaves extract in appropriate doses to the germination and multiplication media of date palm. a small cluster of 7-10 matured somatic embryos of date palm Hayani cv. were grown for 12 weeks on MS medium with 0.5 mg l^{-1} BA, 0.1 mg l^{-1} NAA and 10.0 ml l^{-1} JLE which produced the highest number of somatic embryos (28.3 embryos/jar), with the maximum number of leaves (34.5 leaf/jar) with the longest leaf length (6.0 cm). furthermore; during shoot multiplication stage, clusters of shoots cultivated on MS basal medium supplemented with 1.0 mg l^{-1} BA, 0.5 mg l^{-1} TDZ and 0.25 mg l^{-1} NAA with 10 ml l^{-1} JLE resulted in the best shoot regeneration, with maximum number of shoots (25.2 shoots/jar), the maximum number of leaves (56.0 leaf/jar), and the longest leaf length (5.0 cm). The regenerated shoots with lengths of 5–7 cm were grown on ½ MS medium with 1.0 mg l^{-1} NAA during the rooting stage.

***Keywords*:** Date palm, jatropha leaves extract, natural growth additive, somatic embryos and shoot multiplication.

Introduction

Date palm (*Phoenix dactylifera* L.) is a particularly important crop in the Middle East and North Africa because of its high output, high nutritional value of its fruits, and ability to grow well in both semi-dry desert conditions and newly cultivated soil. Throughout human history, the date palm has played an important role in agriculture and economics. It's one among the world's oldest cultivated fruit trees (El-Sharabasy *et al.,* 2017).

Moreover, date palm fruits have a high content of carbohydrates, minerals and vitamins, and hence considered a significant source of nutrition in hot and dry climates (Sedra, 2015). One of the most excellent soft dates in Egypt is a cultivar called Hayani. It has various commercial benefits in both the Khalal and Rutab stages. It is the most widely planted and significant commercial date variety in Lower Egypt. Date yield and quality may vary based on the cultivar, soil type and agricultural techniques (Ageez and Madboly, 2011).

Generally, date palms are often reproduced by vegetative propagation through offshoots and accordingly; the absence of offshoots hampered rapid and large-scale expansion. On the other hand, seed palms often produce low-quality, fluctuating dates that are unsuited for commercial market (Asemota *et al.*, 2007). Furthermore, the offshoots were difficult to root in the field, with a surviving rate of about 60% (Eke *et al.,* 2005) indicating that date palm micro-propagation is superior to conventionally generated propagated plants (Jain, 2012). Accordingly; tissue culture techniques are employed to accomplish mass and quick propagation to bypass this restriction. Several species have been successfully introduced to plant production through *in vitro* culture (Ghazzawy *et al.,* 2017). It has a number of advantages including year-round large-scale multiplication of genetically uniform plantlets, development of healthier female cultivars (free of disease and pests) and the generation of genetically uniform male cultivars (Cubbin *et al.,* 2004).

Tissue culture is used to create date palm plantlets through somatic embryogenesis or organogenesis. Somatic embryogenesis is thought to be the most successful way for creating uniform *in vitro* plants and fast multiplication of date palm cultivars (Al-Khayri, 2011). Plantlets may be also developed through organogenesis, which creates many buds without the need for callus mediation (Al-Khateeb, 2006) and has the same genetic make-up as the mother palm (Al-Khateeb, 2008).

A few studies have looked into the impact of complex chemical compounds on micropropagation of date palms. Low-cost natural sources of nutrients and growth stimulation are occasionally added to plant tissue culture media, which have been shown to benefit *in vitro* cultures of a variety of

plant species and have played an important role in the history of plant tissue culture.

Jatropha curcas has the potential to become a major energy crop across the globe. It's an Euphorbiaceae shrub or tree that can withstand drought. It originated in North and Central America, however, it is currently expanding to tropical and subtropical areas around the world. Saponins, steroids, tannins, glycosides, alkaloids, and flavonoids were discovered in *J. curcas* stem bark and leaf extracts (Namuli *et al.,* 2011). The leaves of *Jatropha curcas* are strong in carbohydrates, protein, minerals, vitamins and amino acids, making jatropha leaves extract (JLE) a good provider of a variety of essential nutrients. However, there have been no previous studies on the utilization of jatropha extract as a natural source of nutrients in tissue culture. Starting from this point; this research was designed to fill this gap and to determine the optimal dosage of jatropha leaves extract required to enhance somatic embryo development and shoot multiplication.

Materials and Methods

This study was conducted between 2022 and 2023 at the Central Lab of Date Palm Researches and Development, ARC, Giza-Egypt.

Jatropha leaves extract preparation

In July 202**1**, *Jatropha curcas* L leaves were collected from plants growing at the Horticulture Research Institute at the Agricultural Research Center in Giza–Egypt.

As shown in (Fig. 1), the newly cut leaves were cleaned by hand to remove any foreign pollutants such as dust, grime or diseased leaves. The leaves were cleaned, air-dried at room temperature, and then ground into powder using an electric blender. 500g of powdered plant material were dissolved in 3.0 L of methanol for 14 days before being filtered using paper filter (Whatman, 125 mm). By evaporating methanol in awater bath and collecting the resulting paste, the concentrated extract was produced (Najda *et al.,* 2013).

Fig. 1 : *Jatropha curcas* plant

Explants material

The somatic embryos required for this experiment were derived from previous research (Rasha *et al.,* 2021) that investigated *in vitro* salt stress and obtained salinity tolerant somatic embryos. Each group contained seven to ten somatic embryos of the Hayani palm cultivar.

Jatropha leaves biochemical characteristics

All chemical analyses for this study were conducted at the Chemistry of Natural and Microbial Products Department, Pharmaceutical Industry Division, National Research Center, Dokki, Giza, Egypt.

Several minerals (nitrogen, phosphorus, potassium, iron, and magnesium) were evaluated using the methodology described in (Apolonia *et al.,* 1991). The protein percentage was estimated using a conversion factor of 6.25 and the total carbohydrates were determined using a modified version of the Luff–Schoorl method (Fortuna *et al.,* 2003). The technique outlined in (Moran, 1982) was utilized to detect photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids). Vitamin, phenolic acid, flavonoids and tannin concentrations were determined using the method described by (Strzelecka *et al.,* 1987). In general, the amino acids were measured using a technique supplied by (Schneider, 1989).

Germination of somatic embryos

The germination medium comprises ¾ MS (Murashige and Skoog, 1962), 200.0 mg l^{-1} glutamine, 100.0 mg l^{-1} myo-inositol, 35.0 mg l^{-1} sucrose, 0.5 mg l^{-1} BA and 0.1 mg l^{-1} NAA (Mazri *et al.,* 2018). There were six treatments of JLE (0.0, 5.0, 10.0, 15.0, 20.0, and 30.0 ml l^{-1}), each with five replicates. Small culture jars (150 ml) were filled with 40 ml of the medium and sealed with polypropylene caps. The medium was then autoclaved for 20 minutes at 121°C and 1.5 kg/cm^2 pressure and incubated for a week. The somatic embryos were then explanted in three subcultures separated by four weeks. Cultures were incubated at 26± 2 °C for 16 hours with a light intensity of 1000 lux. Following the three subcultures, the number of embryos per explant, leaves per jar and leaf length were recorded.

Shoot multiplication

In the multiplication stage, clusters of shoots were cultivated for three subcultures, with a re-culture every four weeks, to MS basal medium supplemented with plant growth regulators as 1.0 mg l^{-1} BA, 0.5 mg l^{-1} TDZ, and 0.25 mg l^{-1}

NAA (Taha *et al.*, 2021) with various doses of JLE (0, 5, 10, 15, 20, and 30 ml l^{-1}). The control condition lacked JLE. Cultures were incubated at 27± 2 °C for 16 hours with a light intensity of 3000 lux. Three months after planting, the number of shoots and leaves were counted and leaf length was measured.

Rooting stage

The shoots (1 to 2 per jar) were cultivated in rooting media supplemented with 0.4 mg l^{-1} Thiamine-HCl, 100 mg l^{-1} myo-inositol, 40 g l^{-1} sucrose, and 6 g l^{-1} agar. The auxin source was NAA at a concentration of 1.0 mgL^{-1} (Khierallah and Bader, 2007) and cultures were incubated in a culture chamber at 26±2 °C and 2000 lux for 16 hours.

Statistical analysis

The experimental design was completely randomized, with five replicates per treatment. The top three results of each treatment were statistically evaluated using the MSTAT computer tool. Using (Duncan's Many Range Test, 1955), the means of multiple treatments were compared to confirm differences.

Results

Chemical composition and biochemical characteristics

In *Jatropha curcas* leaves, the highest concentrations of total phenols, tannins, and free amino acids were discovered in summer-collected leaves (Tomar *et al.*, 2015). The proximate biochemical composition of jatropha leaves revealed that they were mostly made of carbohydrates (36.50%) and proteins (26.14%), as is the case with most plants (Fig. 2).

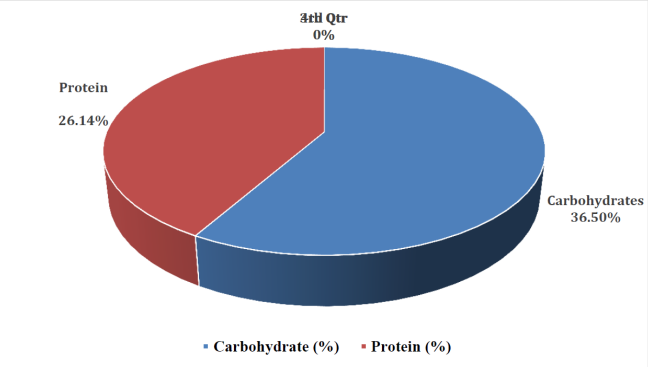

Fig. 2 : Carbohydrate and protein content (%) in jatropha leaves.

Mineral composition (N, P, K, Ca, Fe and Mg) results as shown in (Fig. 3) indicated that most of the minerals have transported from the leaves to other sections of the plant, such as the flowers.

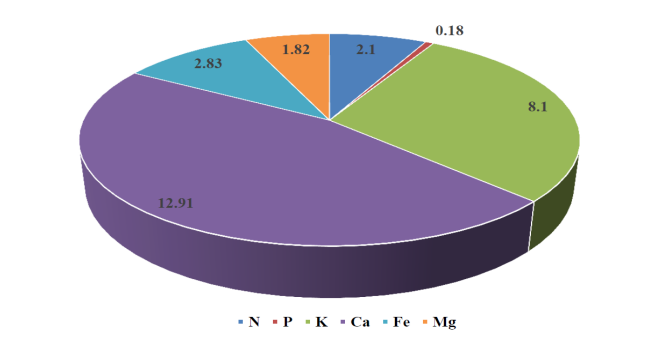

Fig. 3 : Mineral content (%) in jatropha leaves.

However, pigment analysis showed that the chlorophyll content in jatropha leaves was relatively low due to the reduction in nitrogen content (Fig. 4).

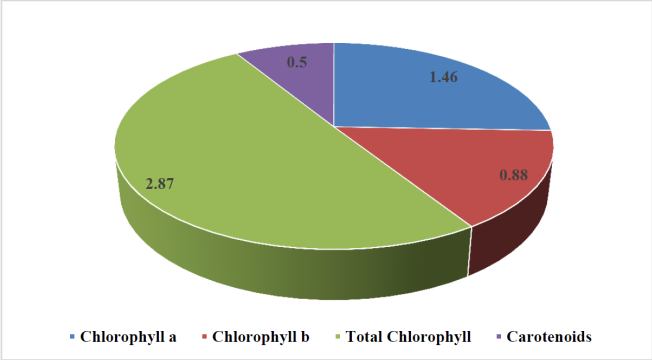

Fig. 4 : Pigment content (mg/g) in jatropha leaves.

Also, vitamin and amino acid analysis showed that jatropha leaves was rich in vitamins as demonstrated in **(Fig. 5)**.

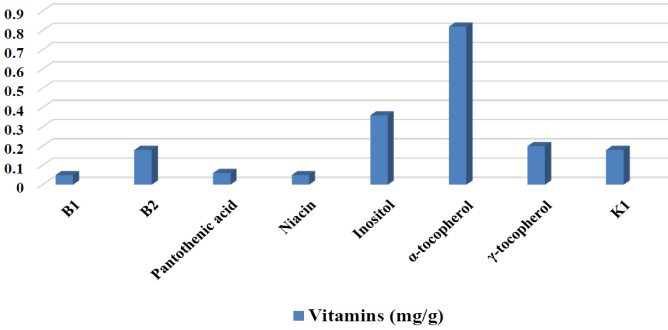

Fig. 5 : Vitamins composition (mg/g) in jatropha leaves.

Somatic embryos germination

Data in Table 1 displays the impact of varying doses of JLE, as a natural additive, on the germination of date palm Hayani cv. somatic embryos. There was a substantial difference between the extract concentrations and the somatic embryos germination of. The number of germinated embryos increased with the increase in JLE concentration added to MS medium supplemented with 0.5 mg l^{-1} BA + 0.1 mg l^{-1} NAA. The treatment with 10.0 ml l^{-1} JLE resulted in the greatest number of somatic embryos (28.3 embryo/jar) and the greatest number of leaves (34.5 leaf/jar) with the longest leaf length (6.0 cm), as indicated in (Table 1) and (Fig. 6). However, as the concentration of JLE increased above 10 m lL-1, the rate of somatic embryo germination decreased. The highest JLE concentration (30 ml l^{-1}) resulted in the lowest number of somatic embryos (7.0 embryo/jar), the lowest number of leaves (3.0 leaf/jar) and the shortest leaf length (0.5 cm) compared to the control treatment which resulted in (7.4 embryo/jar), (2.1 leaf/jar) and 0.5 cm leaf length.

Table 1 : Effect of 0.5 BA and 0.1 NAA (mg l^{-1}) with different concentrations of JLE on somatic embryos germination after three subcultures.

Treatment ($ml l^{-1}$)	No. of somatic embryos	No. of leaves	Leaf length (cm)
0.0	7.4 e	2.1 ef	0.5 e
5.0	12.2 c	7.3 cd	1.5 cd
10.0	28.3 a	34.5 a	6.0 a
15.0	16.0 b	26.7 b	4.3 b
20.0	11.5 d	10.0 c	2.0 c
30.0	7.0 ef	3.0 e	0.5 e

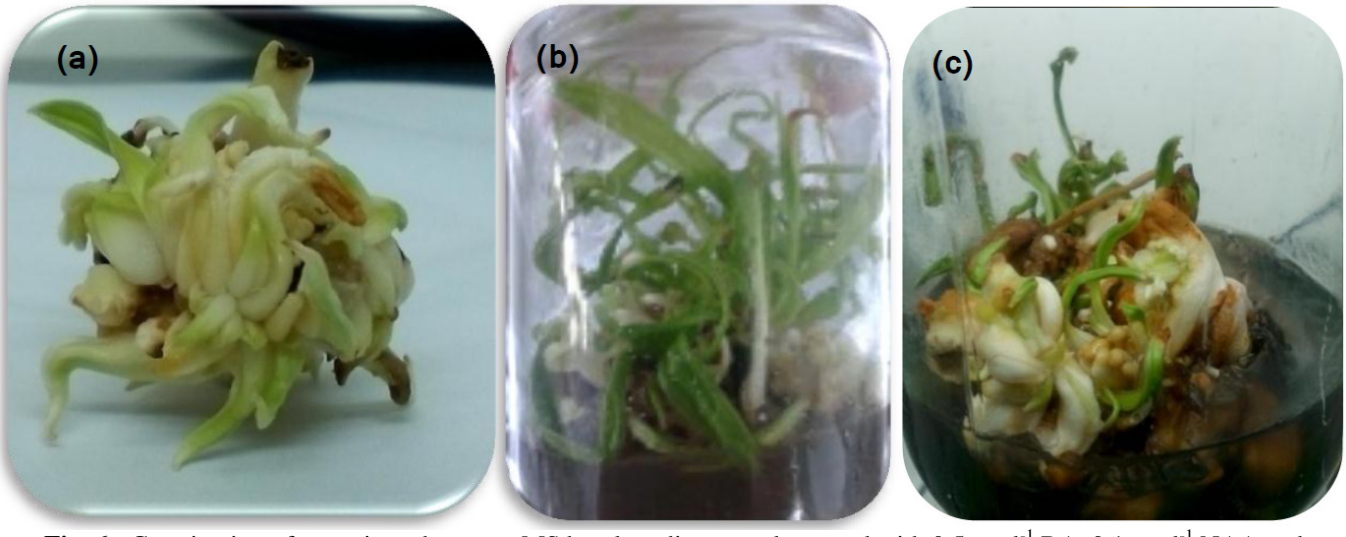

Fig. 6 : Germination of somatic embryos on MS basal medium supplemented with 0.5 mg l^{-1} BA, 0.1 mg l^{-1} NAA and concentrations of JLE (a) control, (b) 10.0 ml l^{-1} and (c) 30.0 ml l^{-1}, after three subcultures.

Shoot multiplication

In this stage, shoot clusters were cultured on MS basal medium supplemented with BA at 1.0, TDZ at 0.5 and NAA at 0.25 mg l^{-1}, with the addition of different concentrations of JLE, as shown in (Fig. 7). Our results showed that adding different concentrations of JLE to the culture medium had a significant effect on shoots regeneration.

The combination of 1.0 mg l^{-1} BA, 0.5 mg l^{-1} TDZ and 0.25 mg l^{-1} NAA with 10.0 ml l^{-1} JLE played an important role in improving and regulating the regeneration of shoots; where it recorded the highest number of shoots (25.2 shoot/jar), the highest number of leaves (56.0 leaf/jar) with the longest leaf length (5.0 cm), as shown in (Table 2). On the other hand, JLE appeared to be less effective when added in high concentrations. The results showed that adding JLE at 30.0 ml l^{-1} recorded the least number of shoots (2.3 shoot/jar), the least number of leaves (5.0 leaf/jar) and the shortest leaf length (2.0 cm) when compared to the control treatment. Moreover, the shoots were very weak and difficult to complete its growth.

Table 2 : Effect of BA at 1.0, TDZ at 0.5 and NAA at 0.25 (mgl^{-1}) with different concentrations of JLE on shoots regeneration after three subcultures.

Treatment (mll^{-1})	No. of shoots	No. of leaves	Leaf length (cm)
0.0	3.3 e	8.2 e	2.0 d
5.0	7.6 d	13.0 d	2.5 bc
10.0	25.2 a	56.0 a	5.0 a
15.0	15.0 b	24.3 b	3.0 b
20.0	10.7 c	18.6 c	2.0 d
30.0	2.3 f	5.0 f	2.0 d

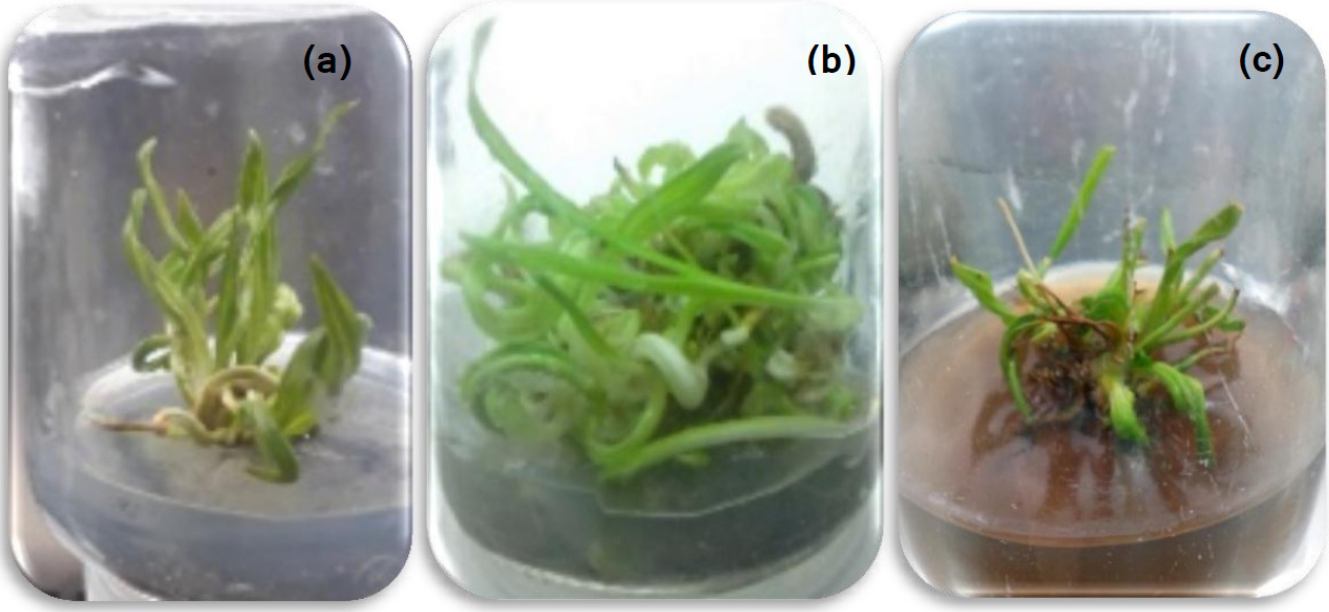

Fig. 7 : The effect of different concentrations of JLE (a) control, (b) 10.0 ml l^{-1} and (c) 30.0 mll^{-1} on multiplication of shoots after three subcultures.

Rooting stage

The regenerated shoots at lengths of 4–6 cm were cultured on ½ MS medium contained 1.0 mg l^{-1} NAA, as shown in **(Fig. 8)**, the low concentration of NAA initiated roots earlier as compared to high concentrations.

Fig. 8. Rooting of date palm shoots in MS basal medium supplemented with NAA at 1.0 mgl^{-1}.

Discussion

Comparing our findings with data from previous researches it was found that the protein content in jatropha leaves (26.14%) was greater than that recorded in Amaranth leaves (3.16%) as indicated by (Olumakaiye, 2011) and that recorded in raw *Moringa stenopetala* leaves (25.5%) as observed by (Abuye *et al.,* 2003). It can be considered a significant source of protein that could be used as an alternative to other protein sources (Hassan *et al.,* 2011). However, jatropha leaves content of carbohydrates (36.5%) was less than *Moringa stenopetala* leaves (39.5%) as recorded by (Abuye *et al.,* 2003). In addition, there were numerous mineral elements abundant in jatropha leaves which accelerate metabolic processes and promote growth and development (Bello *et al.,* 2008). On the other hand, the levels of essential amino acids in jatropha leaves were lower compared to those in other plants (Makkar *et al.,* 1998) which agreed with the findings of this research and in contrast to the findings of (Chigozie *et al.,* 2018) who observed that *Jatropha tanjorensis* leaves were abundant in essential amino acids.

In a number of earlier studies; numerous variables influenced the germination of somatic embryos such as; the size of somatic embryos (Al-Khayri and Bahrani, 2012) and tissue culture basal media (Boufis *et al.,* 2014). However, genotype influences embryo germination in palm trees as demonstrated by (Ibrahim *et al.,* 2012).

Based on the findings of (Zouine and Hadrami, 2007) who employed a combination of NAA, IBA and BA to promote somatic embryo germination in date palms; it was observed that plant growth regulators like cytokinins (BA, TDZ, kinetin or 2iP) are typical media supplements for the development of somatic embryos which increased the germination rate of tissues (Nguyen *et al.,* 2015). There are accumulating data confirming the addition of 0.5 mg l^{-1} NAA and 0.5 mg l^{-1} BA to date palm resulted in a greater proliferation of shoot buds (24.1 shoot buds/explant) such as that reported by (Mazri and Meziani, 2013) and (Mazri *et al.,* 2019) in whicha combination of NAA and BA at 5.0 µM each was observed to be optimal for the germination of date palm cv. Al-Fayda somatic embryos. However, high cytokinin concentrations induce unfavorable physiological conditions, such as hyperhydricity and tissue browning which restrict adventitious shoot bud multiplication and regeneration Khierallah and Bader, 2007; Aslam and Khan, 2009). Consequently, it is crucial to select the optimal cytokinin concentration in the culture medium.

Findings of this research demonstrated that the addition of BA and TDZ to the medium increases reproduction by speeding the phase of new shoot multiplication. As determined by (Bairu *et al.,* 2007) and (Aslam and Khan, 2009), BA is the most often employed cytokinin in micro-propagation methods because of its effectiveness and physiological significance in promoting enhanced bud proliferation. It is more potent than kinetin in inducing numerous shoots in date palm. Furthermore, TDZ encourages cells in the apical meristem to divide, proliferate and develop so that buds differentiate (Dei *et al.,* 2012).

The research work of (Fernandez *et al.,* 2000) demonstrated thatthe addition of auxins and cytokinins to a media is necessary for an *in vitro* morphogenetic response, such as somatic embryogenesis, organogenesis, or both. Some other authors argue that a combination of auxin and cytokinin is necessary because the exogenous plant growth regulators of these two categories alter endogenous hormone levels (Gaspar *et al.,* 2003). According to (Othmani *et al.,* 2009), exogenous plant growth regulators interact with endogenous ones to stimulate plant tissue development. When cv. Deglet Nour was cultivated on a medium supplemented with 1.0 mg l^{-1} NAA and 1.0 mg l^{-1} BA, substantial rates of shoot proliferation were observed.

Regeneration of shoot buds is influenced by many variables such as medium composition, genotype and plant growth regulators. The date palm cv. Maktoom generated the greatest shoots when grown in MS medium containing 1.0 mg l^{-1} NAA, 1.0 mg l^{-1} NOA, 4.0 mg l^{-1} 2iP and 2.0 mg l^{-1} BA, according to (Khierallah and Bader, 2007). After 3 months of growth on MS medium with 0.5 mg l^{-1} NOA and 0.5 mg l^{-1} Kin, date palm cv. Najda generated 23.5 shoot buds/explant, while cv. Hillawi cultivated on MS medium with 1.0 mg mg l^{-1} BA and 0.5 mg TDZ produced 18.2 buds/culture Mazri and Meziani, 2013; Al-Mayahi, 2014). To evaluate the multiplication rate of the Medjool cultivar, the shoots were grown on MS medium supplemented with 0.25 mg l^{-1} NAA, 0.5 mg l^{-1} TDZ, and varying doses of BA from which 1.0 mg l^{-1} resulted in the best significant results. Increasing BA from 1.0 to 2.0 mg l^{-1} significantly reduced the number of shoots (Taha *et al.,* 2021).

The experiments conducted in this study revealed that the addition of JLE at a dosage of 10 ml l^{-1} to MS medium coupled with plant growth regulators promoted the germination of somatic embryos and the proliferation of shoots. It may be possible to lessen the danger of genetic instability in plants by substituting plant growth stimulants with natural additives (Beshir *et al.,* 2012). Moreover, the use of plant-derived products may contribute significantly to the management of insect pests, while maintaining ecological stability, leaving no residues and having no adverse effects on animals and people (Machado *et al.,* 2007).

The leaves of jatropha are a great source of macro and micronutrients. It is rich in ash, accessible carbohydrates and crude protein (Bello *et al.,* 2019). It was about four-folds higher in crude protein content (26.14%) than that found by (El-Hammady, 1999) in *Ipomoea batatus* leaves (6.37%).

Plant phenolics are one of the most significant antioxidant and free radical terminator groups. Jatropha summer-leaves have the highest concentration of phenols, alkaloids, flavonoids, tannins, glycosides and saponins while winter-leaves have more phytic acid according to Khierallah and Bader (2007).

Tannins are phenolic substances associated with growth retardation and nutrient absorption issues, whereas tannins and flavonoids were shown to inhibit fungus growth Eke *et al.* (2013). In enough quantities and under favorable circumstances, phenolic acids exert allelopathic effects, even low quantities of phenolics in aqueous extracts of *J. curcas* observed to improve wheat seed germination and growth characteristics Bekheet *et al.* (2013). It may lead to the creation of novel, sustainable, environmentally friendly and commercially viable bio-regulators Saifullah and Tabassum (2012).

The leaf carbohydrate content was found to be much greater than that of the stem and root. Carbohydrates were utilized for energy and to aid digestion and absorption of other nutrients Lemos *et al.* (2009).

Furthermore, the mineral content of jatropha leaves was analyzed, and the presence of calcium, magnesium, potassium and sodium was determined (Bello *et al.,* 2019). According to Ma *et al.* (2011), jatropha leaves have the highest calcium content, which modulates nutrient transfer across cell walls. In addition, jatropha leaves have the greatest magnesium content, which is essential for the production of DNA and RNA during cell division Sanderson *et al.* (2013). Other determinations done by Chigozie *et al.* (2018) recorded that jatropha leaves are rich in vitamin A, deficient in vitamins B1, C and vitamin E.

Moreover, allelopathic effects may be found in plants and microbes, although in vegetables they are stronger. The allelopathic effect is a natural intervention in which a plant creates chemicals and metabolites that, when released, may be beneficial or detrimental to other plants/organisms Rejilia and Vijaykumar (2011), (Mongelli *et al.,* 1997). The allelopathic impact of plant leftovers, including leaves and roots, is greater than that of any other plant component.

The effect of linear decrease with increasing concentration of the extract is consistent with (Tomar and Agarwal, 2013), who worked with concentrations ranging from 0 to 100% of physical nut aqueous extract, and observed a negative effect, as well as morphological changes on the root with thickening and reduction of the absorption zone. In the same concern, Reichel *et al.* (2013) demonstrated that germination and initial growth of maize and tobacco are reduced when the concentration of aqueous extracts of Jatropha is raised owing to the presence of Azelaic acid in leaves and roots which inhibits germination at doses greater than 500 g/ml while aerial and root growth is hindered at quantities greater than 100 g/ml. However, (Igbinosa *et al.,* 2009) demonstrated that aqueous JLE (1, 5, 10 and 15%) had no deleterious impact on the germination of lettuce, whereas increasing the dose after germination greatly hindered the development of seedlings. Another bioassay conducted by Abugre and Sam (2010) showed that leaf extract inhibits the germination and growth of green pepper and sesame seedlings. Similarly, 100% suppression of wheat roots was reported at 10% *Jatropha macarentana* concentration (Maharjan *et al.,* 2007).

Triticum aestivum was negatively affected by JLE in terms of plant height, leaf area, biomass and spike length (Ashrafi *et al.,* 2008). In addition, they found greater quantities of phenols, phytic acid and free amino acids in the leaf filters than in the ovary extracts, which suggests a substantial interaction. (Goldfarb *et al.,* 2009) also showed that JLE interfered with the early development of *Triticum aestivum*. These investigations of jatropha leaf and root extracts demonstrate that they permanently impair crop germination and seedling development, with the amount of their impact varying with test yield and jatropha extract concentration.

The phytochemical analysis of *J. curcas* (leaves, root and stem bark extracts) revealed the presence of saponins, steroids, tannin glycosides, alkaloids and flavonoids. Limitations on germination and plant height may be due to these phenolic chemicals (Ubazi *et al.,* 2020).

On the other hand, (Baruah *et al.,* 2018) found that plant section had a significant influence on germination, feather length and root length. This was further reinforced by the findings of (Khattak *et al.,* 2015), which revealed that the leaf extract had the most favorable impact of allelopathic effect on seed germination. Similarly, bioassays and analysis of different extracts of the leaves and roots of *J. curcas* demonstrated that Azelaic acid is the primary allelopathic component. In addition, it was discovered that a larger concentration of this substance inhibited the spore and shoot development of the test crops.

It has been shown that low quantities of JLE accelerate the development of wheat seedlings (Tomar *et al.,* 2015). While, Abugre and Sam (2010) examined the effects of *Jatropha curcas* leaf extract on the germination and growth of*Capsicum annual* seedlings and found that when utilizing high quantities of the extract, germination and development of seedlings were inhibited; this may be owing to the high concentrations of allelochemicals present in the extracts as a result of the huge quantity of phenolic compounds in jatropha leaf extract. It is hypothesized that allelochemicals interact with enzymes involved in the mobilization of nutrients essential for germination and cell division, hence limiting seedling elongation Nadaletti *et al.* (2014).

The aqueous extract of jatropha had an effect on the number of leaves as well as the morphological properties of the roots, with the thickness, lack of an absorption area and dry weight decreasing dramatically with increasing concentration (Lemos *et al.*, 2009). The observed rise in features such as the fresh and dry weight of roots and leaves may be attributable to the kind of chemicals, functional group, chemical properties, and concentration of the leaf extract in the medium Bonamigo *et al.* (2009). Similar to the findings of Ubazi *et al.* (2020), it was determined that the restriction of pepper seeds germination and plant length was concentration-dependent, with the exception of Red (*Capsicum frutescens*), which recorded 75.0% (inhibition) at a 12% dose. Compared to *Vigna radiata*, (Baruah *et al.,* 2018) jatropha-extract had a less sensitive allelopathic effect

on *Capsicum annum* germination and seedling growth. Due to the study findings, it has been recommended that *Capsicum annum* and Jatropha be planted as a mixed crop. Compared to the control, Khattak *et al.* (2015) recorded that the stimulating effect of *J. curcas* extract on the percentage of wheat seed germination was greatest at low dosages while fresh weight and dry weight of shoots increased significantly. However, there was a positive and significant effect of jatropha aqueous extracts on cauliflower seedling growth, as indicated by the diameter, length of stem, length of leaf, area of leaf, fresh and dry weight of leaves and roots being positively influenced by high concentrations of jatropha aqueous extract as mentioned by (Nadaletti *et al.,* 2014).

On the contrary, Sanderson *et al.* (2013) found that increasing the concentration of *J. curcas* aqueous extract from 0% to 20% had no allelopathic effect on the lettuce root system. Whereas, Reichel *et al.* (2013) demonstrated that the aqueous extract of jatropha leaves at concentrations of 20, 25, 30 and 35 % enhanced the growth of wheat roots. Bonamigo *et al.* (2009) discovered that the administration of jatropha aqueous extract increased the average root length of *Brassica napus* seedlings. Furthermore, Ashrafi *et al.* (2008) found that low concentrations of jatropha aqueous extracts had no effect on the germination and growth of seedlings, but high concentrations diminished these activities.

The experiments conducted in the current study revealed that the addition of JLE at a dosage of 10 ml l^{-1} to MS medium supplemented with plant growth regulators promoted the germination of somatic embryos and the proliferation of shoots. It may be possible to lessen the danger of genetic instability in plants by substituting plant growth stimulants with natural additives (Beshir*et al.*, 2012).

The leaves of jatropha are rich in ash, crude protein and accessible carbohydrates (Bello *et al.*, 2019). This result was consistent with the findings of El-Hammady (1999), who observed that increasing the auxin concentration decreased root length, whereas applying 1.0 mg l^{-1} NAA promoted root formation. Khierallah and Bader (2007) reported that shoots cultured in a medium containing 1.0 mg l^{-1} NAA exhibited the highest rooting percentage of 90%. After 8 weeks in culture, there were 5.4 roots measuring 9.0 cm in length. On the other hand, Eke *et al.* (2013) reported that shoots grown in media supplemented with 0.1 mg l^{-1} NAA generated the most rooting. The results demonstrated that media supplemented with 1.0 mg l^{-1} NAA had the highest number of roots per shootlet (4.6 root/jar), indicating the effect of NAA concentrations on roots formation and confirming the findings of (Bekheet, 2013), who suggested that 1.0 mg l^{-1} NAA induced superior and optimal rooting. According to Saifullah and Tabassum (2012), NAA is the most commonly used exogenous hormone for the development of date palm roots.

Conclusion

Jatropha curcas leaf extract is a very important source of different substances to support embryogenesis germination. The optimum concentration of jatropha leaf extract is 10.0 ml l^{-1} and it's recommended as an additive to MS basal medium with plant growth regulators to enhance somatic embryo germination and offshoot regeneration.

Significance' statement

The leaves of *Jatropha curcas* are contained high amount of carbohydrate, protein, minerals, vitamins and amino acids, making jatropha leaves extract (JLE) a good provider of a variety of essential nutrients. However, there have been no previous studies on the utilization of jatropha extract as a natural source of nutrients in tissue culture. Starting from this point; this research was designed to fill this gap and to determine the optimal dosage of jatropha leaves extract to enhance somatic embryo development and shoot multiplication.

Key message

Jatropha leaves contained carbohydrates, proteins, minerals, vitamins and amino acids, making Jatropha leaves extract a good source of a variety of essential nutrients. As a result, we investigated the effect of jatropha extract as a natural nutrient on date palm development in vitro. Author contribution

I did this work with my team work.

Abbreviations:

JLE: jatropha leaves extract.

MS: Murashige and Skoog.

BA: Benzyl adenine.

NAA: Naphthalene acetic acid.

TDZ: Thidiazuron.

Eman H. Afifi, Marwa M. Abdalgaleel and Rabab W. El Aramany: Conducting tissue culture experiments.

Sayed, A. A. Elsayh and I. M. Shams El-Din: Write the initial data.

Rasha, N. Arafa and Ghada, A. Ali: write the final copy of the manuscript.

Tamer I. M. Ragab: statistical analysis.

Salwa El-Habashy and Emadeldin A.H. Ahmed: Reference collected.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Compliance with Ethical Standard

There was not ethical issues in this manuscript.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

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