

## ***In Vitro* Propagation of Date Palm (*Phoenix Dactylifera* L.) “Lakramat” Date Palms in Aswan Governorate**

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### **Abstract**

Lakramat cultivar is mainly cultivated in the Siwa Oasis. It is one of the promising Egyptian date palm cultivars, and its good traits make it appropriate for export. This paper describes the micropropagation process utilizing Lakramat cv. shoot tip explants. Explants were grown for 36 weeks on MS medium supplemented with picloram at 2.0 mg/l mixed with 2iP at 0.5 mg/l and 0.5 g/l AC for initiation stage. The obtained embryonic cultures were placed for further development and embryo formation in maturation media containing 0.25 mg/l picloram in combination with 2iP at 1.0 mg/l. The obtained somatic embryos were shifted to multiplication medium containing putrescine at the concentrations of 0, 50, 100, and 150 mg/l to study its effect on multiplication rate. Various levels of light intensity during germination stage were also investigated. Indeed, a new system of acclimatization was discussed. Date palm (*Phoenix dactylifera* L.) “Lakramat” direct globular somatic embryos spontaneously formed on shoot tip sections. The most successful somatic embryo multiplication was produced during treatment with 100 mg/l Putrescine. On the other side, the higher intensity (6000 lux) promoted germination and shoot formation. During rooting stage, the addition of 1.5 mg/l IBA and 1mg/l NAA in rooting medium achieved the highest significant root number. New system of acclimatization used in the present protocol surpassed the traditional methods of acclimatization. The maximum levels of ex vitro plant growth vigor, leaf number/plant, leaf width, root number, and root thickness were stimulated by irrigation with the MS full strength solution.

**Keywords:** Direct somatic embryogenesis, new system of acclimatization, *Phoenix dactylifera*, Putrescin.

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### **Introduction**

Recently, much attention has been paid to the genetic resources of domesticated plants and their wild counterparts. To fulfill future crop demand, genetic resources are unavoidable. For instance, they serve as a repository for new qualitative features or biotic and abiotic resistance genes. Due to a number of causes, including monoculture and urbanization, genetic diversity in clonally propagated crops, such as date palm, is declining. A perennial dioecious monocot plant species in the family Arecaceae is the date palm (*Phoenix dactylifera* L.). It is one of the first fruit crops to be widely planted in North Africa and the Middle East. It is also one of the most economically significant plants in desert and warm locations. The vegetative propagation of date palms generally uses offshoots. Tissue culture techniques, on the other hand, are the most popular technological approach for large-scale plant replication that is healthy and true to type (Sane *et al.*, 2006 and Johnson *et al.*, 2015). Lakramat cultivars are mainly cultivated in the Siwa Oasis. It is one of the

promising Egyptian date palm cultivars, and its good traits make it appropriate for export. The variety produces between 80 and 100 kg per palm tree. The fruit is large, 4.32 cm in length and 2.5 cm in width, fruit shape is ovate. Orange is the color of the fruit when it is in the khalal phase; when ripe, it turns brownish black. The flesh is low in fibre, tasty, and medium in thickness (El-Sharabasy and Rizk 2019) Semi dry. Somatic embryogenesis is viewed as a model for comprehending the biochemical and physiological alterations that take place during plant growth processes (Taha 2017). Rapid, extensive plant production has a great deal of potential with somatic embryogenesis. Date palms may be widely propagated with this approach (Fki *et al.*, 2003; Al-Khayri, 2005). Somatic embryogenesis has been exploited for date palm micropropagation from apical shoot tips and lateral buds for decades (Hassan and Taha 2012; Taha *et al.*, 2016), but recently it has been expedited from inflorescence (Taha *et al.*, 2021). Moreover, somatic embryos can develop either directly, starting with bud differentiation in the first instance, or indirectly, starting with callus induction (Jain 2012; Hassan *et al.*, 2016). In vitro growth, differentiation, and plant regeneration of the date palm depend heavily on the nutrient medium and exogenous application of plant growth regulators (PGRs) (Hapsoro *et al.*, 2020). The auxin 2, 4-dichlorophenoxy acetic acid (2,4-D) has been successfully used to stimulate somatic embryogenesis in date palms (Al-Khayri, 2010). High levels of 2,4-D, however, have reportedly been associated with the possibility of somaclonal variation in regenerants (Fki *et al.*, 2011). Therefore, using other auxins such as picloram and dicamba at low levels could be alternative auxins to induce somatic embryogenesis with no negative effects on regenerated plants (Abahmane, 2010). Picloram was used for induction of peach palm (Steinmacher *et al.*, 2007), African oil palm (Teixeira *et al.*, 1995), and areca nut palm (Karun *et al.*, 2004). In date palm, picloram has been used to induce somatic embryogenesis from inflorescence explants using 1.0 and 2.0 mg/l picloram with 2ip or 2ip+ BA (Hassan *et al.*, 2021). Polyamines have been characterized as crucial induction signals in plants during the somatic embryogenesis process. For various species, the effects of polyamines on somatic embryogenesis have been documented (Silveira *et al.*, 2006 and Wu *et al.*, 2009). Small, polycationic aliphatic compounds with amino groups called polyamines have been thought of as a type of plant growth regulators. They can interact electrostatically with macromolecules like proteins, phospholipids, cell wall components, and nucleic acids (Baron and Stasolla 2008; Tiburcio *et al.*, 2014). The primary polyamines in plants are putrescine, spermidine, and spermine, and studies have linked them to the control of physiological processes such organogenesis, embryogenesis, floral development, senescence, fruit maturation and development, as well as reactions to biotic and abiotic stresses (Ahmad *et al.*, 2012). Many plants grown in vitro still encounter a substantial obstacle after being transplanted. In vitro-grown plantlets have been continuously exposed to a special, stress-free aseptic milieu. Plantlets were cultivated in test tubes or flasks under carefully regulated circumstances, such as high humidity, low light, asepsis, and environments rich in sugar and nutrients that promoted heterotrophic development (Phillips and Garda, 2019). If these plantlets are continuously cultivated in such a situation, they will eventually have aberrant morphology, anatomy, and physiology. When planted immediately in a glasshouse, these plantlets generate culture-induced phenotype that is

unable to resist the surrounding environmental conditions. The plantlets in this environment transition from a heterotrophic to an autotrophic metabolism, which makes them especially susceptible to environmental conditions including disease and climate (Mahendra *et al.*, 2020). In vitro recalcitrance severely hinders the production of many economically valuable crop species (Bidabadi and Jain, 2020). However there has been a lot of study done to make the in vitro environment better. How to adapt plants cultivated in vitro to the ex vitro soil environment, however, is not well understood. The process by which a plant acclimates to the climatic or environmental circumstances of a new home is known as acclimatization. It is the final and most important step in the tissue culture process and the basis for effective plant propagation (Hazarika, 2003). Before transitioning to an in vivo environment, plantlets must correct their morphological, anatomical, and physiological defects after being moved from an in vitro to an ex vitro environment. As a tool for sustainable development in the agricultural sector, innovative technologies are becoming more and more necessary (Mozas-Moral *et al.*, 2020). Automation in agriculture has improved environmental sustainability, decreased labor costs, and increased product quality. Agricultural output was greatly increased by automated farming equipment, glasshouse climate control, and irrigation systems, soil fertility, and pest and disease management (Rehman *et al.*, 2022). In vitro propagation requires a great deal of repetitive work and takes a lot of time, especially if production is limited during the acclimation period due to plantlet death. Automation is a realistic solution in this situation. Two further benefits of automating the acclimation process are reduced contamination risk and personnel cost (Huang and Lee, 2010). The goal of the current work is to improve direct somatic embryogenesis in date palm (*Phoenix dactylifera* L.) cv. Lakramat by utilizing shoot tip explants. Numerous physiological processes, including the development of embryogenic competence and somatic embryogenesis multiplication, have been linked to picloram and polyamine (putrescine) concentrations control. Also, we examined the impacts of light intensity levels on shoot formation. IBA and NAA combination was tested for good root formation. A new system for acclimatization process was also applied to maximize survival percentage under greenhouse conditions.

## Materials and methods

This research was carried out in tissue culture laboratory of date palm researches and production Lab. of Plant Genetic Resources Department, Desert Research Center, Egypt.

The research was done as follow:

### Explant source

Female field-grown date palm Lakramat trees with offshoots that are two to three years old and have high-quality fruits are grown in the Siwa Oasis. Offshoots were carefully isolated and then moved right away to the lab.

### Surface sterilization and explant preparation

The first stage of preparation involved removing exterior leaves, fibrous material, and roots. For 30 minutes, shoot tips that were between 3 and 5 cm wide and 7 to 10 cm long were washed with soap and running water. In order to reduce tissue browning, the shoot tips were then maintained in an antioxidant solution containing 150 mg/l each of citric and ascorbic acid for an hour at 4-5° C. Explants were sterilized by immersing them in a solution of 3% sodium hypochlorite and 1-2 drops of tween 20 for 25 minutes while vigorously shaking the container. This was followed by three rinses with distilled water that had been sterilized.

### Nutrient medium composition and culture conditions

Sterilized shoot tips were divided longitudinally into several parts and grown on induction medium consists of MS formula amended with 2.0 mg/l picloram + 0.5 mg/l 2iP, 100 mg/l glutamine, 2.0 g/l polyvinylpyrrolidone (PVP), 30 g/l sucrose, 0.5 g/l activated charcoal and solidified with 2 g/l gelrite. All cultured explants were kept in a controlled growth room at 27 ± 2°C under dark condition for 36 weeks with regular re-culture to fresh medium (6 weeks intervals).

### Embryo formation

The emergence of embryonic culture ( somatic embryo at globular stage) took place in most cultured explants after 36 weeks, then responded explants were shifted to maturation medium contains 0.25 mg/l Picloram + 1mg/l 2ip for 6 weeks, kept in complete darkness and re-cultured three times to form somatic embryo clusters.

### Multiplication medium

#### Effect of putrescine on embryos multiplication rate and growth value

Clusters of embryos from the maturation stage (each one contains 2-3 embryos) were grown on half-strength MS medium supplemented with 0.1 mg/l NAA+ 0.2 mg/l ABA and 0.25 mg/l BA ( Hassan *et al.*, 2021) along with putrescine at the concentrations of 0, 50, 100, and 150 mg/l to study its effect on multiplication rate. All culture media were dispensed at 30 ml to glass jars (200 ml). Cultured jars were kept for three re-cultures at three-week intervals in low light conditions (500 lux). After re-cultures we evaluated the quantities of new embryos/culture, germinated embryo/culture, culture fresh weight and growth value. Growth value (GV), GV is calculated according to the formula of Ziv (1992):

$$\frac{(FwF-Fwi)}{Fwi}$$

### Germination stage

#### The effect of light intensity

Cluster of matured embryos (2-3 embryo/ cluster) were cultured on MS medium containing 0.1 mg/l NAA + 0.05 mg/l BA + 2 g/l gelrite and incubated at different light intensities (500, 1000, 2000 and 4000 lux) to record light intensity effects on secondary embryo, germinated embryo (shoot) and vitrified shoots.

### Rooting stage

Plantlets of Lakramat cv. 5-7 cm in length resulted from germination stage were cultured on solid half strength MS rooting medium supplemented with IBA at 0.0, 0.5, 1.5 and 2.0 mg/l, each concentration was combined with NAA at 0.0 and 1.0 mg/l to study their impacts on root formation. The media were supplemented with 0.5 g/l activated charcoal, solidified with 6 g/l

agar and dispensed into test tubes 2.5×25 cm. at rate of 25 ml /tube. Mean values of roots number, root length, root thickness and plantlets length were recorded after 3 months (1.5 month interval). Root thickness was estimated visually by degree according to Pottino, as followed: thin (1), low thick (2), average thick (3) and thick (4).

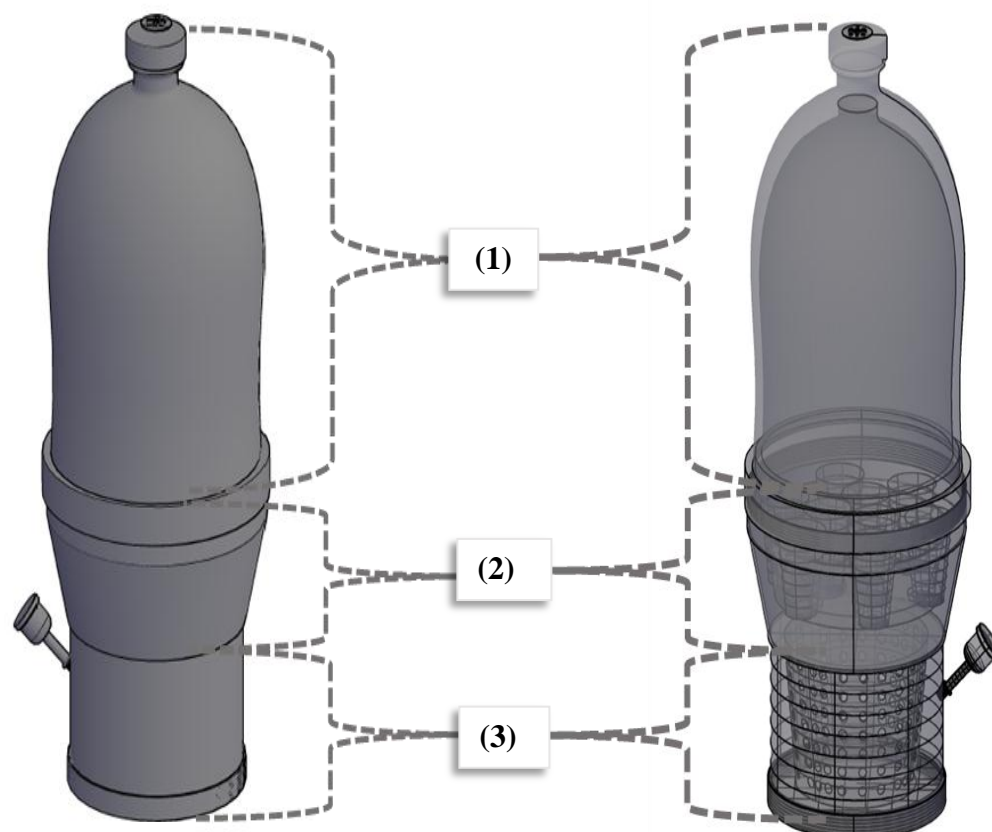
**Acclimatization process/ this stage include two experiments:**

**First experiment: Effect of acclimatization system**

To validate the performance of new system for the improvement of the plants' survival rate and their morpho-physiological characteristics, we set up this experiment to compare the growth of plants using new acclimatization system (Fig.1) with the plants acclimatized traditionally using plastic pots covered with a white polyethylene or under plastic tunnel. All acclimatization treatments were kept at greenhouse conditions.

Vigorous shoots (10–12 cm) with 1-3 roots and 2-3 leaves were transferred to a greenhouse. From test tubes, rooted plantlets were carefully removed, and they were then washed with tap water. For 20 minutes, plantlets were submerged in a 1% (w/v) fungicide solution (Moncut 25%). After that, the plantlets were cultured on acclimatization treatments. All plants in different treatments were cultured on planting medium consists of peat: perlite v/v 2:1

After 3 months, the percentage of plant mortality, plant survival, leaf number and growth vigor in the environmental compartments were recorded. Growth vigor was estimated visually according to Pottino's rate of scaling (1981) the following scores were assigned: poor performance = 1, below average = 2, average = 3, above average = 4 and excellent = 5



**Fig. (1):** shows the multi-layer device; (A) External details of the device, (B) X-ray view of the device showing the internal details, (1) the first layer of the device (the shoot system growth zone), (2) the second layer of the device (the root system growth zone), (3) the third layer (tank zone).

### **The new in system**

The system creates conditions that are similar to those inside planting containers in *in vitro* conditions, especially the degree of humidity, which is the determining factor for the success of the acclimatization process. As a result, the system provides protection for the plantlets during the acclimatization process. The system also provides soil-like circumstances that are similar to *ex vitro* conditions, making it a transitional stage (incubator) between *in vitro* and *ex vitro* environments. All of this is accomplished without the use of energy-intensive machinery or the need to remove the plants' shock-protecting covering. Because the system may be utilized in an uncontrolled environment, it combines all the benefits of earlier acclimatization systems and eliminates all their drawbacks. The invention is a brand-new, creative method for acclimating date palm plantlets. It is distinguished by being cost-effective when compared to other conventional methods that do not require any energy to deliver water and nutrients to plantlets, as well as being highly efficient in acclimatization and water and nutrient consumption.

### **Exploitation method**

The adaptation of *in vitro* propagated plantlets, which is then employed to maintain plantlets survival and avoid their loss, is the focus of technological innovation. The invention is a multi-layer technique used to acclimatize tissue culture plantlets, especially date palms, which are among the hardest plants to do so. When the plantlets are placed in deep torpedo pots with a



suction porous wick that draws water and nutrients from the tank below to the plantlets, controlled humidity and an appropriate temperature are provided, allowing the plantlets to survive.

### Detailed description

As shown in Fig. 2, this invention relates to a multi-layer device for acclimatization and adaptation of date palm plantlets propagated under *in vitro* conditions to *ex vitro* conditions. The invention consists of three layers as follows: -

#### 1. The first layer (the shoot system growth zone)

This clear cover has a diameter of 40 cm and a height of 50 cm; it resembles a water bottle but lacks the bottom base and has a fastening area (screw) for securing to the lower part. The section of the container where the shoot system develops has a humidity-regulating opening at the top.

#### 2. The second layer (the root system growth zone)

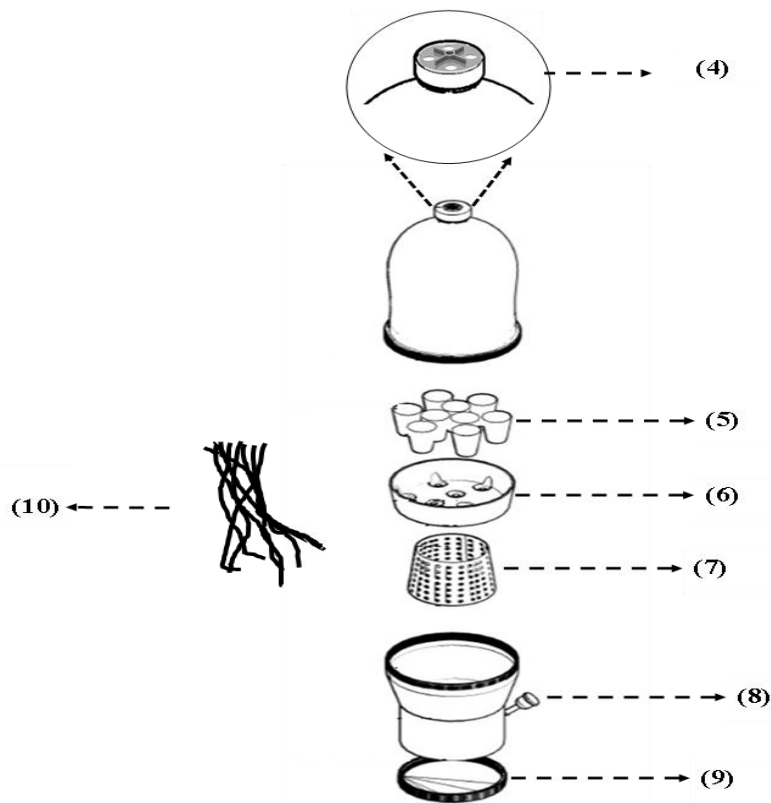
It consists of an external component, the device's body, which encloses the planting pots, soil, and the pots holder.

#### 3. The third layer (water and nutrient storage zone)

That is made up of an exterior component, the device's body, an interior component, an open-ended cylinder that serves as the pots' holder, as well as a bottom cover.

### Method of operation

Building the apparatus as depicted in Fig (1). Connected the tank's bottom cap serves as the device's base (9). (8) The funnel was used to attach the device's body to the water and nutrient inlet. In the water and nutrient storage zone, position the stand that holds up the open-ended cylinder that serves as the pot's holder (3). Positioning the pots' installation base (6) so, that it is supported by the pot's holder (7). One section of the suction porous wick (10) is introduced via a hole in the bottom of the pot (5) to the third layer (3), where it ingests water, while the other portion remains in the second layer (2). Putting planting mixture in the second layer (2) (agricultural soil). Using the funnel (8), fill the tank (3) with water until it is two-thirds full (about 3 to 4 liters). Next, from the top, pour about 2 liters of water onto the soil in the second layer (2). Cover the device with the transparent cover (1) and enclose it in the second layer (2) with the humidity regulation hole closed (4). Allow the device to reach moisture saturation in the first layer (1) after two days. On the third day, the planting process is completed by removing the transparent cover and planting one plantlet in each pot (5). The transparent cover is then replaced, and the second layer (2) is secured with the humidity regulation hole closed (4). The plantlets inside the gadget last for 5 months (1) without having to remove the transparent cover, with humidity being controlled by a top humidity regulating hole (4). The plantlets stay exposed in the device for a full year once the lid is totally removed after 5 months, when fertilization starts through the funnel (8) place plants in large pots a year after planting.



**Fig. (2):** shows the parts of the device separately and the details of each piece separately: (4) Shows humidity regulating hole in the device cover, (5) shows planting pots (root incubator), which are deep torpedo pots, about 18 cm deep and 5 cm in diameter, perforated from the bottom and the side, (6) shows a base with wells in which the planting pots are fixed, for each pot has a special hole in which it is fixed (7) shows a stand to support the base that holds the planting pots, (8) shows the inlet of water and nutrients (the funnel), (9) Shows the device base (tank bottom cover), (10) shows a suction porous wick it is used to transfer a water and nutrient medium from the third layer (tank zone) to the second layer (the root system zone).

### Second experiment

Successfully acclimatized plants aged 3 months were irrigated twice-a week with MS solution at full, half and quarter strength for 4 months and the following data were recorded; leaf number, leaf width, root number and plant length.

### Statistical analysis

Complete randomization was used to arrange the treatments, and each treatment was reproduced three times with five (jars and test tubes in rooting stage) and one explant in each. According to Snedecor and Cochran's technique, means were compared.



## RESULTS

The present work was resulted in successful large scale *in vitro* propagation protocol of date palm cv. Lakramat using shoot tip explants. Most explants responded well to the MS induction medium.

### Multiplication stage

#### Effect of Putrescine on Embryos Multiplication Rate and Growth Value

Results shown in (Table 1 and Fig. 3e) showed that after 9 weeks of incubation, somatic embryos cultured on medium with putrescine (100 mg/l) scored the highest significant values of multiplication rate and growth value in comparison to the other studied concentrations, namely 0, 50, and 150 mg/l. Moreover, putrescine at increased concentration (150 mg/l) had a significant reversible influence on all development parameters. On the other hand; germinated embryo number was higher on control treatment. While, increasing putrescine concentration in culture media reduced gradually germinated embryo number.

**Table (1):** Effects of putrescine on embryos multiplication rate and growth value of date palm “Lakramat” embryos cultured *in vitro* for 6 weeks

Treatment (mg/l)	No. of embryos	Germinated embryo	Culture fresh weight (g)		Growth value
			Started	Produced	
0.00	18 <sup>d</sup>	12 <sup>b</sup>	0.51 <sup>d</sup>	1.8 <sup>d</sup>	2.5 <sup>d</sup>
50	23.2 <sup>c</sup>	15 <sup>a</sup>	0.64 <sup>a</sup>	2.7 <sup>c</sup>	3.21 <sup>c</sup>
100	27 <sup>a</sup>	13 <sup>b</sup>	0.53 <sup>c</sup>	3.4 <sup>a</sup>	5.00 <sup>a</sup>
150	21 <sup>b</sup>	9 <sup>c</sup>	0.6 <sup>b</sup>	2.1 <sup>b</sup>	3.85 <sup>b</sup>

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

### Germination stage

#### The effect of light intensity on embryo growth

Data presented in Table (2) indicated the impact of light intensity on embryo cultures of date palm cv. Lakramat. It is noticed that, incubating date palm embryo cultures on low light intensity achieved the highest significant value of secondary embryo number (9 embryo/culture). While gradual increase in light intensity to 4000 lux decreased significantly secondary embryo number. Meanwhile, increasing light intensity enhanced significantly number of germinated embryos and higher intensity surpassed other treatments (Fig.3f). It is obvious from data in this table that low light intensity has a significant effect on number of vitrified shoots as the highest significant number was noticed with low intensity.

**Table (2):** Effect of light intensity on embryo, germinated embryo and vitrified shoot numbers during germination stage

Light intensity (Lux)	Number of embryos	Number of germinated embryo	Number of vitrified shoots
500	9.66 <sup>a</sup>	9.00 <sup>d</sup>	4.16 <sup>a</sup>
1000	8.00 <sup>b</sup>	25.00 <sup>c</sup>	3.00 <sup>b</sup>
2000	6.00 <sup>c</sup>	42.33 <sup>b</sup>	2.00 <sup>c</sup>
4000	3.00 <sup>d</sup>	64.00 <sup>a</sup>	1.00 <sup>d</sup>

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

### Rooting stage

Data in Table 3 showed the effect of IBA and NAA on plant growth during rooting stage which expressed as root numbers, length, thickness and plant length. It is clear that the addition of 1.5 mg/l IBA and 1mg/l NAA in rooting medium encouraged the highest significant root number/ shoot (8.00) (**Fig.3h**), followed significantly by the addition of 1mg/l from both (6.33). While control treatment showed the lowest significant number (1). In addition, roots mean length and root thickness were higher in rooting medium supplemented with 1.5 mg/l IBA + 1 mg/l NAA followed significantly by other treatments. The lowest averages were related to the control treatment. It is noticed from this table that the presence of auxin in culture media is necessary to increase plant length during rooting stage, as all treatments caused a significant increase compared with control and the highest length was achieved in rooting medium containing 1.5 mg/l IBA and 1 mg/l NAA (10.63 cm).

**Table (3):** Effect of IBA and NAA on root formation of date palm cv. Lakramat plantlets

Auxin conc. (mg/l)		Number of root	Mean length of roots (cm)	Root thickness	Mean plant length (cm)
IBA	NAA				
0.0	0.0	1.00 <sup>h</sup>	2.00 <sup>f</sup>	0.70 <sup>g</sup>	7.43 <sup>e</sup>
0.5	0.0	1.66 <sup>g</sup>	3.60 <sup>e</sup>	0.96 <sup>f</sup>	8.30 <sup>d</sup>
1.0	0.0	3.00 <sup>f</sup>	4.50 <sup>d</sup>	1.33 <sup>e</sup>	9.23 <sup>c</sup>
1.5	0.0	5.66 <sup>c</sup>	6.10 <sup>b</sup>	1.56 <sup>d</sup>	9.46 <sup>c</sup>
2.0	0.0	4.33 <sup>e</sup>	5.30 <sup>c</sup>	1.40 <sup>e</sup>	8.33 <sup>d</sup>
0.5	1.00	3.00 <sup>f</sup>	3.20 <sup>e</sup>	1.26 <sup>e</sup>	8.10 <sup>d</sup>
1.0	1.00	6.33 <sup>b</sup>	5.00 <sup>c</sup>	1.96 <sup>c</sup>	10.06 <sup>b</sup>
1.5	1.00	8.00 <sup>a</sup>	6.70 <sup>a</sup>	2.86 <sup>a</sup>	10.63 <sup>a</sup>
2.0	1.00	5.00 <sup>d</sup>	6.00 <sup>b</sup>	2.53 <sup>b</sup>	10.00 <sup>b</sup>

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

### Acclimatization stage

#### Effect of acclimatization treatments

Healthy rooted shoots were transferred to the greenhouse for acclimatization process. Plants moved to the new system environment had highest significant values of survival percentage (95%), leaf number/ plant and plant growth vigor after 3 months, significantly followed by the the plants acclimatized under tunnel treatments. On the other hand, plants moved to the plastic pots covered with a white polyethylene environment recorded the lowest significant values of all tested parameters.

**Table (4):** Effect of acclimatization methods on survival percentage, leaf number and plant growth vigor after 3 months of acclimatization

Acclimatization method	Mortality	Survival percentage	leaf number	Growth vigor
New system	5 <sup>c</sup>	95 <sup>a</sup>	4.7 <sup>a</sup>	5 <sup>a</sup>
Plastic tunnel in greenhouse	29 <sup>b</sup>	71 <sup>b</sup>	4.3 <sup>b</sup>	4 <sup>b</sup>
Plastic pots covered with a white polyethylene	43.33 <sup>a</sup>	56.67 <sup>c</sup>	3.7 <sup>c</sup>	3.2 <sup>c</sup>

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

Table 5 and Fig. 4 show clearly that changing the MS strength, which is utilized as a fertilizer solution for date palm plants during the acclimation period, has a significant impact on the majority of parameter measurements. The most significant leaf number/plant was observed with MS full strength, followed by MS half strength insignificantly, while the lowest leaf number was observed with quarter strength,. The maximum leaf width was produced by the full-strength solution, which was significantly followed by the other strengths in that order. Full strength MS produced the longest significant plant length, with half strength MS producing the smallest significant plant length. From Table 4's data, it can be inferred that irrigation with full-strength solution resulted in the highest significant mean values of growth vigor degree and root number. The lowest values, however, were found in the 1/4 strength solution.

**Table (5):** Effect of Murashige and Skooge salts (MS) solution strength on growth measurements of date palm plants after 24 weeks in greenhouse conditions

MS strength (cm)	Leaf number	Leaf width	Plant length (cm)	Root number	Growth vigor
¼ MS	4.3 <sup>c</sup>	1.5 <sup>c</sup>	30 <sup>c</sup>	3.4 <sup>c</sup>	3.5 <sup>c</sup>
½ MS	5.1 <sup>b</sup>	2.1 <sup>b</sup>	32 <sup>b</sup>	5.1 <sup>b</sup>	4.3 <sup>b</sup>
Full MS	5.7 <sup>a</sup>	2.5 <sup>a</sup>	36 <sup>a</sup>	7.1 <sup>a</sup>	5.0 <sup>a</sup>

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

## Discussion

Through SE or organogenesis, plant cells have the capacity for totipotent growth to produce a new plantlet. By exposing a variety of explants to suitable growth conditions, SE can be induced in vitro (Yang and Zhang, 2010; Horstman *et al.*, 2017). The use of SE as a method for plant species' propagation in place of seed reproduction has increased recently (Ipekci and Gozukirmizi, 2003; Li *et al.*, 2012), particularly for plants with incredibly low reproduction rates or sustaining agronomic features. Exogenous polyamines have been investigated as growth regulators in tissue culture and as stress-relieving agents in plants or seeds of various species. They have been evaluated in in vitro cultures on somatic embryos (Chiancone *et al.*, 2006; Paul *et al.*, 2009), organogenesis induction (Viu *et al.*, 2009; Arun *et al.*, 2014), as well as during plant development under various stressors (Yin *et al.*, 2014; Hu *et al.*, 2012). Use of 100 mg/l during treatment for the purpose of somatic embryo multiplication, putrescine demonstrated the best results. A high intracellular content of free putrescine produced by the incorporation of exogenous putrescine may be required to induce the best results during the maturation of somatic embryos in sugarcane. Due to the presence of putrescine, which is abundant, endogenous polyamines appear to display patterns that are similar across a number of species when somatic embryogenesis is induced. In cultures of *Araucaria angustifolia* under pre-maturation treatment in culture medium containing polyethylene glycol and maltose as osmotic agents, Farias-Soares *et al.* (2014) reported higher contents of polyamines and higher conversion rates of pro-embryogenic masses to somatic embryos, with putrescine observed at higher contents. Hegazy (2008) observed that embryos cultured on modified MS medium in addition to putrescine (100 mg/l) gained substantial values of multiplication rate and growth

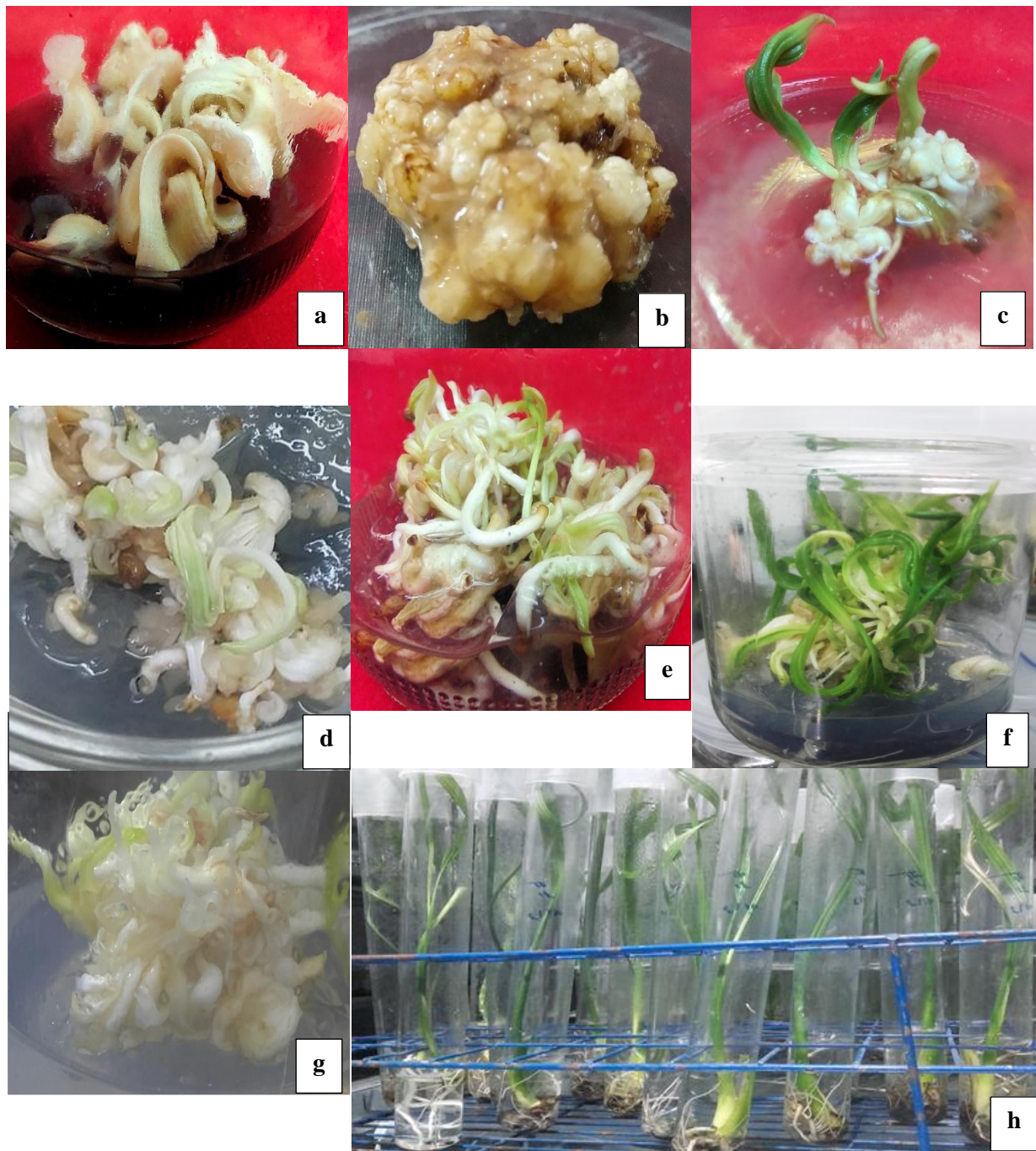
value as well as total soluble protein and phenylalanine ammonia-lyase (PAL) activity on date palm floral buds of the variety "Selmy." In numerous plant systems, auxins, cytokinins, and gibberellins have an impact on the metabolism of PAs, and Srivastava (2002) found that PAs are crucial for many of the growth responses associated with these hormones. Environmental conditions like light, temperature, culture vessels and density of the medium have clear impact on somatic embryogenesis. In general, light regulates physiological processes such as metabolism, gene expression, photosynthesis and morphogenesis (George and Davies, 2008). Moreover, light quality, exposure period and light intensity are very important, and plant species varied in their light requirements needed for optimal growth (Miler *et al.*, 2019). In fact, *in vitro* morphogenesis of most plant species has been affected by the light intensity (Singh and Patel, 2014). Results of the present investigation morphogenesis of Lakramat somatic embryo cultures expressed as secondary embryo and germinated embryos could be explored by using different light intensities. Low light intensities stimulated multiplication process while higher intensity improved germination. At the same direction, Ibrahim and Hassan (2006) induced direct somatic embryo formation of date palm dry cultivar inflorescence under low light intensities (500 and 1000 lux). Meziani *et al.* (2015) showed that high light intensities (2,000 - 3,000 lux) decreased shoot bud proliferation and stimulated shoot length and greening.

*In vitro* rooting of date palm is controlled by nutrient media types, carbohydrate source, activated charcoal and PGRs exogenous application especially auxins (Hassan *et al.*, 2005). Based on the aforementioned results, data showed that the addition of different auxins in various combinations achieved higher values of root number, root length and root thickness and the best treatment was 1.5 mg/l IBA and 1.0 mg/l NAA compared with control treatment. On the same direction, IBA is an auxin precursor; it may work to control IAA levels through –oxidation. By controlling adventitious root growth, oxidation can occasionally irreversibly inactivate this conjugation mechanism (Aryal *et al.*, 2019). IBA causes alterations in the metabolism of enzymes, carbohydrates, RNA, DNA, and proteins in the rooting zone during external contact with the cell, which may either restrict or enhance root growth, particularly during cell division and differentiation. Due to its nontoxicity at a variety of concentrations, indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) are the most commonly utilized root-promoting chemicals in the nursery industry (Kentelky *et al.*, 2021). Moreover, Mazri and Meziani (2013) found that the highest value of root formation of date palm cv. Najda shoot was noticed on medium contained NAA at 1.0 mg/l and 0.5 mg/l NAA when compared to hormone free medium. Arafa (2020) showed that maximize root number of date palm shoots by the addition of NAA at 1.0 mg/l to culture medium. Moreover, since low light intensity, low osmotic and water potentials in the medium, limited carbon dioxide and oxygen gas exchange, high concentrations of micro- and macronutrients, RH and sugar content, plant growth regulators, and low light intensity are all elements that benefit *in vitro* growing plants (Mahendra *et al.*, 2020). High proliferation rates are achieved by these factors, but they also typically lead to physiological, anatomical, and morphological issues that hinder *ex vitro* acclimation and lower plant survival rates (Ruffoni *et al.*, 2013). Plant tissue culture microenvironment variables like temperature, RH, and light are regulated using Internet of

Things-based automation technologies (Widiawan *et al.*, 2021). However only a little amount of study has been done on the application of this technique at the plantlet acclimation stage, where the death rate is quite significant. Effective commercial *in vitro* propagation requires a combination of factors, including high plant proliferation rates during the *in vitro* multiplication stage, high plant survival rates, and some level of automation (Lee *et al.*, 2019). The optimum environment is set up by a successful acclimatization. The findings of the present study showed that plantlets transferred from *in vitro* conditions to new system for acclimatization had a lower mortality rate, higher survival percentage, maximum growth value and leaf number than plastic pots covered with a white polyethylene or plastic tunnel in greenhouse. Similarly, the morpho-physiological characteristics of the plantlets also improved in new system. This indicates that if appropriate automated technology is not followed, plantlets may desiccate quickly and die due to the environmental changes when they are transferred from an *in vitro* culture room to an *ex vitro* greenhouse (Kumar and Rao, 2016).

Full MS strength fertilizer solution utilized throughout the date palm plants' acclimation stage had a significant impact on the majority of parameter measurements in this study. A later technique proved successful in producing micropropagated date palm plantlets in huge quantities. Hoagland solution improved plant height, leaf width, stem base diameter, chlorophyll A and B, carotenoids, and total indoles, among other indices of growth and development (Hassan, 2017).





**Fig. 3:** *In vitro* regeneration of Lakramat cv. a shoot tip explants cultured on 2.0 mg/l picloram + 0.5 mg/l 2iP /l; b Globular embryo formation; c and d Regeneration proceedings of somatic embryos; e Multiplication stage as vigorous secondary embryos during treatment with 100 mg/l putrescine, f Higher intensity (6000 lux) promoted germination and shoot formation; g Vitrified shoots in low light intensity and h Well rooted plants in rooting medium contains 1.5 mg/l IBA and 1mg/l NAA.



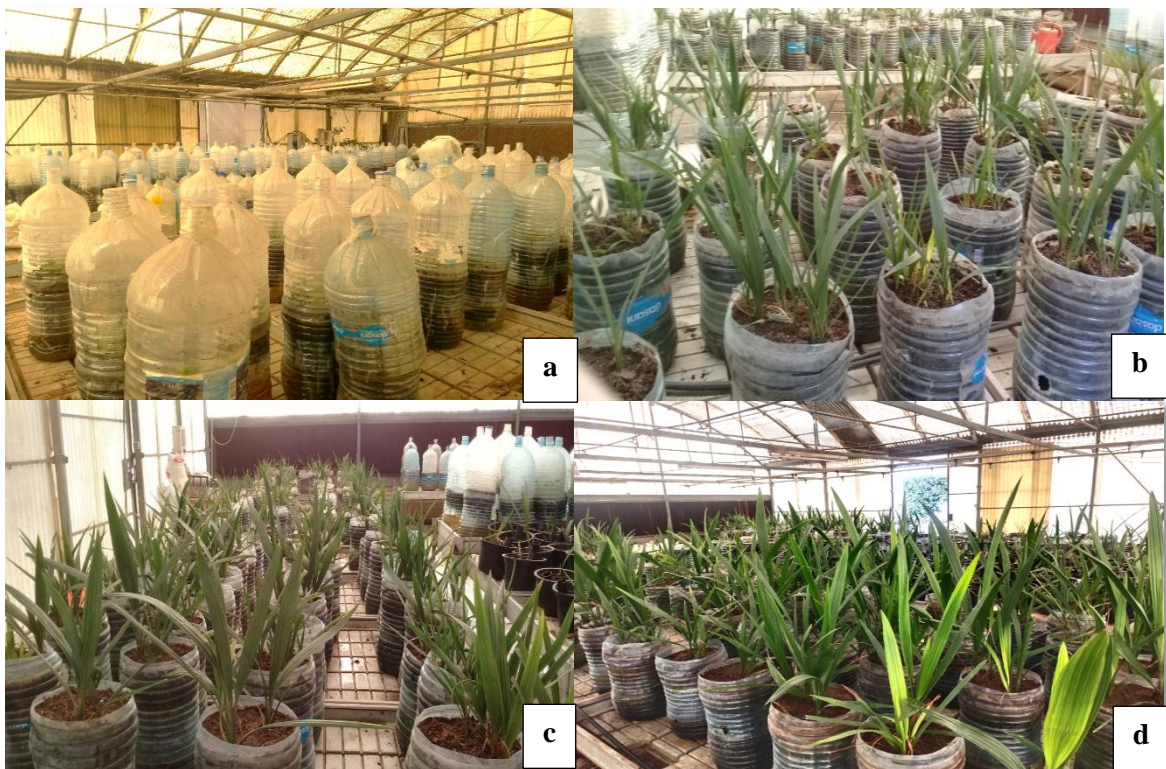


**Full MS**

**1/2 MS**

**1/4 MS**

**Fig. 4:** Effect of MS strength on growth vigor after four months under greenhouse conditions



**Fig. 5:** Advanced protocol for date palm acclimatization. a Acclimatized plants after 1 week irrigated with full MS strength; b Acclimatized plants after 24 week; c Acclimatized plants after 36 week; d Acclimatized plants after 40 week

## Conclusions

An effective methodology for plant large-scale production is provided by the current work. Picloram is required for the creation of somatic embryos at the right concentration during the initiation and maturation stages. Moreover, putrescine was used to create a high multiplication rate. At the germination stage, increasing light intensity improved healthy shoot emergence. The current methodology further emphasized the significance of auxin concentration and combination during the rooting stage. In fact, the innovative acclimatisation method employed here has a great deal of promise to solve acclimatization issues and increase the survival rate. As a tool for sustainable development in the agricultural sector, innovative technologies are becoming more and more necessary. Automation in agriculture has improved environmental sustainability, decreased labor costs, and increased product quality. Agricultural output was greatly increased by automated farming equipment, glasshouse climate control, irrigation and fertigation systems, soil fertility, and pest and disease management. In vitro propagation requires a great deal of repetitive work and takes a lot of time, especially if production is limited during the acclimation period due to plantlet death. Automation is a realistic solution in this situation. Two further benefits of automating the acclimation process are reduced contamination risk and personnel cost.

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## الإكثار المعلمي لنخيل البلح صنف (لاكرامت)

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### الملخص العربي

يزرع نخيل التمر صنف لاکرامت بشكل رئيسي في واحة سيوة. هذا ويعتبر لاکرامت من أصناف نخيل التمر المصرية الواعدة، وخصائصه الجيدة تجعله مناسباً للتصدير. توضح هذه الدراسة عملية الإكثار الدقيق لصنف لاکرامت بطريقة مباشرة أي بدون تكوين الكالس وذلك باستخدام القمة النامية كمنفصل نباتي. تم زراعة المنفصلات النباتية لمدة ٣٦ أسبوع علي بيئة مورايشيچ وسكوج MS والمحتوية علي كلاً من picloram بتركيز ٢,٠٠ ملجم/لتر وكذلك ٢ iP بتركيز ٠,٥ ملجم/لتر في وجود ٠,٥ جم/لتر فحم نشط وذلك خلال مرحلة البداية. تم وضع الخلايا الجنينية التي تم الحصول عليها لمزيد من التطوير وتكوين الأجنة في بيئة نضج الأجنة والمحتوية علي picloram بتركيز ٠,٢٥ ملجم/لتر بالتبادل مع ٢ iP بتركيز ١,٠٠ ملجم/لتر. تم نقل الأجنة الجسدية التي تم الحصول عليها إلى بيئة التضاعف والتي تحتوي علي putrescine بتركيزات ٠ و ٥٠ و ١٠٠ و ١٥٠ ملجم / لتر وذلك لدراسة تأثيره على معدل التضاعف. كما تم دراسة تأثير مستويات مختلفة من شدة الإضاءة خلال مرحلة الإنبات. في الواقع، وفي النهاية تمت مناقشة تأثير استخدام نظام جديد لأقلمة النباتات علي علي نسبة الأقلمة. تم تكوين أجنة جسدية كروية مباشرة علي المنفصل النباتي القمة النامية لنخيل التمر صنف لاکرامت. تم الحصول علي أعلى معدل لتضاعف الأجنة عند استخدام Putrescine بتركيز ١٠٠ ملجم/لتر. على الجانب الآخر، فقد شجع المستوي الأعلى من شدة الأضاءة (٦٠٠٠ لأكس) عملية أنبات وتكوين النموات الخضرية. خلال مرحلة التجذير، حققت إضافة ١.٥ مجلم / لتر IBA في وجود ١ مجم / لتر NAA في بيئة التجذير أعلى عدد معنوي لعدد الجذور. تفوق النظام الجديد لأقلمة النباتات المستخدم في هذا البروتوكول علي الطريقة التقليدية للأقلمة. تم تحقيق المستويات القصوى لحيوية نمو النبات خارج المعمل، وعدد الأوراق / النبات، عرض الورقة، وعدد الجذر وكذلك سمك الجذر عن طريق الري باستخدام محلول مورايشيچ وسكوج MS كامل القوة.

الكلمات الدالة: التطور الجنيني الجسدي المباشر، نظام الأقلمة الجديد، *Phoenix dactylifera* L. Putrescine.