Available online at http://bjas.bajas.edu.iq
https://doi.org/10.37077/25200860.2023.36.2.21
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 **Basrah J. Agric. Sci., 36(2), 267-284, 2023** **E-ISSN: 2520-0860**

In Vitro Propagation of Date Palm (*Phoenix dactylifera* L.) Cultivar Jawzi Using Shoot Tip

Ahmed M. Ibrahim[1*], Mohammed K. Hameed[2] & Arifullah Mohammed[3]

[1]Biotechnology Department, College of Applied Science, University of Fallujah, Iraq

[2]Department of Horticulture, College of Agriculture, University of Anbar, Iraq
[3]Faculty of Agro - Based Industry University of Malaysia Kelantan 17600 Jeli-Malaysia

*__Corresponding author email: A.M__: ahmed.m.ibraheem@uofallujah.edu.iq; **M.K.**: moh.khazaal@uoanbar.edu.iq; **A.M.**: aurifullah@umk.edu.my

Received 13th August 2023, Accepted 1st December 2023, Available online 30th December 2023

Abstract: This study reports the successful establishment of a somatic embryogenesis technique for the mass production of a date palm Jawzi cultivar using shoot tip explants. The shoot tip, leaf primordia, and the ground of apical meristems were utilised as explants in the research. The study involved the utilisation of various types of media. It began with initiation media (IM), which had two stages for inducing embryogenesis. This was followed by multiplication media (MM), then elongation shoot media (EM), and finally rooting media (RM) and acclimatization. Embryo induction in the different media types required 50-53 weeks. The first stage, IM2, (3.0 mg.l^{-1} 2iP, 10.0 mg.l^{-1} NAA and 5.0 mg.l^{-1} 2,4-D) for 12 weeks. This was followed by the second stage, IM3, which lasted for 38-41 weeks and included 1.0 mg.l^{-1} BAP, 1.5 mg.l-l 2iP, and 1.0 mg.l-l NAA. These stages enabled us to achieve the optimal value for embryo induction. Afterward the MM3 (0.5. mg.l^{-1} BAP, 0.5 mg.l^{-1} 2iP and 0.5 mg.l^{-1} KIN) showed the highest percentage of total counts of embryo multiplication, while the highest shoot length was attained on EM1 (1.0. mg.l^{-1} BAP, 1.0 mg.l^{-1} KIN and 0.1 mg.l^{-1} IBA). The results also highlighted that RM1 (0.1 mg.l^{-1} BAP, 0.1 mg.l^{-1} KIN,1.0 mg.l^{-1} NAA and 0.5 mg.l^{-1} IBA) showed the highest roots length and roots number, in conclusion, these findings emphasise the importance of media composition in tissue culture protocols. Evaluating the effects of specific media components on different aspects of plant development can optimise tissue culture protocols for plant propagation.

Keywords: Multiplication, Rooting, Somatic embryogenesis, Tissue culture.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops in the world, cultivated in arid and semi-arid regions for its valuable fruit and ornamental value (Al-Mayahi, 2022). The date palm is an iconic crop of the Middle East, with Iraq being the birthplace of date cultivation and major dates producing zones in the world (Abass & Awad, 2019; Al-Qatrani *et al*., 2021). The fruit is not only high in carbohydrates, but it also contains a variety of essential nutrients, making it an important food source for millions of people

worldwide (Hussain *et al*., 2020). However, traditional methods of propagating date palms through seeds are inefficient and unreliable. The use of seeds leads to a high degree of genetic variation and a long juvenile period before fruiting, making it difficult to maintain desirable traits of the parent tree. To overcome these limitations, micropropagation techniques have been developed as an alternative means of propagating date palms (Mazri *et al*., 2019; Alansi *et al*., 2020; Abdelghaffar *et al*., 2023; Solangi *et al*., 2023).

Plant growth regulators play a significant role in date palm micropropagation, the results on callus induction of date palm cultivar Barhee revealed that a concentration of 0.5 mg.l^{-1} TDZ was ideal for all the traits examined (Al-Asadi *et al*., 2019).

Micropropagation is a technique used to produce large numbers of genetically identical plants from a small piece of tissue (e.g., shoot tip, node, or callus) in a sterile, controlled environment (Al-Qatrani *et al*., 2021). This method has been widely used for the mass production of plants, including fruit trees, ornamentals, and forest trees. Micropropagation of date palms has been successfully achieved using various tissue culture techniques, such as somatic embryogenesis, organogenesis, and callus culture (Abdulhafiz *et al*., 2020; Meira *et al*., 2020; Taha *et al*., 2021). The technique has been shown to be effective in producing high-quality, disease-free, and genetically identical plantlets. Moreover, the method allows for the rapid production of a large number of plantlets, reducing the time required for the multiplication of desirable traits and the establishment of new plantations (Alansi *et al*., 2020; Intha & Chaiprasart, 2020; Maher *et al*., 2021). To organised and expedite the response of all stages of date palm embryogenesis in culture, there is a need for a straightforward and swift method that can enhance efficiency and reduce both time and cost in commercial production. Incorporating low levels of salinity has been found to promote the growth and development of *in vitro* date palm callus cultures (Al-Khayri, 2002).

The development of new and improved date palm varieties is crucial for the sustainability and competitiveness of the date palm industry. With the increasing demand for high-quality dates and the challenges posed by climate change and pests and diseases, there is a need for new date palm varieties that are more productive, disease-resistant, and adaptable to changing environmental conditions (Hazzouri *et al*., 2020). In a study of *Dianthus caryophyllus*, Zeatin demonstrated superior efficacy compared to Kin or 2,4-D, whereas alternative combinations led to direct shoot development. (Mahood *et al.,* 2001).

In this study, we have successfully developed somatic embryogenesis techniques for date palm varieties, specifically cv. Jawzi without going through the callus stage and shoot development, until reaching the rooting and acclimatisation stages. For the first time in the world, we are developing a new protocol.

Materials & Methods

Plant materials

The research was conducted in the laboratories of Janat Al-Nakheel for Plant Tissue Culture Company, located in Alkhadimyia, Baghdad, Iraq, from January 2021 to December 2022. The shoot tips of the date palm variety Jawzi were excised from a 3-4-year-old offshoot and were used as explants (Fig. 1A, B).

Surface sterilization and explant preparation

The shoot tips (12-15 cm long), were soaked in the fungicide solution for 15 min and then rinsed three times with sterile distilled water. After being immersed in ethanol (95%) for 1 min and washed with sterilised water, the excised parts were placed into an antioxidant solution. This solution consisted of 150 $mg.l^{-1}$ of citric acid and 150 $mg.l^{-1}$ of ascorbic acid. Its purpose was to stop the oxidation process and prevent the browning of the tissues. The explants were then transferred to a laminar hood and were treated with a solution of 3% sodium hypochlorite (NaOCl) that was mixed with a few drops of tween 20 for a duration of 15 min. After sterilisation, it was finally rinsed three times with sterilised distilled water for three min each time (Fig. 1C).

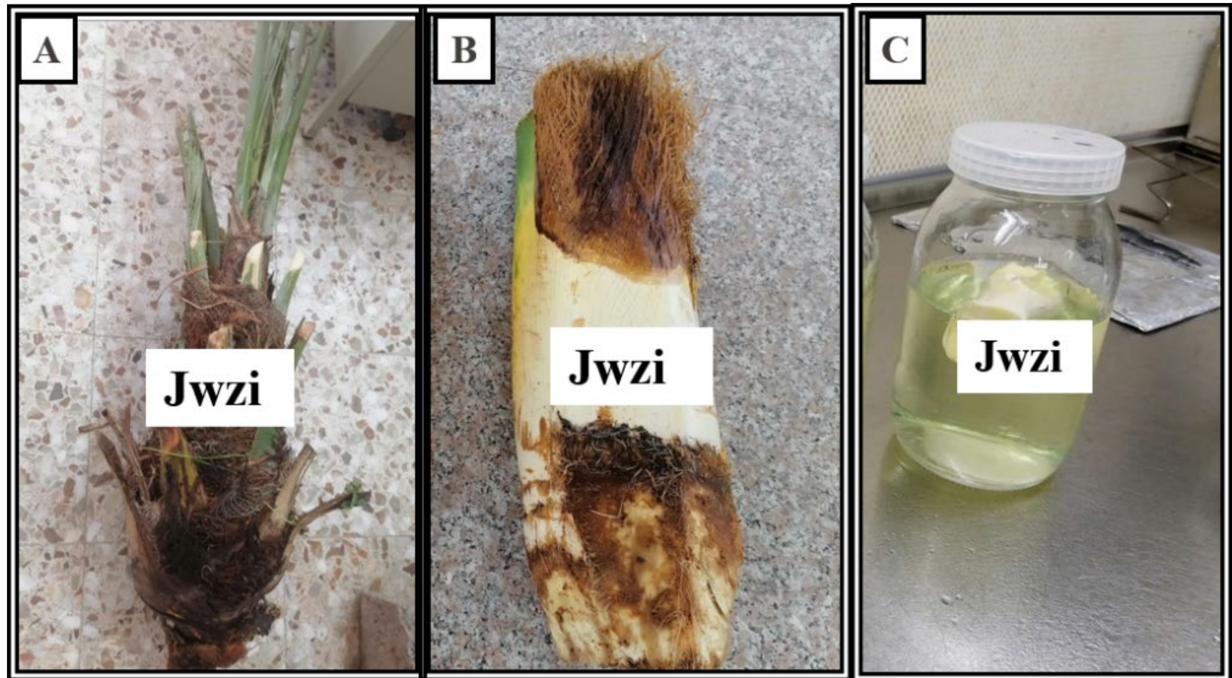

Fig. (1): (A) Offshoot of Jawzi 3-4 years old, (B) Offshoot after removing the outer leaves before extraction the shoot tip (C) Shoot tips socked in surfactant.

The outer covers of the offshoot were gently removed using sterile forceps and scalpel (Fig. 2A-C). The extracted shoot tip was divided into four parts longitudinally (Fig. 2D-F). All the explants were cultured onto initiation media and incubated in a regulated growth room at a constant temperature of 25 ± 2 °C in complete darkness for 8hrs and with evaluations with a 16-h photoperiod provided by cool white fluorescent lights conducted at four week intervals. The swelling of the explant was measured after 8 weeks, and the percentage of explants showing embryonic culture emergence out of the total number of cultured explants was recorded after 50 weeks. In addition, the percentage of browning was assessed after the 16-week period as well (see Fig. 3).

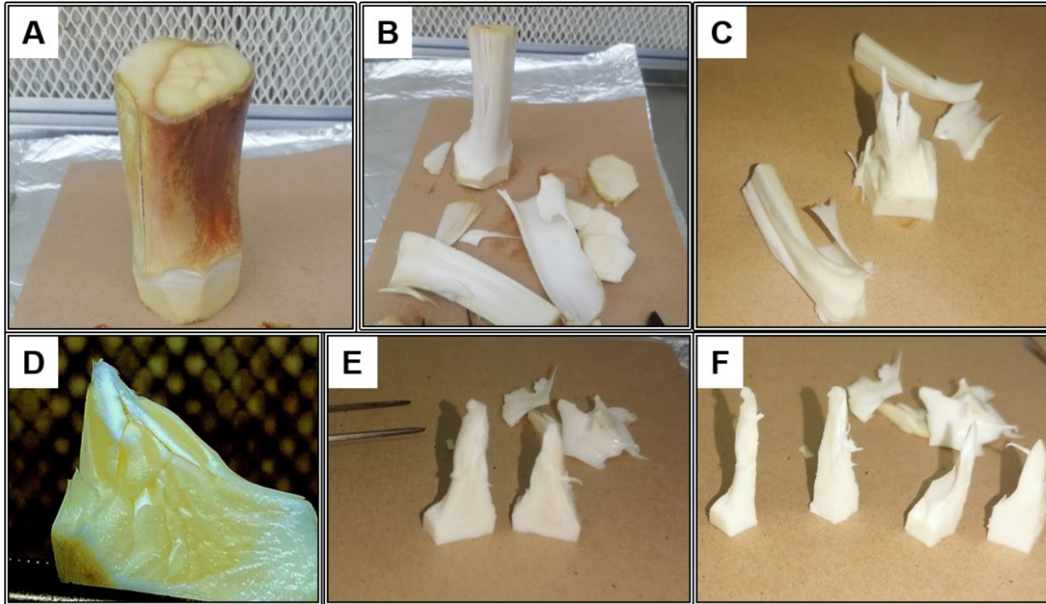

Fig. (2): (A) Offshoot after surface sterilization, (B) Removal of the outer covers (C) 3-4 cm long shoot tip with base of leaf primordia (D) Longitudinal section of shoot tip (E) Shoot tip divided into two parts longitudinally (F) Shoot tip divided into four parts longitudinally.

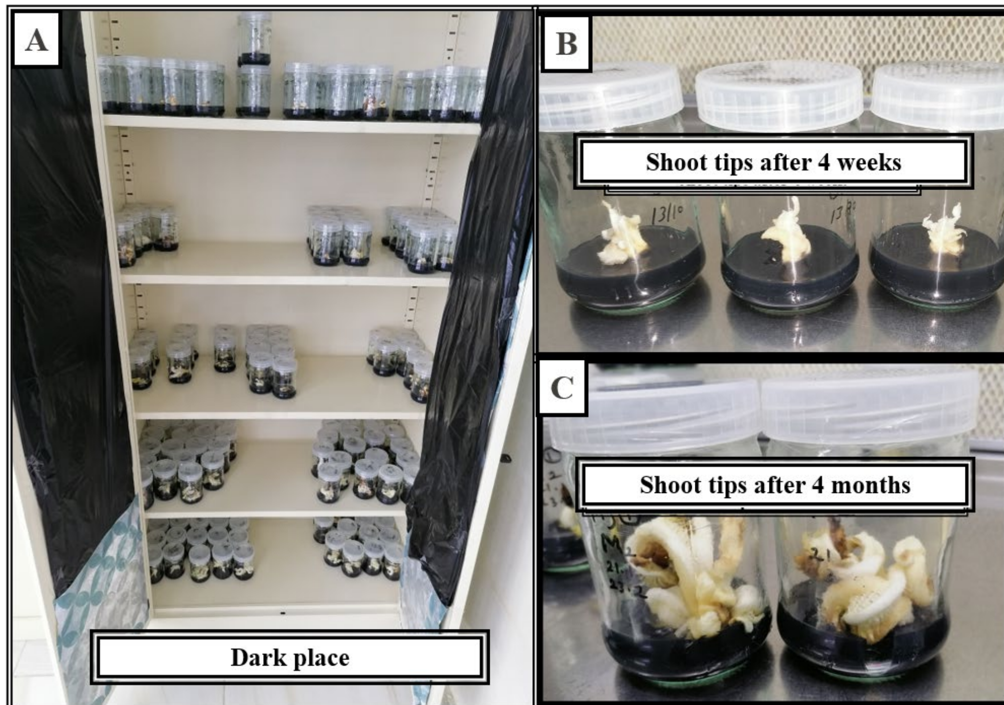

Fig. (3): (A) Explants cultured in a growth room at a constant temperature of 25 ± 2 °C in complete darkness, (B) Swelled shoot tip explants after 4 weeks, (C)More swelling with browning of shoot tips explant after 16 weeks,

Initiation medium (IM)

Explants from different sources such as the shoot tip, the base of leaf primordia and the ground of apical meristems were initially cultured on media consisting of MS medium (Murashige & Skoog, 1962) supplemented with different components (Table 1) and plant growth regulators (PGRs) (Table 3). Two types

of initiation media were used in this study. Initially, the explants were inoculated onto three distinct initiation media (IM) during the initial three-month stage, Afterward, the explants were moved to another three types of initiation media (IM) second stage (Table 2). They were cultured in this medium for eight months until the induction of embryos commenced.

Table (1): Components of initiation media (IM).

Components	First stage (month)			Second stage (month)							
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
MS $gm.l^{-1}$	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4	4.4
Sucrose $gm.l^{-1}$	30	30	30	40	40	40	40	40	40	40	40
Glutamine $mg.l^{-1}$	100	100	100	200	200	200	200	200	200	200	200
Myo-inositol $mg.l^{-1}$	100	100	100	150	150	150	150	150	150	150	150
Ascorbic acid $mg.l^{-1}$	250	250	250	0	0	0	0	0	0	0	0
Citric acid $mg.l^{-1}$	250	250	250	0	0	0	0	0	0	0	0
PVP $mg.l^{-1}$	1000	1000	1000	500	500	500	500	125	125	125	125
NaH_2Po_4 $mg.l^{-1}$	200	200	200	200	200	200	200	200	200	200	200
KH_2Po_4 $mg.l^{-1}$	0	0	0	120	120	120	120	120	120	120	120
$Ca(NO_3)_2$ $mg.l^{-1}$	0	0	0	200	200	200	200	200	200	200	200
PMP $ml.l^{-1}$	1	1	1	1	1	1	1	1	1	1	1
Pyridoxine $mg.l^{-1}$	1	1	1	1	1	1	1	1	1	1	1
Nicotine $mg.l^{-1}$	1	1	1	1	1	1	1	1	1	1	1
Thiamine $mg.l^{-1}$	1	1	1	1	1	1	1	1	1	1	1
Agar $gm.l^{-1}$	5	5	5	5	5	5	5	5	5	5	5
charcoal $gm.l^{-1}$	2	2	2	2	2	2	2	2	2	2	2
pH	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7

Multiplication medium (MM)

To achieve mass production of embryos, bud formation, and plantlet development, we used three different types of multiplication media, each containing different types and concentrations of plant growth regulators (PGRs) during the embryo multiplication process (Table 2).

Elongation shoots medium (EM)

After the multiplication stage, the developed shoots were grouped into clusters. Afterward, the explants were transferred to three distinct elongation media in order to promote the growth of vigorous individual shoots for the subsequent rooting stage (Table 2). The culture medium consisted of MS medium supplemented with 50 $gm.l^{-1}$ sucrose. The medium was dispensed into large jars, each with a volume of 800 ml. The volume of the medium in each jar was 25 ml, and the total amount of activated charcoal AC was 0.5 $g.l^{-1}$. The cultures were maintained in the liquid medium for a duration of 8 weeks, with a 4-week interval between each period. They were incubated at a temperature of 25 ± 2°C, with a light intensity of 3000 lux.

Hardening and acclimatization

The healthy rooted plantlets were chosen, 2-3 leaves with a length of about 10-15 cm, and the roots were rinsed with flowing tap water to remove any attached solidified culture medium. Afterward, the plantlets should be immersed in a fungicide solution called Beltanol for a period of 15 minutes. Afterwards, the plantlets should be relocated into plastic pot trays that are filled with a blended mixture consisting of autoclaved peat moss, perlite, and vermiculite in a ratio of 3:1:1. The container should then be placed under a plastic-sheet tunnel in the greenhouse. During a period of 4-6 weeks, the seedlings were maintained at 25 2 °C normal daylight temperature and high relative humidity levels of 90-95% by using a covering made of white, transparent polyethylene sheets. After seven days, the tunnel is opened for ventilation for 10 to 15 minutes, 1 $ml.l^{-1}$ of fungicide and insecticide is sprayed, and the openings are progressively widened over the next two weeks additionally, deceased plants were eliminated and leaves afflicted by fungus were trimmed away. After a planting period of 2-3 months, ensure that the plants receive suitable fertilisation and irrigation. A dosage of 1-2 gm. l^{-1} of N-P-K fertiliser (1-2-1) yields favourable outcomes when combined with irrigation and should only be administered when deemed necessary. After five months, plants that had acclimatised began to develop new roots and leaves *in vitro* conditions.

Rooting media (RM)

Healthy shoots from individuals were placed on a solid MS salt medium that was supplemented with three types of rooting media components (as shown in table 2) and contained 60 $gm.l^{-1}$ sucrose. The medium was solidified by adding 5.0 $g.l^{-1}$ agar and then distributed into culture tubes (measuring 2.5 × 25 cm) at a volume of 25 ml per tube. The shoots were then allowed to grow and develop roots for a period of three months. Following the rooting stage, the shoots that had been rooted were subsequently transferred to a half-strength liquid MS medium for an additional duration of one month, in order to undergo a pre-acclimatization stage (Kadhim & Abdulhussein, 2021). During the entire process, the plantlets on solid medium and those in the culture media were incubated at a temperature of 25 ± 2 °C, with a light intensity of 2000 lux in a 16/8 light/dark cycle.

Data analysis

After three months of shoot tip culture, the experiments were designed using a Completely Randomised Design (CRD), four explants from each part of shoot tip were used for each treatment with four replications, The data were analysed using One-Way Analysis of Variance (ANOVA), Significant differentiations were compared by Duncan Multiple Range Test (DMRT). The variables observed for data analysis were the time required to induce embryos, number of embryo induction and multiplication, elongation shoot, number and length of root induction with different levels of PGR. Data were calculated and analyzed by using the Statistical Packages for Social Science (SPSS)

Abbreviations

(MS) Murashige and Skoog
(IM) Initiation media
(MM) Multiplication media
(EM) Elongation shoot media
(RM) Rooting media
(2-iP) 2-isopenteMyladenine
(2, 4-D) 2,4-Dichlorophenoxyacetic acid

(NAA) Naphthaleneacetic acid
(IBA) Indole-3-butyric acid
(KIN) Kinetin
(BAP) 6-Benzylaminopurine
(AC) Activated charcoal
(MS) Murashige and Skoog
(TDZ)Thidiazuron

Table (2): Components of plant growth regulator during different stages.

Type of stage	Type of media	Type of PGR $mg.l^{-1}$					
		BAP	2iP	KIN	NAA	2,4-D	IBA
Initiation media first stage (3 months)	IM1		3		15	10	
	IM2		3		10	5	
	IM3		3		5	2.5	
Initiation media (second stage)	IM1	1	5		3		
	IM2	1	2.5		2		
	IM3	1	1.5		1		
Multiplication media	MM1	0.5	0.5	2			
	MM2	0.5	0.5	1			
	MM3	0.5	0.5	0.5			
Elongation shoot media	EM1	1		1			0.1
	EM2	1		2			0.1
	EM3	1		3			0.1
Rooting Media	RM1	0.1		0.1	0.5		0.5
	RM2	0.1		0.1	1		1
	RM3	0.1		0.1	1.5		1.5

Results & Discussion

Effect types of media on embryo induction

The specific hormone combination and concentration of media played a crucial role in promoting embryogenic cell development and subsequent embryo formation.

As shown in table (3), the time required for embryo induction for two stages is affected by the different media types and type of tissue. For the shoot tip tissue, the IM2 medium in the first stage (3.0 $mg.l^{-1}$ 2iP, 10.0 $mg.l^{-1}$ NAA and 5.0 $mg.l^{-1}$ 2-4,D) followed by IM3 (1.0 $mg.l^{-1}$ BAP, 1.5 $mg.l^{-1}$ 2iP and 1.0 $mg.l^{-1}$ NAA) exhibited the shortest duration of embryo induction, with an average of 50 weeks. While the IM1 and IM3 media required 54 and 53 weeks, respectively, for embryo induction in Jawzi variety (Fig. 4).

In the case of the base of leaf primordia, the IM2 medium showed the quickest response, with an average embryo induction time of 60 weeks, the IM1 medium followed closely behind with an average time of 66 weeks. While the IM3 medium required the longest time for embryo induction in base of leaf primordia averaging 68 weeks.

A positive response was not observed across all types of media for embryo induction in the context of apical meristem explants. However, after a duration of 50 weeks, the tissue underwent a transition. It first turned brown and subsequently black, leading to its eventual death.

Table (3): Effect of media types on embryo induction.

Type of Media	Weeks to embryo induction		
	Shoot tip	Base of leaf primordia	Ground of apical meristems
IM1	54 ±1^{b}	66 ±1^{b}	0
IM2	50 ±1^{a}	60 ±1^{a}	0
IM3	53 ±1^{b}	68 ±1^{b}	0

Letter (s) within a column are not statistically significant difference ($P \leq 0.05$, DMRT)

The critical elements influencing the micropropagation of date palms are primarily the constituents of the culture medium, specifically the growth hormones, and the timing of nutrient media application throughout the micropropagation procedure (Abul-Soad, 2011). The initiation of embryogenic processes in date palm is characterised by a notably slow pace. The duration of this initiation phase oscillates according to the specific genotype and can span from a few months to several months. The findings show that increasing the concentration of 2, 4-D and NAA at the first stage of initiation, which increases the frequency of embryo induction, may result in a minor increase in the time required for callus induction (Solangi *et al*., 2020). The observed differences in the number of weeks required for embryo induction across media types suggest that the specific composition and concentration of these components play a critical role in the efficiency of embryo formation. Similar findings have been reported in previous studies on tissue culture of other plant species (Mangena, 2020). The positive influence of macronutrients on embryo induction can be attributed to their roles in supporting cellular metabolism and growth (Neelakandan & Wang, 2012). Furthermore, the effects of plant growth regulators on cell division and organogenesis during embryo induction have been well-documented (Yaseen *et al*., 2013).

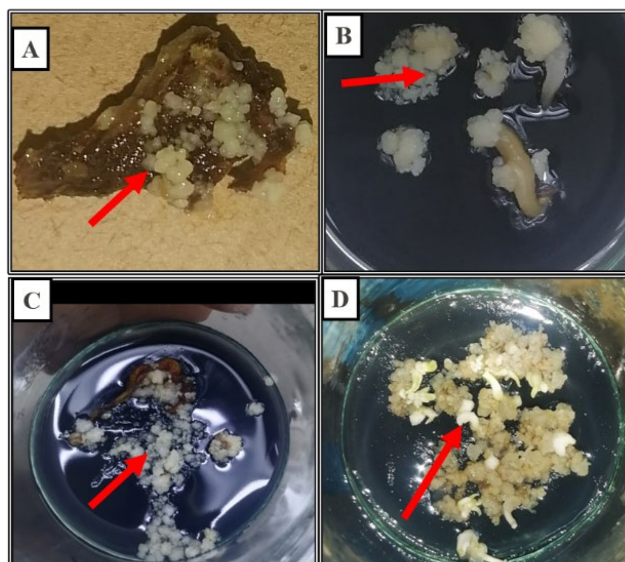

Fig. (4): (A) The beginning of the emergence of embryos from shoot tip tissue after 15 months (B, C) Embryo multiplication after 16-18 months. (D) The embryos were started to development into bud.

Effect type of media on Embryo Formation and multiplication

The results showed variations in the number of somatic embryos formed among the different subculture cycles and explants (Table 4). For the Sub1, the highest number of somatic embryos was obtained from MM3 (0.5. mg.l^{-1} BAP, 0.5 mg.l^{-1} 2iP and 0.5 mg.l^{-1} KIN) with a total count of 55 embryos, while MM1 yielded 30 somatic embryos and MM2 40 embryos (Figs. 5 and 6). In the case of the Sub2, MM3 displayed the highest somatic embryo count, totaling 120. The result of MM2 was 80 somatic embryos, whereas MM1 produced 100 somatic embryos. In the case of Sub3, MM1 displayed the highest number of somatic embryos, amounting to a total count of 300. MM3 yielded 250 somatic embryos, while MM2 resulted in 55 somatic embryos. The study showed that a higher frequency of subculture was positively correlated with a greater number of somatic embryos for all three types of media. The trend was consistent across three types of multiplication media, which indicates the importance of regular subculturing in promoting somatic embryo formation.

These findings are consistent with previous studies on tissue culture of various plant species (Sun *et al*., 2022). Regular subculturing plays a crucial role in maintaining the embryogenic potential of the cultures by eliminating senescent tissues and providing fresh nutrients and growth regulators (Konan *et al*., 2010). The concentration of 0.4 mg.l^{-1} NAA proved to be highly effective in achieving the longest average branch length, reaching an impressive 3.92 cm. The presence of sufficient nutrients and growth regulators at each subculture stage promotes the development and proliferation of somatic embryos. The findings emphasise the significance of including regular subculturing in tissue culture protocols for efficient somatic embryo production. The ability to produce a greater number of somatic embryos through increased subculture frequency opens up possibilities for large-scale tissue culture plant propagation and accelerated breeding programmes. The trend observed across all

three types of media suggests a positive relationship between increased subculture frequency and a higher number of somatic embryos (Ku *et al.*, 2023).

Table (4): Effect of media types on frequency on somatic embryo multiplication

No. of subculture	Total number of somatic embryos		
	1st subculture	2nd subculture	3rd subculture
MM1	30 ±2[c]	100 ±5[b]	300 ±5[a]
MM2	40 ±2[b]	80 ±5[c]	180 ±5[b]
MM3	55 ±2[a]	120 ±5[a]	250 ±5[ab]

Letter (s) within a column are not statistically significant difference ($P \leq 0.05$, DMRT).

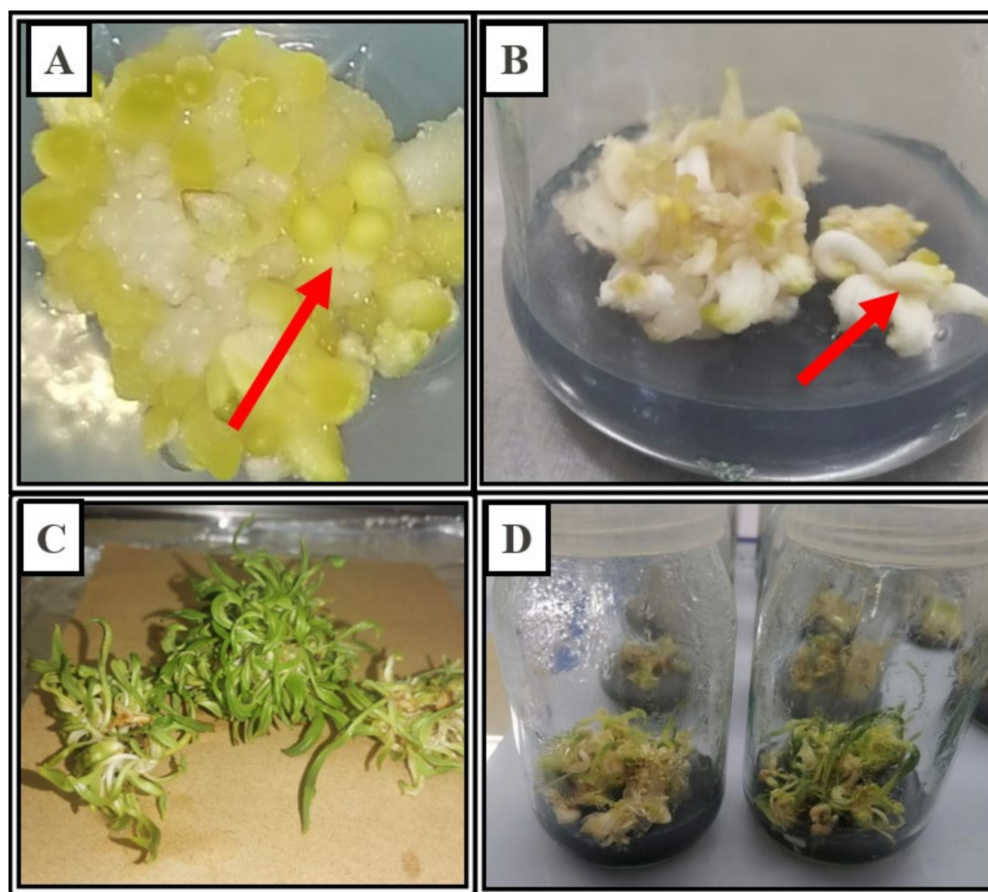

Fig. (5): (A) Stages of buds Multiplication (B) Stage of bud development into shoot (C) stage of shoot multiplication (D, E) Stage of shoot elongation.

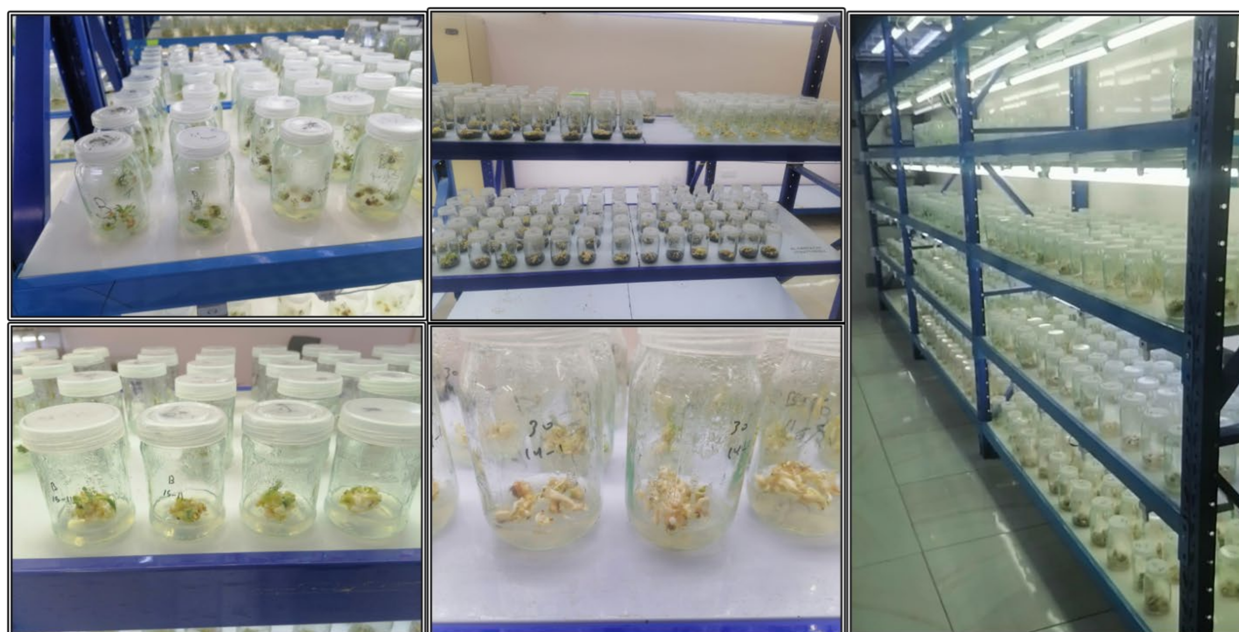

Fig. (6): Incubation room condition, different stages of buds multiplication.

Elongation shoot stage

The results revealed that the length of the shoot varied between media types and varieties. The shoots grown in EM1 medium had the longest length, measuring 18 cm (Table 5), while those grown in EM2 and EM3 medium had slightly shorter lengths of 10 and 15 cm, respectively. EM1 media types produced four leaves, followed by EM3 (three leaves) and EM2 (two leaves) in terms of the number of leaves. With regard to diameter of shoot base, the shoots cultured on EM1 and EM3 exhibited the largest diameter of shoot base of 4mm, while the shoots on EM2 media had length 2mm (Fig. 7). Overall, the study indicated that the choice of media type had a significant impact on shoot length, Number of leaves and diameter of shoot base in tissue culture plants. Each media responded differently to the shoot formation. The response of different plant varieties to media composition can be attributed to variations in their genetic makeup, physiological characteristics, and developmental pathways (Bita & Gerats, 2013). These genotype-specific responses highlight the importance of customised media formulations that are tailored to the needs of each plant variety.

Table (5): Effect of different Elongation media type on plantlets.

Type of Media	Shoot size		
	leaf length (cm)	No. of leaves/ plantlets	Diameter of shoot base (mm)
EM1	18 ±1^{a}	4±1^{a}	4 ±1^{a}
EM2	10 ±1^{b}	2±1^{b}	2 ±1^{b}
EM3	15 ±1^{ab}	3±1^{ab}	4 ±1^{a}

Letter (s) within a column are not statistically significant difference ($P \leq 0.05$, DMRT).

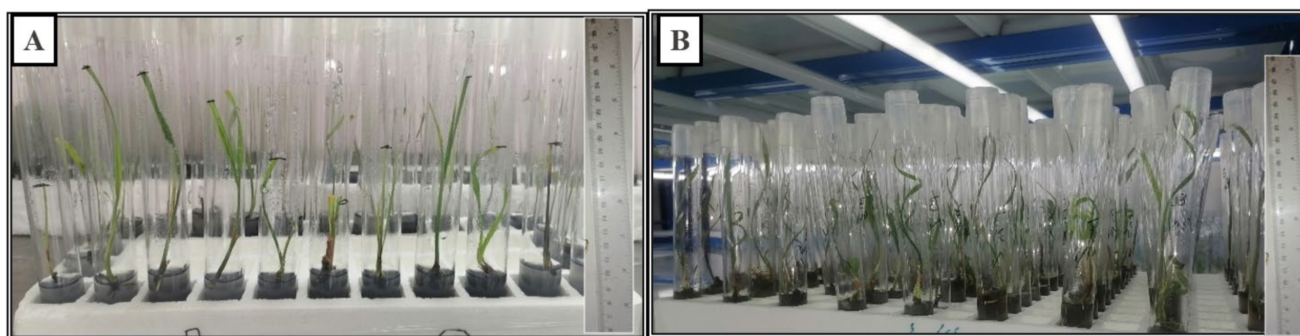

Fig. (7): The plantlets produced under the influence of different elongation mediums type

Rooting and acclimatization

Two phases of the rooting process were undertaken in order to cultivate healthy plantlets suitable for acclimatization. The experiment involved transferring elongated shoots to three distinct rooting media (RM) in order to promote root growth. The outcomes revealed distinct differences among the three types of RM in terms of root length, count of roots, and the number of root hairs per root. According to data analysis, RM1 was identified as the variety that exhibited the most significant root length, with an average of 10 cm (Table 10). The observation of the greatest root count was made in RM2, which had 9 roots. On the other hand, RM1 produced the most favourable outcome in terms of the number of root hairs per root. Figs. (8 and 9) were subsequently transferred to the pre-acclimatization liquid medium. In fig. (8), plantlets with well-established roots and robust shoots were effectively acclimated within the greenhouse environment. Subsequently, irrigation was performed employing half the strength of MS salts. The augmentation of MS strength, which was utilised as a fertilisation solution for date palm plants during the acclimatization phase, significantly influences a majority of the measured parameters (Fig. 10). The success of date palm tissue culture during the rooting phase is critical in the micropropagation process (Al-Khalifah *et al*., 2012). In the present study, a gradual increase in root number and root length was observed as the strength of the rooting MS media reduced concentration of NAA and IBA. This significant finding underscores the enhanced root formation and growth on a high-sucrose medium as opposed to a low-sucrose one. This outcome aligns with previous findings as reported by (Thakur *et al*., 2015). The plantlets with established roots were delicately retrieved from the tubes, followed by a thorough rinse under flowing water and treatment with a Rezolex fungicide (100 g) solution (Generic) for a duration of 10 minutes. Subsequently, these plantlets were carefully placed into pots containing a mixture of peat and perlite in a 2:1 v/v ratio (Rohim *et al*., 2021), Enhancing date palm production relies not exclusively on *in vitro* propagation methods, but also on the application of chemical and physical mutagens (Ghareeb *et al*., 2022), modern genetic strategies and phylogenetic analysis (Raza *et al*., 2022) and molecular markers (Al-Khayri *et al*., 2022).

Table (6): Effect of media types on roots formation.

Type of Media	Root structure		
	Root length (cm)	No. of root	No. of root hairs/root
RM1	10 ±1[a]	7 ±1[a]	8 ±1[a]
RM2	8 ±1[ab]	9 ±1[a]	5 ±1[b]
RM3	5 ±1[b]	4 ±1[b]	2 ±1[c]

Letter (s) within a column are not statistically significant difference ($P \leq 0.05$, DMRT).

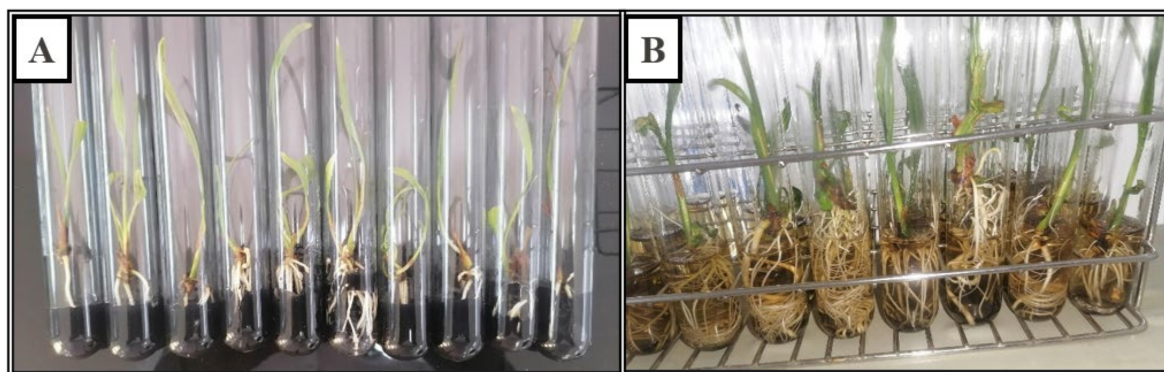

Fig. (8): (A) New root initiated after 8 weeks, (B) High number of root induction for RM2 after 12 weeks.

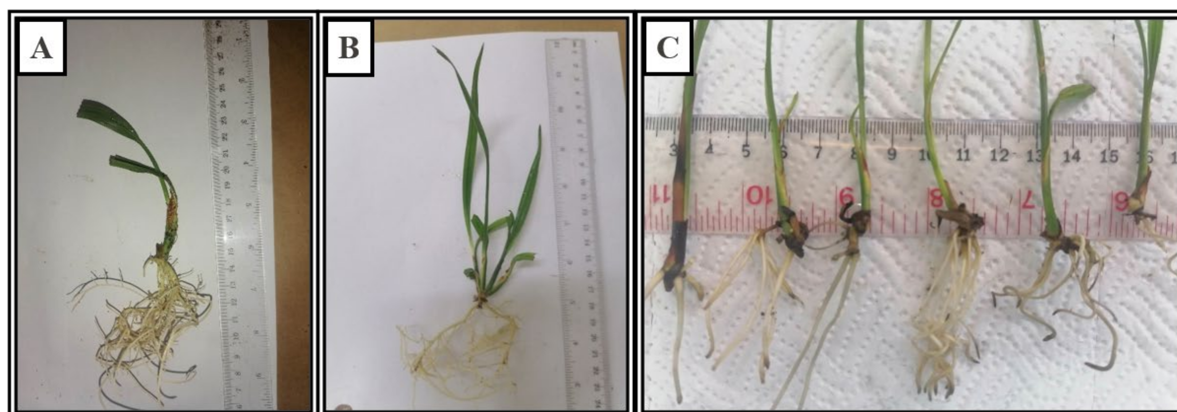

Fig. (9): Select the healthy rooted plantlets with 2–3 leaves and about 9-13 cm in length.

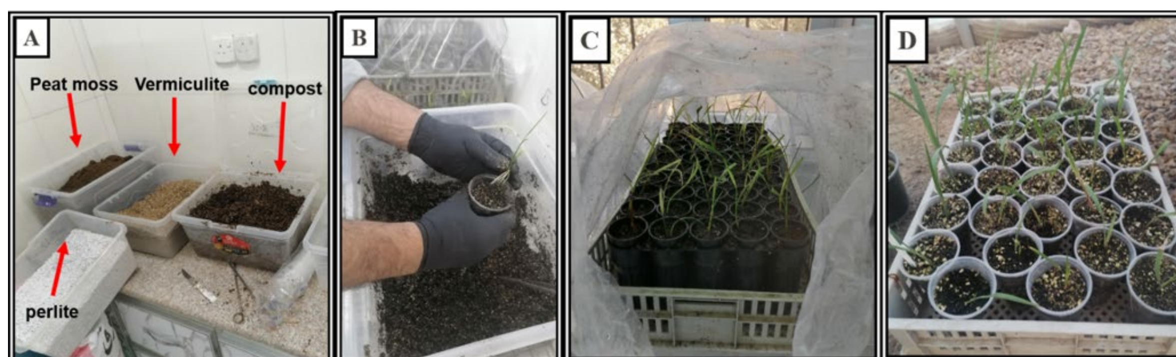

Fig. (10): (A, B) Transfer the plantlets into plastic pots trays, filled with composed mixture contained autoclaved peat moss, perlite and vermiculite (3:1:1). (C) Place the container in the greenhouse under plastic-sheet tunnel (D) Final acclimatization stage after four months.

Conclusion

The results revealed that media composition had a significant effect on date palm embryo induction. The multiplication media (MM1) yielded the most somatic embryos after three subcultures, with a total count of 300. The shoots cultured on EM1 were the longest, measuring 18 cm in length. These findings emphasise the significance of selecting appropriate media types and optimising their composition in tissue culture protocols to achieve desired results. Regular subculturing was found to promote somatic embryo formation, with the number of somatic embryos increasing with each subculture cycle. These results indicate the importance of maintaining the embryogenic potential of cultures through proper nutrient and growth regulator supply. The observed variations in shoot length among media types and varieties further underscore the influence of media composition on shoot growth and development. These findings provide valuable insights for refining tissue culture protocols, enhancing plant propagation, and accelerating breeding programmes, where customised media formulations and regular subculturing can play pivotal roles in achieving desired outcomes.

Acknowledgments

We would like to express our sincere gratitude to the laboratories of Janat Al-Nakheel and the University of Fallujah for their valuable assistance and provision of other facilities used to conduct this work.

Contributions of authors

A.M.I.: writing paper, Laboratory, methodology.

M.K.H.: Suggest a title of the paper, writing paper.

A.M.: writing paper, data analysis.

All authors reviewed the manuscript

ORCID

A.M: https://orcid.org/0000-0001-9670-5360

M.K: https://orcid.org/0000-0001-8389-8845

A.M: https://orcid.org/0000-0001-7793-8134

Conflicts of interest

The authors state that they do not have any conflicts of interest.

Ethical approval

The article adhered to all ethical guidelines concerning the welfare and treatment of fish, as

they were outlined by national and international organisations.

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اكثار نخيل التمر (*Phoenix dactylifera* L.) صنف الجوزي بواسطة استخدام القمة النامية خارج الجسم الحي

احمد محمود ابراهيم ومحمد خزعل حميد و عارف الله محمد

[1]قسم التقانة الاحيائية، كلية العلوم التطبيقية، جامعة الفلوجة، العراق

[2]قسم البستنة، كلية الزراعة، جامعة الانبار، العراق

[3]كلية الزراعة الصناعية، جامعة كلنتان، ماليزيا

المستخلص: تُظهر هذه الدراسة نجاح الانتاج الواسع في الحصول على الاجنة الجسمية لنخيل التمر صنف الجوزي (من الانواع التجارية في العراق) باستخدام زراعة القمه النامية وبطريقة الانتاج المباشر للاجنة الجسمية . تم استخدام القمة النامية وبادئات الأوراق والمناطق التي اسفل المرستيم القمي كأجزاء نباتية. استخدم في البحث أنواع مختلفة من الاوساط الزرعية، ابتداءً من وسط النشوء (IM) بمرحلتين لتحفيز تكوين الاجنة الجسمية، تلاها وسائط التضاعف للاجنة (MM)، ثم وسائط الاستطالة (EM)، واختتم بوسائط التجذير (RM) والاقلمة. استغرقت افضل مدة لتحفيز الاجنة الجسمية من 50 إلى 53 أسبوعًا، حيث استخدم وسط النشوء IM2 المرحلة الأولى (3.0 ملغم.لتر$^{-1}$ 2iP، 10.0 ملغم.لتر$^{-1}$ NAA و 5.0 ملغم.لتر$^{-1}$ 2,4–D) لمدة 12 اسبوع، ثم الوسط IM3 في المرحلة الثانية (1.0 ملغم.لتر$^{-1}$ BAP، 1.5 ملغم.لتر$^{-1}$ 2iP و 1.0 ملغم.لتر$^{-1}$ NAA) لمدة 38–41 اسبوع والتي سمحت لنا بتحقيق افضل نسبة لتكوين الاجنة الجسمية. بعد ذلك، أظهروسط التضاعف MM3 (0.5 ملغم.لتر$^{-1}$ BAP، 0.5 ملغم.لتر$^{-1}$ 2iP و 0.5 ملغم.لتر$^{-1}$ KIN) أعلى نسبة لإجمالي عدد الاجنة الجسمية، بينما تم الحصول على افضل استطالة من الوسط EM1 (1.0 ملغم.لتر$^{-1}$ BAP، 1.0 ملغم.لتر$^{-1}$ KIN و 0.1 ملغم.لتر$^{-1}$ IBA). كما أظهرت النتائج أن الوسط RM1 (0.1 ملغم.لتر$^{-1}$ BAP، 0.1 ملغم.لتر$^{-1}$ KIN، 1.0 ملغم.لتر$^{-1}$ NAA و 0.5 ملغم.لتر$^{-1}$ IBA) أظهر أعلى معدل طول وعدد للجذور. في الختام، تؤكد هذه النتائج أهمية الاوساط الزراعية المستخدمة في بروتوكولات زراعة الأنسجة. وتقييم تأثير مكونات الاوساط الزراعية على جوانب مختلفة من تطوير النباتات يمكن أن يحسن بروتوكولات زراعة الأنسجة للحصول على النباتات.

الكلمات المفتاحية: التضاعف، التجذير، الاجنة الجسمية، زراعة الانسجة.