Effect of media type, Cytokinins and Auxins on the formation of embryogenesis of date palm *Phoenix dactylifera* L. in vitro

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Abstract

The study was carried out in the tissue culture laboratory of the Date Palm Research Center, University of Basrah in 2022-2023 Ad, three types of nutritional media were used, Murashige and Skooge,(1962)(MS) , Woody plant media (WPM) (Lloyd and McCown, 1981), and Gamborg, (1968) (B5) supplied with two types of cytokines Zeatin and 2ip at four concentrations for each (0, 0.5 ,1 ,2) mg L^{-1} and two types of auxins NAA and IBA at three concentrations for each (0, 0.1, 0.5) mg L^{-1} to formation embryogenesis of Barhi cultivar *in vitro*. The results showed that the WPM and MS media gave the highest rate of the number of embryos, which amounted to 7.45 and 7 embryos, with a significant difference from the B5 media which reached (4.92 embryos) Also, no significant differences were observed in the number of embryos for the cytokine type trait, while the results showed that the concentrations of 0 and 0.5 mg.L^{-1} were excelled in the rate of the number of embryos, which amounted to 7.13 embryos, with a significant difference from the rest of the concentrations. the WPM nutrient media recorded the highest percentage of embryos, number, and length of roots, which amounted to 41.7%, 1.6 roots, and 5.48 cm, respectively, with a significant difference from the MS media, which amounted to 30% percentage of embryo, and the number and length of roots amounted to 1.36 roots and 5.06 cm, respectively, while the type of auxin did not record any significant effect on the embryo percentage and root length, while IBA was excelled in the rate The number of roots reached 1.6 roots compared with NAA concentration exceeded 0.5 mg L^{-1} significantly in the percentage of embryo, number of roots and their length at a rate of 48.8% and 1.7 roots and 6.125 cm compared to the control treatment, while the effect of the double and triple interactions did not record any significant differences with statistical significance

Keywords: Media type, Auxins, Cytokinines, Date palm, in vitro

Introduction

Date palm. *Phoenix dactylifera* L. is a monocotyledonous, diploid, unisexual, dioecious plant. It is one of the most important fruit crops because it has nutritional and economic value [1]. Date palm culture in Iraq is limited to the area extending between Mandali and Tikrit districts at latitude 35 degrees north to the city of Al-Faw at latitude 30 degrees south, Its culture is widespread in most governorates of Iraq [2] and is cultivated in many countries in the arid regions of West Asia, North Africa and the Middle East [3]. Plant tissue culture is one of the most important and recent scientific and applied specializations, and its importance has emerged as an alternative method to traditional methods of propagation and genetic improvement of plants of various types [4]. Researchers in most countries of the world have been able to harness this technology for the widespread propagation of plants, and tissue culture technology has proven its efficiency in terms of the number of plants that can be produced from one plant and the genetic matching of the resulting plants to their origins [5]. Palm trees are propagated by tissue culture either by organogenesis from the culture of the growing tip and axillary buds or by the formation of somatic embryos (Somatic embryogenesis) by passing through the callus stage, from which embryogenesis are formed, by culture plant tissues in sterile artificial nutritional media [6]. Callus and the production of embryogenesis have been stimulated by many researchers for date palms [7 , 8]. Nutritional media are among the most important factors that determine the success of tissue culture because of their effective role in the processes of growth, specialization and division by providing the plant part with all the nutritional elements needed, provided that the balance in the components of those media is taken into account [9]. The nature of the nutritional media and the concentrations of added growth regulators affect the success of the plant tissue culture process [10]. Auxins and cytokinins are among the most important components of the nutritional media that affect the success of tissue culture [11]. The type and concentration of cytokinins are among the most important main factors that affect the success of plant tissue culture, and the balance between internal plant hormones and added growth regulators is essential for the success of palm tissue culture [12]. Through the above, we decided to use three nutritional media, namely Woody plant media, B5 media, and MS media, in order to compare them, in order to know the difference in the results that will be obtained compared to the MS nutritional media, as it is the most widely used nutritional media in tissue culture in producing and germinating embryos.

Materials and Methods

The study was conducted in the Plant Tissue Culture Laboratory of the Palm Research Center, University of Basra during the period 2022-2023**.**

Preparation and sterilization of cultural media

Three nutrient media were used in agricultureMS [13] ,WPM Woody plant media [14], and B5 [15], manufactured by the American company Caisson Lab. The media salts and growth regulators were added after they were prepared as basic solutions, sucrose and agar Table (1). The pH of the nutrient media was adjusted to 5.7 by add-

ing drops of sodium hydroxide solution or 0.1 N hydrochloric acid. The concentrations of the added cytokinins were (0, 0.5, 1, 2) mg L^{-1} for each of Zeatin and 2ip separately with a fixed concentration of auxin (NAA) in all research experiments in the nutrient media at a concentration of 1 mg L^{-1}. The nutrient media was heated to 90 °C using a heater equipped with a magnetic mixer, then the nutrient media was distributed in 500 ml jars and the media was distributed in them at a rate of 50 ml/jar and closed with plastic covers, then placed in the autoclave and sterilized at a temperature of 121 °C and an atmospheric pressure of 1.05 kg cm^{-2} for 20 minutes. Then it was extracted from the sterilizer, shaken and left to cool and solidify until planting.

Culture of the primary callus

Culture of the primary callus resulting from the culture of the flower spikes [16] of the Barhi cultivar on the nutritional media MS, WPM, B5 mentioned in Table (1) and prepared with concentrations of 0, 0.5, 1 and 2 mg L^{-1} for each of Zeatin and 2ip, the culture was carried out in the air flow table that was sterilized before planting and the crops were incubated in the growth room at a temperature of 1±27 °C and lighting intensity of 1000 lux for 16 hours and 8 hours of darkness, where the replanting process was once every 4-5 weeks until embryos were formed, where their measurement was taken through daily observations.

Table(1) : The media prepartions

Material	Quantity/ mg.L^{-1}
Sucrose	30000
Sodium hydrogen ortho phosphates	170
Adenine sulphates	40
Riboflavin	1
Glutamine	100
Activated charcoal	1000
Agar	6000

Formation of embryos

For the purpose of embryos formation from callus mentioned in paragraph (1), embryos were randomly selected that were identical in length and size as much as possible. They were grown on the nutritional media mentioned in Table (1) and provided with two types of auxins NAA and IBA at three concentrations for each (0, 0.1 and 0.5) mg L^{-1}, with the addition of materials as in Table (1). The following measurements were taken:

1- percentage of embryos
2- Number of roots
3- Root length

Statistical analysis

All experiments were implemented using the Completely Randomized Design (CRD) and factorial experiments. The significance of the averages was tested according to the Least Significant Difference (L.S.D) test at a probability level of 0.05. The statistical analysis program Genstat V.3 was used to analyze the results according to what was stated in [17].

Results and Discussion

The effect of the type of media and cytokines and its concentration on the rate of the number of embryos

The results shown in Table (2) the effect of the nutritional media and cytokines and the interaction between them on the rate of the number of embryos. The results showed that the WPM and MS media gave the highest rate of the number of embryos produced, which amounted to 7.45 and 7 embryos, with a significant difference from the B5 media, which gave the lowest rate, which amounted to 4.92 embryos. The results also showed that there were no significant differences between the type of cytokines in the rate of the number of embryos produced, while the results showed that the concentrations of 0 and 0.5 mg.L^{-1} were superior in the rate of the number of embryos, which amounted to 7.13 embryos, with a significant difference from the rest of the concentrations. The results also showed that there were no significant differences with statistical significance in the interaction between the type of the nutritional media and cytokines, while the results of the interaction between the type of media and the concentration of the growth regulator showed significant differences in the rate of the number of embryos produced, as the WPM nutritional media provided with a concentration of growth regulator 0 and 0.5 mg.L^{-1} gave the highest rate of the number of embryos, which amounted to 8.6 and 8.7 embryos, respectively, followed by the MS nutritional media provided with the same concentrations, with a rate of 7.4 and 8 embryos. There was a significant difference from the rest of the interactions of the same two media and the B5 media with a concentration of 2 mg L^{-1}, which gave the lowest rate of 4.5 embryos. As for the effect of the triple interaction between the type of media and the type and concentration of growth regulator, there were no significant differences with statistical significance in the average number of embryos produced. The plant parts grown in vitro are a waste in their nutrition, depending on what the nutrient media provides them with of organic compounds that lead to their growth and development [18]. The development of embryogenesis occurs as a result of reducing the concentration of cytokines due to the long period of culture or as a result of its consumption and spread in the cells of the growing tissue or its absorption by activated charcoal [19 , 20]. After the regulator runs out in the nutrient media, the development of spherical embryos occurs by stopping division, as its meristematic pole begins to divide and grow, accompanied by the rupture of the hard shell of the spherical embryo, thus elongating the cotyledon and the appearance of the cylindrical shape of the embryo [21]. The reason for the increase in the rate of embryogenesis in the MS and WPM media may be due to their content of nutrients such as nitrates, po-

tassium sulfate, magnesium sulfate and manganese sulfate compared to the B5 media, and the importance of Sulfate salts in the formation and maturation of embryos, as [22]. stated that the nutritional media containing sulfate salts was suitable for the differentiation of embryonic callus into embryogenesis and their maturation compared to the media that were free of them, as the role of sulfates in the differentiation of embryogenesis of date palms may be due to the role of sulfur in the composition of proteins that contain a high percentage of cysteine amino acid units [23]. in addition to the presence of the nitrogen element that helps embryonic callus cells to divide and grow because it enters into the construction of amino acids, nucleic acids and proteins or through its contribution to increasing the respiration processes and energy production (ATP) that is used in the growth and development processes [24] as [25] stated that reducing the source of nitrogen in the nutritional media leads to competition between cells for food, and increasing the concentration of ammonium nitrate in the media negatively affects cell growth because ammonium From a toxic effect when its concentration increases [26]. These results were consistent with what [22,27] reached, and [28].

Table (2): the effect of the type of nutritional media, cytokines concentration, and their interactions on the number of embryos.

Media type (A)	Cytokinin type (B)	Cytokinin concentration (mg/L C)			averag A e	averag B e	A B
		0	1	2			
MS	2ip	7.4	6	6	7	6.42	6.8
	Z	7.4	6.6	6.6		6.5	7.2
MSC		7.4	6.3	6.3			
WPM	2ip	8.6	6.2	6.2	7.45		7.45
	Z	8.6	5.6	7			7.45
WPMC		8.6	5.9	6.6			
B5	2ip	5.4	5.2	4.4	4.92		5
	Z	5.4	5	4.6			4.85
B5C		5.4	5.1	4.5			
BC	2ip	7.13	5.8	5.53			
	Z	7.13	5.73	6.07			
C average		7.13	5.77	5.8			
L.S.D.= 0.05	A=0.521		AB=NS				
	B=NS		AC=1.043				
	C=0.602		BC=NS				
	ABC=NS						

Effect of the type of media and auxin on the percentage of embryos (%)

The results shown in Table (3) indicate the effect of the type of nutrient media, auxin, its concentration, and the interaction between them on the germination percentage rate of embryos. The results showed that the WPM nutrient media recorded the highest germination percentage of embryos, reaching 41.7%, with a significant difference from the MS media, which recorded the lowest percentage of 30%. In contrast, the type of auxin added to the media did not record any significant effect, while the concentration of 0.5 mg L-1 significantly exceeded the germination percentage rate, reaching 48.8% compared to the comparison treatment, which gave the lowest percentage of 15%. In contrast, the effect of the binary and triple interactions did not record any significant differences with statistical significance in the germination percentage rate. Many studies related to plant tissue culture have indicated that reducing the concentration of major salts in the MS media leads to a significant improvement in the germination or rooting percentage. In this study, two types of nutrient media were used, and the WPM media has a low content of ammonium nitrate compared to the MS media. The occurrence of rooting may be due to the reduction in the concentration of the total nitrogen element [29]. The reason for the superiority of embryos from the media supplied with plant growth regulators in the percentage of embryo germination may be due to the effect of the auxin concentration in the media in which they were formed, which stimulated the embryo cells to divide and grow [30]. This result was consistent with what was reached by [8 , 31].

Table (3): The effect of the type of nutrient media, auxin, its concentration and their interactions on the percentage of germination

Media type ((A)	Auxin type(B)	Auxin concentration mg L-1(C)			average A	average B	A B
		0	0.1	0.5			
MS	NAA	10	35	45	30	34.2	30
	IBA	10	40	40		37.5	30
MSC		10	37.5	42.5			
WPM	NAA	20	40	55	41.7		38
	IBA	20	60	55			45
WPMC		20	50	55			
BC	NAA	15	37.5	50			
	IBA	15	50	47			
C average		15	43.8	48.8			
L.S.D.= 0.05	A=6.50			AB=NS			
	B=NS			AC=NS			
	C=7.97			BC=NS			
	ABC=NS						

Effect of the type of media, auxin and their concentration on the average number of roots

The results shown in Table (4) indicate the effect of the type of nutrient media, auxin and its concentration and the interaction between them on the average number of roots. The results of the statistical analysis showed that the type of nutrient media had a significant effect on the average number of roots formed, as the WPM nutrient media was excelled in giving the highest average number of roots, which amounted to 1.6 roots, with a significant difference from the MS nutrient media, which gave the lowest average of 1.38 roots. As for the effect of the type of growth regulator, the IBA auxin was excelled in giving the highest average of 1.6 compared to the NAA auxin, which gave an average of 1.38 roots. While the effect of the growth regulator concentration, the concentration of 0.1 and 0.5 mg L^{-1} was excelled in giving the highest average of 1.7 and 1.65, respectively, with a significant difference from the control treatment. As for the effect of the double and triple interactions, no significant differences were recorded in the average number of roots. Rooting occurs well in a nutrient media that contains a full concentration of nutrients, but there are many common experiments to transfer plant parts from a media with a high concentration of salts to a media with a low concentration for the rooting process [32]. Some researchers in their experiments have achieved a good germination rate, number and length of roots by reducing the concentrations of minerals in the MS media to half [8 , 31]. This difference in the results obtained may be because we used two types of the WPM and MS nutrient media in our experiments at full strength. [33] explained that the positive effect of reducing the concentration of salts in the nutrient media is due to reducing the nitrogen element and considering that the WPM nutrient media has a low concentration compared to the MS media, it improved the rooting process. This result was consistent with what [34].

Table (4): Effect of the type of media, auxin, its concentration and their interactions on the number of roots

Media type (A)	Auxin type(B)	Auxin concentration mg L-1(C)			average A	average B	AB
		0	0.1	0.5			
MS	NAA	1	1.4	1.4	1.367	1.367	1.267
	IBA	1	1.6	1.8		1.6	1.467
MSC		1	1.5	1.6			
WPM	NAA	1.2	1.6	1.6	1.6		1.467
	IBA	1.2	2.2	1.8			1.733
WPMC		1.2	1.9	1.7			
BC	NAA	1.1	1.5	1.5			
	IBA	1.1	1.9	1.8			

C average		1.1	1.7	1.65			
L.S.D.= 0.05	**A=0.191**			AB=NS			
	B=0.191			AC=NS			
	C=0.234			BC=NS			
	ABC=NS						

Effect of the type of media, auxin and their concentration on the average root length (cm)

The results shown in Table (5) indicate the effect of the type of nutrient media, auxin and its concentration, and their interaction on the average root length. The results of the statistical analysis showed that the type of nutrient media had a significant effect on the average root length formed, as the WPM nutrient media excelled in giving the highest average root length of 5.48 cm, with a significant difference from the MS nutrient media, which gave the lowest average of 5.07 cm. As for the effect of the type of growth regulator, no significant effect was recorded on the average root length, while the auxin concentration of 0.5 mg L^{-1} added to the nutrient media excelled in giving the highest average of 6.125 cm compared to the control treatment concentration, which gave the lowest average of 4.4 cm with auxin NAA, which gave an average of 1.38 roots. As for the effect of the interaction between the type of media and auxin, no significant differences were recorded in the average root length. While the effect of the double interaction between the type of media and the concentration of auxin added to the nutrient media showed a significant effect, as the WPM nutrient media provided with a concentration of 0.5 mg L^{-1} of auxin outperformed at a rate of 6.2 cm, followed by the MS media provided with the same concentration of auxin at a rate of 6.05 cm, with a significant difference from the rest of the interactions for both media. As for the effect of the triple interaction, no significant differences were recorded in the average root length. The rooting process occurs well in the nutrient media that contains a full concentration of nutrients, but there are many common experiments to transfer plant parts from a media with a high concentration of salt content to a media with a low concentration for the rooting process [32]. Some researchers in their experiments have reached a good germination rate, number and length of roots by reducing the concentrations of minerals in the MS media to half [8 , 31]. This difference in the results obtained may be due to the fact that we used two types of the WPM and MS nutrient media in our experiments at full strength. (33) explained that the positive effect of reducing the concentration of salts in the nutrient media is due to reducing the nitrogen element and considering that the WPM nutrient media has a low concentration compared to the MS media, it improved the rooting process. This result was consistent with what [34 , 35] .

Table (5): Effect of the type of media, auxin, its concentration and their interactions on the length of roots (cm)

Media type ((A)	Auxin type (B)	Auxin concentration mg L-1 (C)			average A	average B	AB
		0	0.1	0.5			
MS	NAA	4.2	5.1	6	5.067	5.35	5.1
	IBA	4.2	4.8	6.1		5.2	5.033
MSC		4.2	4.95	6.05			
WPM	NAA	4.6	5.9	6.3	5.487		5.6
	IBA	4.6	5.4	6.1			5.367
WPMC		4.6	5.65	6.2			
BC	NAA	4.4	5.5	6.15			
	IBA	4.4	5.1	6.1			
C average		4.4	5.3	6.125			
L.S.D.= 0.05	A=0.157			AB=NS			
	B=NS			AC=0.272			
	C=0.193			BC=NS			
	ABC=NS						

The results demonstrated that the food medium WPM gave an average of the number of embryos, the highest percentage of embryo germination, the number and length of the roots, with a significant difference from the MS mean, which recorded the lowest germination rate, number and length of the root, while it did not differ significantly from the medium wpm in the number of embryos.

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