AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

Effect Type of Nutrient Medium and Cytokinin on the Production and Development of the Embryonic Callus of *Phoenix dactylifera* L., Albarhi date Palm Variety, in Vitro

Mohammad H. Tuaimah[1], Osama N. Jaafar[2] and Manal Z. Sabti[3]

[1,2]Palm Research Centre, University of Basrah, Basrah, Iraq.
[3]College of Agriculture, University of Basrah, Basrah, Iraq.

[1]E-mail: mhady4822@gmail.com
[2]E-mail: dr.osama74@gmail.com
[3]E-mail: manal.al_myhi@uobasrah.edu.iq

Abstract. The study was carried out in the tissue culture laboratory of the Palm Research Center, University of Basra for the year 2022-2023, as three types of food media were used, namely MS, WPM and B5, with two types of cytokines Zeatin and 2ip with four concentrations of each (0, 0.5, 1, 2) mg L^{-1} in the production of fetal callus and its development into vegetative embryos for the Barhi variety outside the vivo. The results showed that the nutrient medium WPM and MS recorded the shortest period for the appearance of embryo callus and period of appearance of embryos and an increase in fresh and dry weight and amounted to 39.22, 40.32 days, 30.4, 31.18 days, 1.55, 1.58 g, 0.067 and 0.087 g, respectively, with a significant difference from the B5 medium in all studied traits. The results showed that the medium WPM and MS equipped with cytokinin 2ip and Zeatin at a concentration of (0 and 0.5) mg $liter^{-1}$ recorded the shortest period of appearance of embryo callus and amounted to 36 and 38.2 days 36.4 and 38.8 days respectively and a significant difference from the rest of the interventions and it was noted that the superiority of the medium MS equipped with 2ip at a concentration of 0.5 mg $liter^{-1}$ and the medium of WPM Zeatin at a concentration of 0.5 mg $liter^{-1}$ in the average fresh weight and amounted to 2.23 g and 2.03 g, While it was noted that the MS medium with 2ip at a concentration of 0.5 mg L^{-1} gave the highest rate of dry weight and a significant difference from the rest of the interactions of another medium.

Keywords. Nutrient medium, WPM; Zeatin, Embryonic callus, Cytokinin.

1. Introduction

Date palm (*Phoenix dactylifera* L.) is a monocotyledonous, diploid, monoecious, dioecious plant and is one of the most important fruit crops due to its nutritional and economic value [1]. It is grown in many countries of the arid regions of West Asia, North Africa, and the Middle East [2]. Plant tissue culture is one of the most important and recent scientific and applied specialties, and its importance has emerged as an alternative method to traditional methods in the multiplication and genetic improvement of plants of all kinds[3,4].

Researchers in most countries of the world have been able to harness this technology for the widespread propagation of plants, and plant tissue culture has proven its efficiency in terms of the number of plants that can be produced from one plant and the matching of the resulting plants to

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

their genetic origins [5]. Date palms are multiplied tissue-cultured either by organogenesis (Organogenesis) from the cultivation of the growing apex and axillary buds or by somatic embryogenesis (Somatic embryogenesis) by passing through the callus stage from which the green embryos are formed by planting plant tissues in sterile industrial nutrient media [6].

The induction of callus and the production of somatic embryos have been stimulated by many researchers for date palms, such as [7,8]. Nutrient media are one of the most important factors that determine the success of tissue culture, as they play an active role in growth, specialization, and division by providing the plant part with all the nutritional needs, provided that the balance in the components of those media is taken into account[9].

The nature of the nutrient medium and the concentrations of growth regulators added affect the success of plant tissue culture [10]. The type and concentration of cytokinin are one of the most important 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 date palm tissue culture[11] . Auxins and cytokines are the most important components of the nutrient medium that affect the success of tissue culture [12]. Auxins play a fundamental role in the formation of callus and its development into green embryos and their germination, while cytokines are important factors in the induction of lateral and adventitious buds[13,14]. Kin. Zeatine, BA, and 2ip are among the most commonly used cytokines[15] .

Based on the above, we decided to use three nutrient media, namely the Woody plant medium, the B5 medium, and the MS medium, for comparison in order to know the difference in the results that will be obtained compared to the MS nutrient medium, as it is the most commonly used nutrient medium in tissue culture, in terms of the period for inducing embryonic callus and the appearance of green embryos and its development into green embryos that later give seedlings ready for acclimatization.

2. Material and Methods

The study was carried out in the Plant Tissue Culture Laboratory of the Palm Research Center at the University of Basrah during the period 2022-2023.

2.1. \ Preparation and Sterilization of Culture Media

Three nutrient media were used in the cultivation, namely the MS medium [13] the Woody plant medium [16] which is denoted as WPM, and the B5 mediumwhich is denoted as B5, and manufactured by the American company Caisson Lab. with vitamins as shown in Table (1). Nutrient medium salts and growth regulators were added after they were prepared as basic solutions, sucrose, and agar, as shown in Table (2). The pH of the nutrient medium was adjusted to 5.7 by adding drops of 0.1 N sodium hydroxide or hydrochloric acid solution. The concentrations of cytokines added were (0, 0.5, 1, 2) mg l^{-1} for each of Zeatin and 2ip, each separately, with a constant concentration of the auxin Naphthalene Acetic Acid (NAA) in all research experiments in the nutrient medium at a concentration of 1 mg l^{-1}. The nutrient medium was heated to 90 °C using a heater equipped with a magnetic stirrer; then the nutrient medium was distributed in 500 ml jars and distributed at a rate of 50 ml/jar, closed with plastic lids, then placed in an autoclave and sterilized at a temperature of 121 °C and atmospheric pressure of 1.05 kg cm-2 for 20 minutes. After that, it was taken out of the sterilization device, shaken, and left to cool and solidify until the time of cultivation.

2.2. Primary Callus Culture

The primary callus resulting from the cultivation of the floral shoots of the Barhi variety obtained from the Date Palm Research Center/Tissue Culture Laboratory was planted on MS, WPM, and

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

B5 nutrient media equipped with concentrations of 0, 0.5, 1, and 2 mg l^{-1} for each of Zeatin and 2ip, with a weight of 50 mg callus per jar. The cultures were incubated in the growth chamber at a temperature of 27 ± 1 °C and a light intensity of 1000 lux for 16 hours of light and 8 hours of darkness. After the formation of the embryonic callus, it was transferred to another medium to stimulate the formation of embryos, which included the same components of the previous medium with the stability of growth regulators to show the effect of the medium in this regard. The replanting process was once every 4-5 weeks. The data and observations were recorded daily, represented by the duration required to start the formation of the embryonic callus, the appearance of the embryos and their number. After two months of the experiment, the following traits were measured:

- The duration required for the start of the formation of the embryonic, callus (days).
- Calculation of the fresh and dry weight of the embryonic callus (g).
- The duration required for the appearance of green embryos (days).

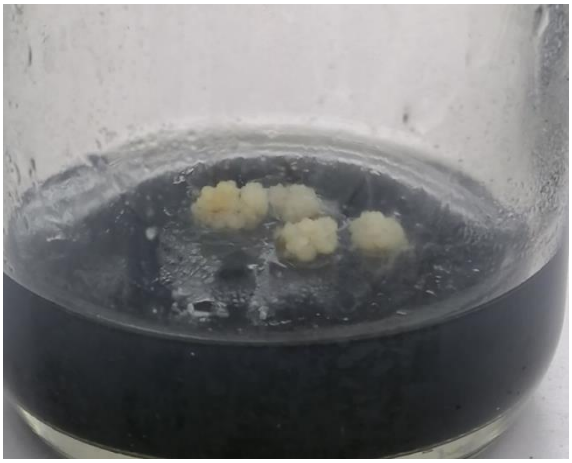

Figure 1. Cultivation of primary callus.

Figure 2. Effect of type of nutrient medium and cytokines at different concentrations On the start of the appearance of embryonic callus in date palms.

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

Table 1. Composition of nutrient media based on (mg l^{-1}).

Substance name	MS	WPM	B5
A- Macronutrients			
NH_4No_3 Ammonium nitrate	1650	400	-
Calcium nitrate $CaNo^3$	-	556	-
Potassium nitrate KNo_3	1900	-	2500
Ammonium sulfate So_4No_3	-	-	134
KSo_4 Potassium sulfate		990	
Magnesium sulfate $MgSo_4.H_2O$	370	180	122
Calcium chloride $CaCl_2$	440	72,47	113,24
Potassium phosphate $KHPo_4$	170	170	-
Sodium phosphate $NaHPo_4$	-	-	150
Micronutrients B-			
Boric acid H_3BO_3	6,20	6,20	3
Cobalt chloride $CoCl_2.6H_2O$	0,025	0,160	0,025
Manganese sulfate $MnSo_4$	22.3	22,30	10
Copper sulfate $CuSo_4.5H_2O$	0,025	0.160	0,025
Sodium molybdate $NaMoO_4.2H_2O$	0,25	0,25	0,25
Potassium iodide KI	0.83	-	0.75
Zinc sulphate $ZnSo_4.7H_2O$	8,60	8,60	2
Aqueous ferrous sulfate $FeSo_4.7H_2O$	27,84	27,85	27,80
chelating substance Na_2EDTA	36,7	37,30	37,26
C- Vitamins and amino acids			
Thiamine HCl	0.5	1	10
Nicotinic acid	0.5	0.5	1
Pyridoxine	0.5	0.5	1
Claesin	2	2	-
My inositol	100	100	100

Table 2. Concentrations of substances added to the nutrient medium.

Substance	Quantitative mg l^{-1}
Sucrose	30000
Sodium hydrogen orthophosphates	170
Adenine sulphates	40
Amino acid glutamine	100
Activated charcoal	1000
Agar	6000

2.3. Statistical Analysis

All experiments carried out a completely randomized design (CRD) with factorial experiments, and the significance of the means was tested according to the least significant difference (L.S.D) test with a probability level of 0.05. The statistical analysis program GenstatV.3 was used to analyze the results, according to what was stated [17].

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

3. Results and Discussion

3.1. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Rate of the Period Required for the Appearance of Embryonic Callus (day)

Table (3) results indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for the appearance of embryonic callus (day). The results showed that the WPM medium recorded the shortest period for the onset of fetal callus, followed by the MS median, amounting to 39.22 and 40.32 days, respectively, with a significant difference from the B5 mean, which recorded the longest period, reaching 54.67 days, while the effect of the growth model type was not recorded, giving any statistically significant differences, as the results showed that the effect of cytokinin concentration in the period required for the appearance of sexual callus, as it was noted that the concentration of 0.5 mg liter^{-1} recorded the shortest period and amounted to 42.20 days in a row compared to the rest of the concentrations. The results of the interference between the type of medium and cytokinin showed that the WPM medium, equipped with cytokinin Zeatin 2ip, had recorded the shortest period required to start fetal callus, amounting to 38.85 and 39.6 days, respectively, followed by MS nutrient medium with cytokinin 2ip and olives, with a rate of 39.8 and 40.85 days, respectively, while the longest period for the appearance of embryonic callus was 56 days recorded for developing callus on the B5 nutrient medium with cytokinin 2ip. As for the effect of interaction between the type of medium and the concentration of cytokinin, the results showed that the WPM medium with a concentration of 0 and 0.5 mg liter^{-1} with the lowest period of embryonic callus appearance was 36 and 37.1 days respectively compared to other interactions of the same medium and the medium B5 and MS except for the MS medium equipped with a concentration of 0.5 mg.L^{-1} gave the shortest period of embryonic callus appearance, reaching 36.3 days. As for the effect of overlapping the type of growth regulator and its concentration, the results showed that there are no significant differences, as for the effect of triple interference for the type of medium, the type of cytokinin, and concentration, it was found through the results that the shortest period of appearance of embryonic callus was recorded at the medium WPM and MS with cytokinin 2ip and olives 0 and 0.5 mg liter^{-1} and amounted to 36, 38.2 days, 40.2 and 36.4 days respectively, with a significant difference from the rest of the interactions for the same two mediums and the medium of B5. The plant parts grown outside the living body are lopsided in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development [18]. In addition, low concentrations were more responsive in the induction of embryonic callus This may be because high concentrations lead to a decrease in the rate of cell division and development[19]. As the medium WPM is considered a suitable nutrient medium for woody plants and its content of major nutrients is ammonium nitrate, calcium nitrate and potassium sulfate in appropriate quantities compared to the medium MS and B5, which lead to the growth and development of plant tissue, especially that the nitrogen element, which enters the construction of amino and nucleic acids and proteins, which encourages the growth and development of the plant part cultivated [20] as well as it was found that high concentrations of growth regulators lead to the death of callus tissue as a result of the release of ethylene in large quantities, which leads to rapid cell divisions and the occurrence of a state of imbalance of growth and lack of development and thus the death of the formed callus tissueIt was also noted that the addition of growth regulators in high concentrations to the nutrient medium higher than the ideal concentration leads to an impact on the work of enzymes responsible for the construction of the cell wall and their decomposition, which affects the mechanical properties. Of cell wall and influence on cell division and embryonic callus formation [21] and these results were consistent with what he found [22,23] .

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

Table 3. Effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for the appearance of embryonic callus (day).

Type Medium A	Type Cytokinin B	C concentration Cytokinin mg L^{-1} 0	1	2	Average A	Average B	A*B
MS	2ip	40.2	37.4	45.2	40.32	45.13	39.8
	Z	40.2	43.2	43.8		44.35	40.85
	MS*C	40.2	40.3	44.5			
WPM	2ip	36	43.2	43.2	39.22		39.6
	Z	36	40.2	41			38.85
	WPM*C	36	41.7	42.1			
B5	2ip	58.2	56.8	55	54.67		56
	Z	58.2	50.2	52.6			53.35
	B5*C	58.2	53.5	53.8			
B*C	2ip	44.8	45.8	47.8			
	Z	44.8	44.53	45.8			
	Average C	44.8	45.17	46.8			
L.S.D.= 0.05	A=1.254		A*B=1.773				
	B=NS		A*C=2.508				
	C=1.448		B*C=NS				
	A*B*C=3.546						

3.2. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Rate of the Fresh Weight of the Embryonic Callus (g)

The results of Table (4) indicate the effect of the type of nutrient medium and cytokinin, its concentration and the interaction between them on the average soft weight of embryonic callus (g). The results showed that the nutrient medium MS and WPM have given the highest rate of soft weight for fetal callus, as it reached 1.58 g and 1.55 g, respectively, with a significant difference from the medium B5, which recorded the lowest average soft weight of 1.256 g, as for the effect of the type of growth regulator, no significant differences were recorded, while the concentration of cytokinin recorded a significant effect, as the concentration exceeded 0.5 mg liter^{-1} for all concentrations at a rate of 1.73 g, The results of the interaction between the type of medium and cytokinin showed that there are no significant differences in the average soft weight of embryonic callus that the medium MS provider 2IP, while the effect of the interaction between the type of nutrient medium and the concentration of cytokinin. The results showed that the nutrient medium MS and WPM with a concentration of 0.5 mg liter^{-1} gave the highest rate of soft weight for fetal callus, as it amounted to 1.96 g and 1.95 g, respectively, with a significant difference from the rest of the interactions for the same two mediums and the medium of B5. The results of the effect of the interaction between the type of growth regulator and its concentration showed the superiority of the growth regulators Zayatin and 2ip at a concentration of 0.5 each by giving the highest rate of the soft weight of 1.723 and 1.796 g respectively and with a significant difference from the rest of the interactions The results of the triple intervention showed that the highest rate of the soft weight of embryonic callus has been observed in the MS nutrient medium with a concentration of 0.5 mg liter-1 of 2ip as it reached 2.23 g, followed by the WPM medium equipped with Zeatin at a concentration of 0.5 Amalgam L^{-1} gave an average soft weight of 2.03 g with a significant difference from the rest of the interventions, while the lowest soft weight was recorded at the medium B5 equipped with both types of cytokinin for all concentrations. Plant parts grown outside the living body are discarded in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development [24] The

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

reason for the superiority of the two mediums MS and WPM may be due to the difference in the composition of the components of the medium and their high content of macro and micronutrients compared to the medium B5, especially the element nitrogen, which is involved in the construction of amino and nucleic acids and proteins, which encourages the growth and development of the cultivated plant part [20] as well as the presence of growth regulators in low concentrations helps the embryonic callus to divide and grow, as auxin stimulates the formation of RNA as it leads to flexible energy that They are exploited by tissues for division and growth [24] and food media with a high content of growth regulators cause cell suppression, resulting in poor growth[25] and these results were in line with what he found [26].

Table 4. Effect of the type of medium, type of cytokinin, and its concentration on the rate of the fresh weight of the embryonic callus (g).

Type Medium	Type Cytokinin B	Cytokinin, Concentration mg L $^{-1}$ C			Average A	Average B	A*B
		0	1	2			
MS	2ip	1.598	1.65	0.936	1.584	1.421	1.603
	Z	1.598	1.394	1.576		1.507	1.565
MS*C		1.598	1.522	1.256			
WPM	2ip	1.488	1.586	0.936	1.552		1.471
	Z	1.488	1.554	1.458			1.633
WPM*C		1.488	1.57	1.197			
B5	2ip	0.995	1.168	1.304	1.256		1.188
	Z	0.995	1.56	1.294			1.324
B5*C		0.995	1.364	1.299			
B*C	2ip	1.36	1.468	1.059			
	Z	1.36	1.503	1.443			
Average C		1.36	1.485	1.251			
L.S.D.= 0.05	A=0.089 B=NS C=0.103	A*B=0.127 A*C=0.179 B*C=NS A*B*C=0.253					

3.3. Effect of the Type of Medium, Type of Cytokinin and its Concentration on the Rate of the Dry Weight of the Embryonic Callus (g)

The results shown in Table (5) indicate that the results of Table (4) indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them on the average dry weight of embryonic callus (g). The results showed that the food medium MS and WPM have given the highest rate of dry weight for fetal callus, as it amounted to 0.087 g and 0.067 g, respectively, with a significant difference from the medium B5, which recorded the lowest dry weight rate of 0.034 g, as for the effect of the type of growth regulator, no significant differences were recorded, while the concentration of cytokinin recorded a significant impact on the rate of dry weight, as the concentration exceeded 0.5 mg liter^{-1} for all concentrations at a rate of 0.10 g. The results of the interaction between the type of medium and cytokinin showed that the medium MS with growth regulator 2ip gave the highest rate of dry weight of 0.119 g compared to the same medium with olive and medium WPM and B5 equipped with both growth regulators, while the results of the effect of the interaction between the type of nutrient medium and the concentration of cytokinin The results showed that the MS nutrient medium with a concentration of 0.5 mg liter^{-1} gave the highest rate of dry weight for fetal callus, as it amounted to 0.167 g And a significant difference from the rest of the interferences for the same medium, WPM medium, and B5 medium. The results of the effect of the interaction between the type of

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

growth regulator and its concentration showed the superiority of the growth regulator 2ip at a concentration of 0.5 mg liter^{-1} by giving the highest rate of dry weight of 0.140 g and a significant difference from the remaining interactions for both growth regulators The results of the triple intervention showed that the highest rate of dry weight of embryonic callus has been observed in the MS nutrient medium with a concentration of 0.5 mg liter^{-1} of 2ip as it reached 0.289 g and a significant difference from the rest of the interventions. Plant parts grown outside the living body are discarded in their nutrition dependent on the organic compounds provided by the nutrient medium that lead to their growth and development [18]. Accordingly, the increase in the soft and dry weight of callus is a reflection of changes in the different contents of its cells depending on its growth in the quality of the food medium used and on the quality and concentration of growth regulators added, and generally accompanied by the process of cell division of callus an increase in the contents of the task to sustain division and growth such as proteins and amino acids with internal changes leading to division and growth and then specialization [29]. The reason for the superiority of the MS medium may be attributed to its high content of macro and micronutrients, especially nitrogen, which is involved in the construction of amino, and nucleic acids and proteins within the plant tissue, which encourages the growth and development of the cultivated plant part [18]. In addition the presence of growth regulators in low concentrations helps the embryonic callus to divide and grow, as auxin stimulates the formation of RNA as it leads to mRNA by providing energy that is exploited by tissues for division and growth[23] and the food media that contain a high content of growth regulators cause inhibition of cells, resulting in poor growth [26] and these results were in line with what he found [27].

Table 5. Effect of the type of medium, type of cytokinin and its concentration on the rate of the dry weight of the embryonic callus (g).

Type of Medium (A)	Type of cytokinin (B)	Concentration Cytokinin mg L^{-1} (C)			Average A	Average	A*B
		0	1	2			
MS	2ip	0.06	0.062	0.056	0.087	0.072	0.119
	Z	0.06	0.06	0.048		0.054	0.056
	MS*C	0.06	0.061	0.052			
WPM	2ip	0.06	0.06	0.056	0.067		0.066
	Z	0.060	0.066	0.064			0.069
	WPM*C	0.06	0.063	0.06			
B5	2ip	0.025	0.031	0.035	0.034		0.032
	Z	0.025	0.042	0.038			0.037
	B5*C	0.025	0.036	0.037			
B*C	2ip	0.048	0.051	0.049			
	Z	0.048	0.056	0.050			
	Average C	0.048	0.054	0.049			
L.S.D.= 0.05	A=0.023 B=NS C=0.027	A*B=0.033 A*C=0.046 B*C=0.038 A*B*C=0.066					

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

3.4. Effect of the Type of Medium, Type of Cytokinin, and its Concentration on the Period Required for the Appearance of Embryos (day)

The results of Table (6) indicate the effect of the type of nutrient medium and cytokinin, its concentration, and the interaction between them in the period required for required of embryos (day) The results showed that the medium WPM and MS recorded the shortest period for required of vegetative embryos amounted to 30.4 and 30.18 days respectively, with a significant difference from the food medium B5, which recorded the longest period of 41.75 days. As for the effect of the type of growth regulator, there were no statistically significant differences, while the effect of concentration of the growth regulator gave a significant effect, as the results showed that the concentrations of 0 and 0.5 mg liter^{-1} had a significant superiority by giving the least period of emergence of vegetative embryos compared to other concentrations, as it reached 33.93 and 33.63 days respectively. As for the effect of the interaction between the type of medium and the growth regulator, no significant differences were recorded, while the results of the interaction between the type of food medium and the concentration of the growth regulator showed significant differences, as it was noted that the MS medium with a concentration of 0 and 0.5 mg liter^{-1} had recorded the shortest period of embryo appearance, amounting to 28.8 and 30.4 days, respectively, followed by the WPM food medium with a concentration of 0.5 and 0 mg liter^{-1} at a rate of 29 30.2 days respectively and a significant difference from the rest of the interventions for the same Middle and middle B5. As for the effect of bilateral interference between the type of growth regulator and its concentration, the results showed no significant differences, and the results of the triple overlap showed no statistically significant differences in the average period of emergence of vegetative embryos. The plant parts grown outside the living body are thrown in their nutrition depending on the organic compounds provided by the nutrient medium that lead to their growth and development, as well as the development of vegetative embryos occurs as a result of the reduction of cytokinin concentration due to the long period of implantation or as a result of its consumption and spread in the cells of the growing tissue or its absorption by activated charcoal [29,30]. After the exhaustion of the growth regulator in the nutrient medium, the development of spherical embryos occurs by stopping them about division as its pole begins to meristem division and growth accompanied by rupture of the solid shell of the embryo spherical and thus occur elongation of cotyledon and required of the cylindrical shape of the embryo [5] The reason for the superiority of the two mediums MS and WPM may be due to the difference in the composition of their components of macro and minor nutrients, for example, the presence of potassium nitrate and calcium nitrate compared to the medium B5 or their high content of elements, especially the element nitrogen, which is involved in the construction of amino and nucleic acids and proteins, which encourages the growth and development of the cultivated plant part [18] as well as these results were in line with what he found [27].

Table 6. Effect type of nutrient medium, cytokinin, its concentration and their interactions on the period required for the appearance of embryonic callus (day).

(A) type of medium	(B) Type of cytokinin	Cytokinin of concentration mg L^{-1} (C)			Average A	Average B	A*B
		0	1	2			
MS	2ip	28.8	32	34.4	31.18	43.63	31.45
	Z	28.8	33.4	31.2		34.25	30.9
MS*C		28.8	32.7	32.8			
WPM	2ip	30.2	32.2	31.2	30.4		30.65
	Z	30.2	30.4	31			30.15
WPM*C		30.2	31.3	32.8			
B5	2ip	42.8	41.6	41.2	41.75		41.8
	Z	42.8	42	40.6			41.7

AL-KUNOOZE SCIENTIFIC JOURNAL
ISSN 2706-6231 (ONLINE)
ISSN 2706-6223 (PRINT)
6th International Conference of Modern Technologies in Agricultural Sciences,
18-19 December 2024, Kufa, Najaf, Iraq

	B5*C		42.8	41.8	40.9
B*C		2ip	33.93	35.27	35.6
		Z	33.93	35.72	34.27
	Average (C)		33.93	35.27	34.75
L.S.D.= 0.05		A=0.657		A*B=NS	
		B=NS		A*C=NS	
		C=0.759		B*C=1.073	
			A*B*C=NS		

Conclusion

The results demonstrated that the shortest embryo callus appearance period was recorded by the WPM and MS mediums supplemented with cytokinin 2ip and Zeatin at concentrations of 0 and 0.5 mg liter^{-1}, respectively, with durations of 36 and 38.2 days. The average fresh weight was 2.23 g for the MS medium and 2.03 g for the WPM medium, while the highest rate of dry weight was observed in the MS medium supplemented with 2ip at a concentration of 0.5 mg L^{-1}, distinguishing it significantly from the other interventions.

References

[1] Vardareli, N., Do˘garo˘ glu, T., Do˘ gaç, E., Ta¸skın, V. and Göçmen Ta¸skın, B. (2019). Genetic characterization of tertiary relict endemic Phoenix theophrasti populations in Turkey and phylogenetic relations of the species with other palm species revealed by SSR markers. Plant Syst. Evol., 305: 415–429.

[2] Trigiano, R.N., and D.J.Gray. (2000). Plant Tissue Culture Concepts and Laboratory Exercises. CRC Press LLC, USA.p.44-60.

[3] Al-Mayahi, Ahmed Wahid Madi (2010). Effect of NAA, 2ip, Inorganic Sulfate, PEG and adenine sulfate on the maturation of vegetative embryos of date palm Alkantar variety abundant outside the vivo. Basra Journal of Date Palm Research.9 (1)2010.

[4] Al-Qatrani, M. K. J., Al Khalifa, A. A. S., & Obaid, N. A. (2021). Effect of Jasmonic acid on stimulating the growth and development of date palm callus (Phoenix dactylifera L.) cultivar Shukar in vitro under salt stress conditions. IOP Conference Series. Earth and Environmental Science, 923(1), 012017. https://doi.org/10.1088/1755-1315/923/1/012017

[5] Al-Khayri, J. M. And Naik, P. M. (2017).Date Palm Micropropagation: Advances and Applications', Ciência E Agrotecnologia, 41(4), Pp. 347–358.

[6] Khan, A. (2009) 'In vitro micropropagation of "Khalas" date palm (phoenix dactylifera l .), an important', in vitro, 17(1), pp. 15–27.

[7] Al-Mir, O. N. J. (2020). The effect of cytokinin and auxin on the growth and rooting of vegetative branches of the two varieties of date palm, Halawi and Ashqar, ex vivo, Basra Journal of Date Palm Research, 19 (1) 2020.

[8] Eke CR, Akomeah P, Asemota. O (2005). Somatic embryogenesis in date palm (Phoenix dactylifera L.) from apical meristem tissues from 'zebra and 'loko' landraces. Afr J Biotechnol. 4: 244-246.

[9] El-Dawayati,M.,Baki,M.A.A And Abdelgalil,L.M.(2018).Effect Of Different Conservation Periods with Different Surface Concentrations On Conserving Somatic Embryo Clusters Of Date Palm (Phoenix Dactylifera L.)Under Minimal Growth Conditions.Applied Science Reports 21(1) 14-21.

[10] Ibrahim, M. A., Waheed, A. M. and Al-Taha, H. (2013). Plantlet regeneration from root segments of Date palm tree (Phoenix dactylifera L. cv. Barhee) produced by in vitro culture. A. A. B. Bioflux., 5(1): 45–50

[11] Al-Asadi, A. Z., Abdul Wahid, A. H., & Al-Mayahi, A. M. (2019). The Effect of Thidiazuron on Callus and in vitro Shoots Development of Date Palm (Phoenix dactylifera L.) cv. Barhee. Basrah Journal of Agricultural Sciences, 32, 258–265. https://doi.org/10.37077/25200860.2019.170

[12] Bhatia, S. And Bera, T. (2015). Somatic Embryogenesis and Organogenesis, Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. Elsevier Inc. Doi: 10.1016/B978-0-12-802221-4.00006-6.
[13] Al-Rawi, K. M., and Khalaf Allah, M. A. (2000) Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul. 488 p.
[14] Corchete, M.P., J.M.Sanchez.C.Cacho, M.Moran, and J.F.Tarrag (1990). Ardionlide content in cultures derived from root and leaf callus of digitalis therapists L.J.Plant Physiol.137.196-200Lioyd, G.; and McCown, B. (1981). Commercially feasible micropropagation of mountain laurel, Lamia Latifolia, by use of shoot tip culture. Proc. Inter Plant Propagation Soc, 30: 421-427.
[15] Al-Rawi, K. M., and Khalaf Allah, M. A. (2000) Design and analysis of agricultural experiments. Ministry of Higher Education and Scientific Research, Dar Al-Kutub Foundation for Printing and Publishing, University of Mosul. 488 p.
[16] Krueger, R. R. (2021). Date Palm (Phoenix dactylifera L.) Biology and Utilization. In The Date Palm Genome, Springer: New York.
[17] Trigiano, R.N. and D .j. Gray.(2005). Plant Development and Biotechnology.CRC PRESS LLC.
[18] Hopkins, W.G., and N.P.A.Huner. (2004). Introduction to Plant Physiology. The University of Western Ontario .PP.67-70.
[19] Taiz, L and E, Zeiger. (2006). Plant Physiology. Sinauer Associates, Inc Publishers. Sunderland. USA .p. 290-300.
[20] Sharif, Hussein Jassim and Al-Mayahi, Ahmed Madi Waheed and Mohsen, L. Q. (2016). Micropropagation of the agricultural date palm Pholifera L. Persian cultivar ex vivo. Dhi Qar University Journal of Agricultural Research 5(1):472-484.
[21] Mohsen, K. A. (2004) Studies on improving the formation of somatic embryos and their germination of date palm Phoenix dactylifera L. Barhi cultivar ex vivo, Master's thesis - College of Agriculture - University of Basra - Iraq 78. s.
[22] Krueger, R.J., and D.P.Carew. (1978). Catharanthus roseus tissue culture: The effects of precursors on growth and alkaloids production.Liodia 41:327-331.
[23] Mohsen, K. A., Abbas, M., Batoul, H., Faleh, A. (2014) The effect of yellow corn extract and naphthalene acetic acid on the development of embryonic callus and the formation of vegetative embryos and their germination of date palm Barhi cultivar ex vivo, Basra Research Journal (Al-Alamiyat), Issue 40, Part B.4: 15-23.
[24] Mohsen, K. A. (2007). Date palm Phoenix dactylifera L. Sharifi variety of various extravivo apical parts. Basra Journal of Date Palm Research.6(1):64-80.
[25] Hamad, Muhammad Shehab and Jassim, Noura Jabr (2011). The effect of components of the nutrient medium and plant part on callus induction of belladonna plants ex vivo. Iraqi Agricultural Sciences Journal.42(3):59-70.
[26] Wiesman, Z.; Riov, J. and Epstein, E. (1989). Characterization and rooting ability of indol-3-butyric acid conjugates formed during rooting of mung bean cuttings.Plant Physiol. 91: 1080-1084.
[27] Saad, A. & Elshahed,A.(2012).plant tissue culture media.In: Leva, A. and Rinaldi, L.M.R.,(eds)recent advanced in plant in Vitro Culture, chap. 2, Intech, Winchester, pp 29-40.
[28] Khalil, A. I. (2002) Using some alternatives to plant growth regulators in propagating the date palm Phoenix dactylifera L. ex vivo. Master's thesis, Department of Horticulture and Palm Trees, College of Agriculture, University of Basra, Iraq. Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture Physiol. Plaut.15:473-497.
[29] Lioyd, G.; and McCown, B. (1981). Commercially feasible micropropagation of mountain laurel, Lamia Latifolia, by use of shoot tip culture. Proc. Inter Plant Propagation Soc, 30: 421-427.
[30] Sen, M. K., Nasrin, S., Rahman, S. and Jamal, A. H. (2014). In vitro callus induction and plantlet regeneration of Achyranthes aspera L., a high-value medicinal plant. Asian Pacific Journal of Tropical Biomedicine, 4(1): 40–46