EFFECT OF SODIUM CHLORIDE ON THE GROWTH OF EMBRYOGENIC CALLUS FOR DATE PALM (BARHI CULTIVAR) AFTER CRYOPRESERVATIVE

Nahla H. Hussein* Hussam S.M. Khierallah**
*Dept. Hort. and Landscape Gardening, College of Agriculture, University of Baghdad.
* Date Palm Research Unit, College of Agriculture, University of Baghdad.

ABSTRACT

The current study was conducted for the period from October 2014 until July 2017 in order to increase the susceptibility of date palm (*phoenix dactylifera* L). to withstand the water stress using plant tissue culture and treated with some materials used for cryopreservative , The embryonic callus from the cultivating of the Shoot tips, planted on the Murashige and Skoog media, was subjected to immersion in preservative solution (Glycerol, PEG and DMSO) and cultivation on a sucrose-containing medium (342.3 $g.L^{-1}$) for seven days at 27ºC in the dark, to save the embryonic callus in liquid nitrogen at -196ºC for 6 and 12 weeks, The callus was cultured on media included sodium chloride at concentrations 0, 1500, 3000 and 4500 ppm. The effect of sodium chloride on the growth rate and fresh weight of embryonic callus and number of embryos. The results showed that the effect of tri-interactions between sodium chloride and preservative solutions and concentrations was significant in increasing the number of embryos. The interaction treatment between sodium chloride 1500 ppm, DMSO and 10% concentration was excelled by recording it as the highest number of embryos was 23.2 embryo. The triple interaction treatment between 3000 ppm sodium chloride, DMSO, 10% concentration has excelled by giving it the highest growth rate 0.0372 $mg.day^{-1}$, while the interaction treatment between 4500 ppm sodium chloride, PEG and 10% concentration recording the lowest growth rate 0.0002 $mg.day^{-1}$. The interaction treatment between control and sodium chloride with its three concentrations, DMSO, PEG and 20% concentration didn't recorded any growth rate.

Keywords: *Phoenix dactylifera*, somatic embryogenesis, cryopreservative.
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تاثير كلوريد الصوديوم في نمو الكالس الجنيني لنخيل التمر صنف برحي بعد الحفظ بالتجميد الفائق

نهلة حمودي حسين حسام سعد الدين محمد
مدرس مساعد استاذ مساعد
قسم البستنة وهندسة الحدائق- كلية الزراعة – جامعة بغدادوحدة أبحاث النخيل والتمور كلية الزراعة - جامعة بغداد

الخلاصة

اجريت الدراسة الحالية للمدةمن تشرين اول 2014 حتى تموز 2017 بهدف زيادة قابلية الكالس الجنيني لنخيل التمر *Phoenix dactylifera* L. على تحمل الاجهاد الملحي وذلك باستخدام زراعة الانسجة النباتية والمعاملة ببعض المواد المستخدمة للحفظ بالتجميد , عُرِضَ الكالس الجنيني الناتج من زراعة البراعم الطرفية(Shoot tips) والمزروعة على وسط Murashige and Skoog الى الغمر بمحاليل الحفظ (الكليسيرول،PEG و DMSO) والزراعة على وسط حاو على السكروز بتركيز (342.3 غم.لتر$^{-1}$) لمدة سبعة ايام وعلى درجة حرارة 27 ^{o} م في الظلام وتم حفظ الكالس الجنيني في النيتروجين السائل على درجة -196 ^{o} م لمدة 6 و 12 اسبوعاً ، تم زراعة الكالس على اوساط شملتكلوريد الصوديوم بتراكيز 0 و 1500 و 3000 و 4500 جزء بالمليون. دُرس تأثير كلوريد الصوديوم في معدل النمو والوزن الطري للكالس الجنيني وعدد الاجنة واظهرت النتائج ان تأثير التداخل الثلاثي بين كلوريد الصوديوم ومحاليل الحفظ وتراكيزها كان معنوياً في زيادة معدل عدد الاجنة ، وتفوقت معاملة التداخل بين كلوريد الصوديوم 1500 ppm و DMSO و التركيز 10 % وسجلت اعلى معدل لعدد الاجنة بلغ 23.2 جنين، وقد تفوقت معاملة التداخل الثلاثي وكلوريد الصوديوم 3000 ppm و DMSO و التركيز 10% وسجلت اعلى معدل للنمو بلغ 0.0372 ملغم . يوم-1 ، بينما سجلت معاملة التداخل بينكلوريد الصوديوم 4500ppm و PEG و التركيز 10% اقل معدل للنمو بلغ 0.0002 ملغم . يوم-1 ولم تسجل معاملات التداخل بين القياس و كلوريد الصوديوم بتراكيزه الثلاثة و DMSO و PEG و التركيز 20% اي معدل للنمو.

الكلمات المفتاحية: *Phoenix dactylifera*، تكوين الأجنة الجسمية ، التجميد الفائق.
البحث مستل من اطروحة دكتوراه للباحث الاول.

1. INTRODUCTION

One of the most important problems facing agriculture on a global scale, particularly in dry and semi-dry regions, is the problem of salinity, whether soil salinity or irrigation water **(Teste and Munns, 2008)**, it affects about 20% of land irrigating in the world. The adverse effects of salinity on plant growth and its productivity are due to ionic toxicity of cell, mainly Na^+, Cl^- and SO_4^-, Osmotic pressure, nutrient deficiency, oxidative stress, as well as hormonal imbalance **(Teste and Munns, 2008)**. Studies indicate that cell culture and plant tissue technology is one of the most common systems that researchers tend to using as a cellular system to study cell response to various environmental stresses, including physiological and biochemical changes, including date palm, cells bearing to salinity are selected and propagated, then grown to salinity-tolerant plants **(Jasim et al., 2010).** Dates palm is one of the most fruit trees to salt-tolerant compared to some fruit trees such as citrus and olives **(Greenway and Munns, 1980),** However, date palm productivity begins to decline when salinity levels reach 48 mM of sodium chloride and there is no production of fruits at salinity levels of 240 mM **(Hassan, 1991),** In vitro cultivation of different types of plants under saline conditions resulted in decreased callus growth and increased proline **accumulation (Kumar, Sharma, 1989; El-Yacoubi et al., 2010; Htwe et al. 2011),** increased concentration of sodium ions and reduction concentration of calcium ions **(Cano et al., 1996; El-Yakoubi et al., 2010)**. **Al-Kaabi and Abd Al-Qader (2007)** reported that increasing the concentration of sodium chloride in the nutrient media of date palms (Barhi cultivar) causing a significant reduction in fresh weight of the first callus was 0.243 g compared to the control treatment with a fresh weight of 0.323 g. Al-Zubaidi (2008) showed that the increase in concentrations of sodium chloride (1, 1.5, 2%) in the nutritional media of embryonic callus for date palm (Al-ashqir cultivar) has led reduced in fresh weight of callus compared to the concentration (0.5%) which resulted in a significant increase in fresh weight After four and eight weeks of cultivating. Abbas (2016) showed that the increase of sodium chloride concentrations was 320 mmol, led to a decrease in the fresh weight of callus for date palm (Al-ashqir cultivar) was 0.021 and 0.058 mg for four and eight weeks, respectively, While there was a significant increase at the concentration of 80 mM of sodium chloride, reaching 0.348 and 1.240 mg after four and eight weeks, respectively. **Al-zubaydi et al (2013)** studied the effect of adding concentrations of sodium chloride to the MS media of embryonic callus for date palm (Barhi cultivar), the results showed that adding 1% sodium chloride resulted in an increase in the time of the cylindrical embryos formation 51.2 days and a decrease in length and fresh weight at the concentrations of 0.5 and 2.0% of sodium chloride and the addition of Proline at a concentration of 25 $mg.L^{-1}$ resulted in an increase in percentage of germination 51.1 % compared with control treatment of 37.0%. Abbas (2016) found that the high concentrations of sodium chloride 204.34 mmol led to reduction of the fresh and dry weight of embryonic callus for date palm (Halawi cultivar), to 0.53 and 0.10 respectively, while the low concentrations of 137 mmol increased the fresh and dry weight of 1.92 and 0.48 g, respectively. Therefore, the current study aims to improve the ability of embryonic callus for date palm (Barhi cultivar) to withstand the salt stress, using the cultivation of plant tissues culture and treatment with some materials used for cry preservative.

2. MATERIALS AND METHODS

The experiments were conducted in the Laboratory of Biotechnologies Which belongs to the palm and dates research unit at the college of Agriculture, University of Baghdad, for the period from October 2014 to July 2017. The work was carried out according to the following stages:

1- Preparation of plant parts

The shoots were selected from Barhi cultivar with age of 2 - 3 years and leaves were gradually removed from the bottom up to reach to the apical meristem area with 10 cm long, It placed in an anti-oxidant solution of citric acid with a concentration of 150 $mg.L^{-1}$

and ascorbic acid at a concentration of 100 mg.L^{-1} together for one hour, The apical meristem was separated with 1 cm length, sterilized With sodium hypochlorite solution (NaOCl) at 3% concentration for 15 minutes With the addition of 20 drops of Tween. After that, it was washed with sterile distilled water three times. The apical meristem was cut into four parts 1 cm length and cultivated on MS media

Components of nutrient media

The nutrient media is composed of a group of salts (Murashige, Skoog, 1962 MS), sucrose, vitamins, growth regulators and other ingredients, in preparation of the nutrient media, the inorganic salts were mixed that prepared by dissolving of 4.3 g of the MS salts prepared by the Dutch company (Duchefa) and its salt concentration is shown in a table (1). Phosphate was added in additional quantities in the form of sodium phosphate (Hydrogenation) (NaH2PO4) and 170 mg.L^{-1}. The types of vitamins added to the nutritional media have been added to the group of vitamins (mg.L^{-1}) containing: Thiamine-HCl (1) and Pydroxin Pyrodoxin-HCl (0.5) and nicotine 1 and clicin (2) as well as biotin (1) Biotin and salt Ca-pantothenic acid (2) A basic solution of these vitamins was prepared and a specific amount of it was withdrawn and added to the nutritional media to obtain the desired concentration, Myosinosol was added directly to the nutritional media at a concentration of 100 mg.L^{-1}. Sugar was added to the nutritional media at a concentration of 30 g.L^{-1}. The amino acid was also included.

Table 1: Concentrations of inorganic salts for the MS media used in the study

Group	Name of compound	Chemical symbol	Quantity (g.L^{-1})
Nitrate	**Ammonium nitrate**	NH_4NO_3	1.650
	Potassium nitrate	KNO_3	1.900
Sulphate	**Magnesium sulfate**	$MgSO_4.7H_2O$	0.370
	Manganese(II) sulfate	$MnSO_4.H_2O$	0.0169
	Zinc sulfate	$ZnSO_4.7H_2O$	0.0086
	Copper(II) sulfate	$CuSO_4.5H_2O$	0.000025
P.B.Mo	**Potassium iodide**	KI	0.170
	Boric acid	H_3BO_3	0.0062
	Potassium Phosphate	KH_2PO_4	0.00025
Halides	**Calcium hydrochloride**	$CaCl_2.2H_2O$	0.440
	Cobalt hydrochloride	$CoCl_2.6H_2O$	0.000025
	Sodium hydroxide	$Na_2MoO_4.2H_2O$	0.00083
Chelating Iron	**Iron sulfate**	$FeSO_4.7H_2O$	0.0278
	The Chelators material in form of sodium (II) salt	EDTA.Na_2	0.03724

Glutamine with a concentration of 200 mg.L^{-1} and amniotic sulphate with a concentration of 40 mg.L^{-1}. For the purpose of hardening the nutrient media, Agar-Agar was added with a concentration of 7 g.L^{-1}. After addition of the components of all nutrient media, pH was changed to 5.7 by addition to 0.5 standard solution of NaOH or HCl, which is suitable for the hardening of the nutrient media to the required degree, as well as the nutrient availability of absorption from the cultivated part, The final size of the nutrient media is Completed after the addition of sugar and heated for the purpose of melting and distribution of pots in agricultural vessels, which is a test tubes of Pyrex with dimensions of 25 × 200 mm. Nutrient media was prepared with 50 mg.L^{-1} of Picloram with 3 mg.L^{-1} of Cytokinin 2ip and 3 mg.L^{-1} activated charcoal, The plant parts were cultivated on the nutrient media, the explant were incubated in the dark at 27.5°C and transferred to a new nutrient media every four weeks, After six months, the first callus was started. This callus was transferred to the center of MS with 10 mg.L^{-1} NAA, 3 mg.L^{-1} 2ip and 2 mg.L^{-1} activated

charcoal [Ibrahim, 2012], After 6 months, the embryonic callus began to formation, cultivation has been repeated on the same medium each month to reproduce the embryonic callus for the purpose of conducting experiments as shown in Fig (1).

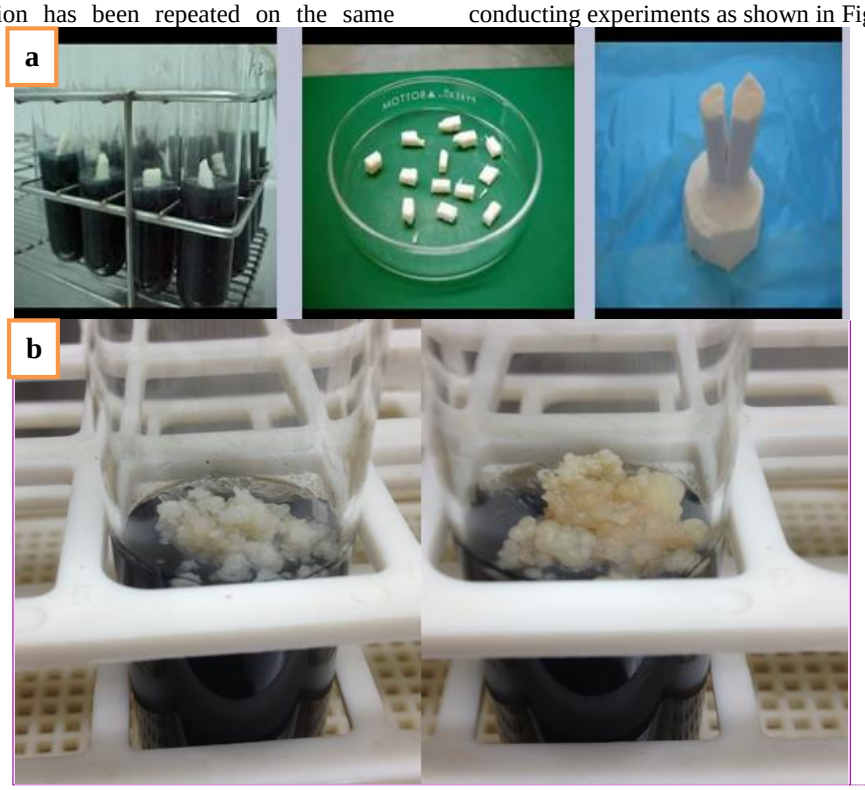

Figure 1: a) The growing apical meristem and plant parts b) the embryonic callus after six months of cultivation

2- Saving embryonic callus in the cryopreservative

The embryonic callus was immersed with a solution of glaze consisting of:

1) Concentrations of 0, 10, 20% Volume / volume of Glycerol
2) Concentrations of 0, 10, 20% weight / volume of Ethylene glycol.
3) Concentrations of 0, 10, 20% Volume / volume of Dimethyl sulfoxide (DMSO)

It has immersed for one hour. The embryonic callus has been cultivated in media containing of 1.0 mol sucrose (342.3 $g.L^{-1}$) for 7 days at 27°C in the dark. The embryonic callus was then transferred to the preservative tubes at 0 °C for two hours to be transferred to liquid nitrogen -196 °C for 6 weeks and 12 weeks, The frozen tubes were then submerged in a 37°C water bath for 90 seconds and then washed with water several times to remove the protective material from Freezing as shown in Fig (2).

1- Effect of sorbitol

250 mg of embryonic callus was cultivated in MS that was provided with 1 $mg.L^{-1}$ NAA and 0.1 $mg.L^{-1}$ BA with different concentrations of NaCl salts (0, 1500,3000, 4500 $mg.L^{-1}$). The explant were incubated at a temperature of 27 ± 1 m and the illumination intensity was 1000 lux for16 hours lighting and 8 hours darkness.

2- Measuring fresh weight

The fresh weight of the callus was calculated by placing the sensitive balance in the lamellar air flow table and in sterile conditions.

التعليق [l1]: ????

Figure 2: Cultivation of the callus on the media containing 1 mol sucrose and preservative of the callus in liquid nitrogen

5- The number of cylindrical embryos was calculated by visual observation of embryonic callus tissue.

6- Relative growth rate

It was calculated according following equation:

$$\text{Relative growth rate} = \frac{\text{Last fresh weight} \times \text{first fresh weight}}{\text{Growth period}}$$

7- Experimental design and statistical analysis

Experimental data were analyzed as global experiments using complete randomized design (CRD). The averages were compared with the least significant difference at the probability level of 0.05 [Al-Sahuki and Wahib, 1990].

3. RESULTS AND DISCUSSION

Table (2) shows that the effect of sodium chloride treatment at concentration of 4500 ppm was significant in reducing the fresh weight. The lowest weight recorded was 0.39 g compared with the control treatment which recorded the highest average of fresh weight (1.01 g). The effect of preservative solutions was significant in the increase in fresh weight, the Glycerol treatment recorded the highest average of fresh weight of 1.043 g, and while the PEG treatment recorded the lowest average of fresh weight was 0.988 g. The concentration of preservative solutions was significant in the increase in fresh weight. The concentration 10% gave the highest average of fresh weight of 1.2 g compared with the concentration of 20%, which recorded the lowest average of weight was 0.3 g. The bi-interaction between sodium chloride and the preservative solution had a significant effect on reducing the average of fresh weight. The interaction treatment between sodium chloride 4500ppm and PEG recorded the lowest average of fresh weight of 0.05 g, while the control interaction treatment and Glycerol recorded the highest average of fresh weight of 1.41 g. The bi-interaction between sodium chloride and preservative solutions, it had a significant effect in reducing the average of fresh weight. The interaction treatment between sodium chloride1500 ppm and 10% concentration recorded the highest average of fresh weight was 1.58 g while the interaction treatment between sodium chloride 4500 ppm and 20 % concentration recorded the lowest average of fresh weight was 0.133 g. The bi-interaction between DMSO and 10% concentration was significant in the average of fresh weight. The interaction treatment between DMSO and 10% concentration was excelled by giving it the highest average of fresh weight 1.928 g while the interaction treatment between DMSO and 10% concentration recorded the lowest average of fresh weight 0.063 g. The effect of the triple interaction between sodium chloride and the preservative solution and their concentration was significant in the average of fresh weight. The interaction treatment between sodium

chloride, DMSO and 10 % concentration was excelled by recording it the highest average of fresh weight 2.52 g. The interaction treatment between sodium chloride 4500 ppm, PEG and 20% Concentration recorded the lowest average of fresh weight of 0.04 g.

Table 2: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the fresh weight of embryonic callus for date palm after 6 weeks of preservative.

Stress treatments	Preservative treatments	Concentrations		Stress treatments × Preservative treatments
		10	20	
Control	DMSO	2.05	0.07	1.06
	GLY	1.43	1.39	1.41
	PEG	1.02	0.09	0.56
NaCl-1500	DMSO	2.52	0.08	1.30
	GLY	2.08	0.61	1.35
	PEG	0.16	0.07	0.11
NaCl-3000	DMSO	1.83	0.06	0.95
	GLY	1.56	0.39	0.98
	PEG	0.10	0.06	0.08
NaCl-4500	DMSO	1.30	0.08	0.67
	GLY	0.56	0.32	0.44
	PEG	0.05	0.04	0.05
LSD		0.289		0.204
Averages Concentration		1.2	0.3	
LSD		0.083		
Stress × Concentration				
		10	20	Averages
Control		1.5	0.515	1.01
NaCl-1500		1.588	0.253	0.92
NaCl-3000		1.165	0.172	0.67
NaCl-4500		0.639	0.133	0.39
LSD		0.1668		0.118
		10	20	Averages of preservative treatments
DMSO		1.928	0.063	0.996
GLY		1.409	0.677	1.043
PEG		0.332	0.064	0.198
LSD		0.1445		0.1022

Table (3) showed that the main effect of sodium chloride at a concentration of 4500 ppm has led to a significant decrease in the average of fresh weight, which was 0.63 g compared to the control treatment, which recorded the highest average of fresh weight of 1.19 g, The effect of preservative solutions was significant in the average of fresh weight, he treatment of Glycerol was excelled by recording it the highest average of fresh weight was 1.449 g, while the treatment of PEG recorded the lowest average of fresh weight was 0.298 g, There was a significant effect of concentration of preservative solutions, a 10% concentration was excelled by recording it the highest average of fresh weight of 1.6 g compared to 20% which recorded the lowest average of fresh weight of 0.5 g. The effect of bi-interaction between sodium chloride and preservative solutions was significant in this trait, the interaction treatment between sodium chloride1500ppm and Glycerol recorded the highest average of fresh weight of 1.78 g. The interaction treatment between sodium chloride 4500 ppm and PEG gave the lowest average of fresh

weight of 0.16 g. There was a significant effect of the bi-interaction between sodium chloride and concentrations of preservative solutions, the interaction treatment between the control and sodium chloride (1500, 3000 ppm) and 10% concentration recorded the highest average of fresh weight was (1.76, 1.73, 1.65 g), respectively. While the interaction treatment between sodium chloride 4500 ppm and 20% concentration gave the lowest average of fresh weight was 0.14 g. The interaction between preservative solutions and their concentrations was significant in this trait, the interaction treatment between DMSO and 10% concentration recorded the highest average of fresh weight was 2.46 g, compared to the treatment of DMSO and 20% concentration, which recorded the lowest average of fresh weight 0.09 g. The tri-interaction between sodium chloride and preservative solutions and its concentrations was significant effect in the average of fresh weight. The interaction treatment between sodium chloride1500 ppm and DMSO, 10% concentration gave the highest average of fresh weight was 2.84 g, while the interaction between sodium chloride 4500 ppm, DMSO and 20% concentration the lowest average of fresh weight. Table (3) indicates that the concentration (1500 ppm) caused a significant increase in fresh weight. This may be due to the fact that the callus cells at the beginning contain small size gaps and spread in the dense cytoplasm. Then, these gaps begin to gradually increase in size as a result of the entry of the materials resulting from the vital functions of the cell that are necessary to withstand the cells to the effects of saline stress, so the cell attempts to limit saline ions inside the gaps and the cell sap serves as an Osmotic regulating unit within the cell, (Jesche, 1984), Thus increasing the cell's susceptibility to salt stress conditions and the occurrence of vital activities necessary for normal growth and agreed, This results agree with (Kaabi and Abdelkader, 2007) showed that the increase in the concentration of sodium chloride (2, 2.5, 3%) caused a decrease in the fresh weight of the primary and genetic calcareous at the concentration of sodium chloride (0.5%). Al-Zubaidi (2008) found that salinity of the nutrient media at 0.5% concentration of sodium chloride resulted in a significant increase in the fresh weight of embryonic callus for date palm (Al-ashqir cultivar), while the fresh weight of the embryonic callus was decreased when the sodium chloride concentrations increased to (1, 1.5, 2%).

Effect of sodium chloride and preservative solutions and their concentrations in relative growth rate

Table (4) and Figure (2) show that the effect of sodium chloride treatment was significant in reducing the relative growth rate of embryonic callus; the sodium chloride 4500 ppm treatment recorded the lowest relative growth rate of 0.0039 $mg.day^{-1}$ compared to the control treatment which recorded the highest relative growth rate of 0.0132 $mg.day^{-1}$. The effect of preservative solutions was significant. DMSO treatment gave the highest relative growth rate of 0.0138 $mg.day^{-1}$, while PEG treatment recorded the lowest relative growth rate of 0.0015 $mg.day^{-1}$. The concentration 10% was excelled by giving it the highest relative growth rate of 0.0165 $mg.day^{-1}$, while the concentration 20% treatment recorded the lowest relative growth rate was 0.0023 $mg.day^{-1}$. The bi-interaction between sodium chloride and preservative solutions had a significant effect in the reducing relative growth rate. The interaction treatment between sodium chloride 4500ppm and Glycerol recorded the lowest relative growth rate was 0.0030 $mg.day^{-1}$, while the interaction treatment between control and Glycerol recorded the highest relative growth rate of 0.0188 $mg.day^{-1}$, it did not differ significantly from the interaction treatment between 1500 ppm sodium chloride, DMSO and Glycerol . The interaction treatment between sodium chloride 1500, 3000, 4500 ppm and PEG did not record any relative growth rates. The effect of the bi-interaction between sodium chloride and the concentrations of the preservative solutions was significant, the interaction treatment between sodium chloride1500 ppm and 10% concentration the highest relative growth rate of 0.0225 $mg.day^{-1}$, while the interaction

treatment between sodium chloride 4500 ppm and 20% concentration gave the lowest relative growth rate of 0.0003 mg.day^{-1}. The effect of the bi-interaction between the preservative solutions and their concentrations was significant. DMSO and 10% concentration recorded the highest growth rate of 0.0276 mg.day^{-1} while PEG and 10% concentration recorded the lowest relative growth rate of 0.0031 mg.day^{-1}, the interaction treatments between DMSO and PEG and 20% concentration did not record any relative growth rate. The effect of triple interaction between sodium chloride and preservative solutions and their concentrations was significant. The interaction treatment between sodium chloride 1500 ppm, DMSO and 10% concentration was excelled by giving it the highest growth rate was 0.0374 mg.day^{-1}, while the interaction treatment between sodium chloride 4500 ppm, Glycerol and 20% concentration was the lowest growth rate of 0.0010 mg.day^{-1}. The interaction treatments between control and sodium chloride with its three concentrations, DMSO and PEG and 20% concentration were not recorded any relative growth rate.

Table 3: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the fresh weight of embryonic callus for date palm after 12 weeks of preservative.

Stress treatments	**Preservative treatments**	**Concentrations**		**Stress treatments × Preservative treatments**
		10	**20**	
Control	**DMSO**	2.3	0.2	1.2
	GLY	1.83	1.52	1.70
	PEG	1.12	0.14	0.63
NaCl-1500	**DMSO**	2.84	0.08	1.46
	GLY	2.03	1.52	1.78
	PEG	0.32	0.10	0.21
NaCl-3000	**DMSO**	2.52	0.07	1.30
	GLY	2.14	1.26	1.70
	PEG	0.30	0.09	0.20
NaCl-4500	**DMSO**	2.16	0.06	1.11
	GLY	0.94	0.30	0.62
	PEG	0.24	0.07	0.16
LSD		**0.355**		**0.251**
Averages Concentration		1.6	0.5	
LSD		**0.103**		
Stress × Concentration				
		10	**20**	**Averages**
Control		1.762	0.622	1.19
NaCl-1500		1.73	0.567	1.15
NaCl-3000		1.655	0.474	1.06
NaCl-4500		1.113	0.144	0.63
LSD		**0.2049**		**0.1449**
		10	**20**	**Averages of preservative treatments**
DMSO		2.464	0.093	1.278
GLY		1.735	1.163	1.449
PEG		0.496	0.1	0.298
LSD		**0.1774**		**0.1255**

Table 4: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the relative growth rate (mg.day^{-1}) of embryonic callus for date palm after 6 weeks of preservative.

Stress treatments	Preservative treatments	Concentrations		Stress treatments × Preservative treatments
		10	20	
Control	**DMSO**	0.0296	0.0000	0.0148
	GLY	0.0192	0.0184	0.0188
	PEG	0.0122	0.0000	0.0061
NaCl-1500	**DMSO**	0.0374	0.0000	0.0187
	GLY	0.0300	0.0058	0.0179
	PEG	0.0000	0.0000	0.0000
NaCl-3000	**DMSO**	0.0260	0.0000	0.0130
	GLY	0.0216	0.0022	0.0119
	PEG	0.0000	0.0000	0.0000
NaCl-4500	**DMSO**	0.0172	0.0000	0.0086
	GLY	0.0050	0.0010	0.0030
	PEG	0.0000	0.0000	0.0000
LSD		**0.0047**		**0.0033**
Averages Concentration		0.0165	0.0023	
LSD		**0.103**		
Stress × Concentration				
		10	**20**	**Averages**
Control		0.0203	0.0061	0.0132
NaCl-1500		0.0225	0.0019	0.0122
NaCl-3000		0.0159	0.0007	0.0083
NaCl-4500		0.0074	0.0003	0.0039
LSD		**0.0027**		**0.0019**
		10	**20**	**Averages of preservative treatments**
DMSO		0.0276	0.0000	0.0138
GLY		0.0190	0.0069	0.0129
PEG		0.0031	0.0000	0.0015
LSD		**0.0024**		**0.0017**

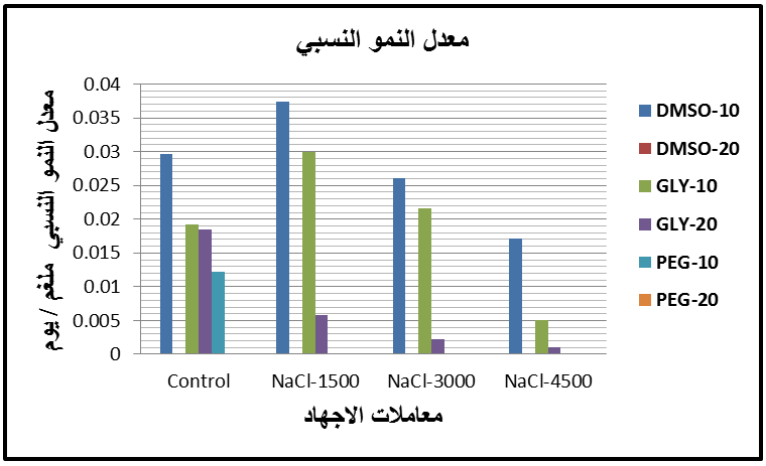

Figure 2: Effect of Sodium Chloride and Preservative Solution and its Concentrations and their Interaction in the Relative Growth Rate (mg.day^{-1}) for embryonic callus of Date Palm after 6 Weeks of Preservation.

Table (5) and Figure (3) show that the effect of sodium chloride was significant after callus preservative for 12 weeks. The treatment of sodium chloride 1500 ppm recorded the highest relative growth rate of 0.0156 mg.day^{-1} compared with sodium chloride 4500 ppm, which recorded the lowest relative growth rate of 0.0074 mg.day^{-1}. The effect of preservative solutions, the treatment of Glycerol and DMSO recorded the highest growth rate was 0.0197 and 0.0182 mg.day^{-1}, respectively, which differed significantly from the PEG treatment, which recorded the lowest growth rate of 0.0020 mg.day^{-1}, 10% concentration treatment was excelled by giving it a growth rate of 0.0126 mg.day^{-1} compared with 20% concentration treatment that recorded the lowest growth rate of 0.0050 mg.day^{-1}. The interaction treatment between sodium chloride and preservative solutions was significant. The interaction treatment between sodium chloride 1500ppm and Glycerol gave the highest growth rate was 0.0249 mg.day^{-1}, compared with the interaction treatment between sodium chloride 4500ppm and PEG, which recorded the lowest growth rate of 0.0001 mg.day^{-1}. The effect of the bi-interaction between sodium chloride and the concentrations of the preservative solutions was significant. The interaction treatment between sodium chloride 1500 ppm and 10% concentration recorded the highest growth rate of 0.0243 mg.day^{-1}, which did not differ significantly from the interaction treatments between the control, sodium chloride 3000 ppm and 10% concentration, which recorded rates of 0.0248 and 0.0230 mg.day^{-1}respectively, it significantly differ from the interaction treatment between sodium chloride 4500 ppm and 20% concentration, which recorded the lowest growth rate of 0.0005 mg.day^{-1}. The bi-interaction between preservative solutions and their concentrations was significant. DMSO and 10% concentration treatment was excelled by giving it the highest growth rate was 0.0365 mg.day^{-1}, while the interaction treatment between PEG and 10% concentration ratio recorded the lowest growth rate of 0.0039 mg.day^{-1}, the interaction treatment between DMSO, PEG and 20% concentration did not record any growth rate. The effect of triple interaction treatment between sodium chloride and preservative solutions and concentrations was significant. Triple interaction treatment between sodium chloride 3000 ppm, DMSO, 10% concentration was excelled by giving it the highest growth rate of 0.0372 mg.day^{-1}, while the interaction treatment between sodium chloride was 4500 ppm, PEG and 10% concentration recorded the lowest growth rate was 0.0002 mg.day^{-1}. The interaction treatments between control, sodium chloride with its three concentrations, DMSO, PEG and

20% concentration were not recorded any growth rate.

Table 5: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the relative growth rate (mg.day^{-1}) of embryonic callus for date palm after 12 weeks of preservative.

Stress treatments	Preservative treatments	Concentrations		Stress treatments × Preservative treatments
		10	20	
Control	DMSO	0.0344	0.0000	0.0172
	GLY	0.0260	0.0216	0.0238
	PEG	0.0140	0.0000	0.0070
NaCl-1500	DMSO	0.0344	0.0000	0.0214
	GLY	0.0292	0.0206	0.0249
	PEG	0.0008	0.0000	0.0004
NaCl-3000	DMSO	0.0372	0.0000	0.0186
	GLY	0.0312	0.0164	0.0238
	PEG	0.0006	0.0000	0.0003
NaCl-4500	DMSO	0.0314	0.0000	0.0157
	GLY	0.0110	0.0016	0.0063
	PEG	0.0002	0.0000	0.0001
LSD		0.0058		0.0041
Averages Concentration		0.0216	0.0050	
LSD		0.0017		
Stress × Concentration				
		10	20	Averages
Control		0.0248	0.0072	0.0160
NaCl-1500		0.0243	0.0069	0.0156
NaCl-3000		0.0230	0.0055	0.0142
NaCl-4500		0.0142	0.0005	0.0074
LSD		0.0034		0.0019
		10	20	Averages of preservative treatments
DMSO		0.0365	0.0000	0.0182
GLY		0.0244	0.0151	0.0197
PEG		0.0039	0.0000	0.0020
LSD		0.0029		0.0017

Effect of sodium chloride, preservative solutions and their concentrations in the number of embryos

Table (6) show that the effect of sodium chloride was significant in the number of embryos. The treatment of sodium chloride 1500 ppm gave the highest average number of embryos of 4.10 embryo compared to the treatment of sodium chloride 4500 ppm, which recorded the lowest average number of embryos reached 0.90. The effect of preservative solutions, Glycerol treatment was excelled by giving it the highest average number of embryos was 4.62, compared to the PEG, which recorded the lowest average number of embryos 0.175 embryo. A significant effect was observed for the concentration of preservative solutions. The 10% concentration treatment recorded the highest average number of embryos was 4.3 embryo compared with the 20% concentration treatment, which recorded the lowest average number of embryos of 1.2 embryo. The bi-interaction between sodium chloride and the preservative solution was significant. The interaction treatment between sodium chloride

1500 ppm and Glycerol, which recorded the highest average number of embryos of 6.8 embryo, while the interaction treatments between control and PEG showed the lowest average number of embryos of 0.7 embryo. The effect of the interaction between sodium chloride and concentrations of the preservative solutions was significant, The interaction treatment between sodium chloride 1500 ppm and 10% concentration recorded the highest average number of embryos of 6.8 embryo compared to the interaction treatment between sodium chloride 4500 ppm and 20% concentration, which recorded the lowest average number of embryos of 0.46 embryo. The bi-interaction between DMSO and 10% concentration gave the highest average number of embryos 6.95 embryo, while the interaction between PEG and 10% concentration recorded the lowest average number of embryos was 0.35. The effect of triple interaction between sodium chloride, preservative solutions and their concentrations was significant in this trait. The interaction treatment between sodium chloride 1500 ppm, DMSO and 10% concentration recorded the highest average number of embryos was 11.0 embryo, while the interaction treatment between sodium chloride 4500 ppm, Glycerol and 20% concentration gave the lowest average number of embryos of 1.4 embryo.

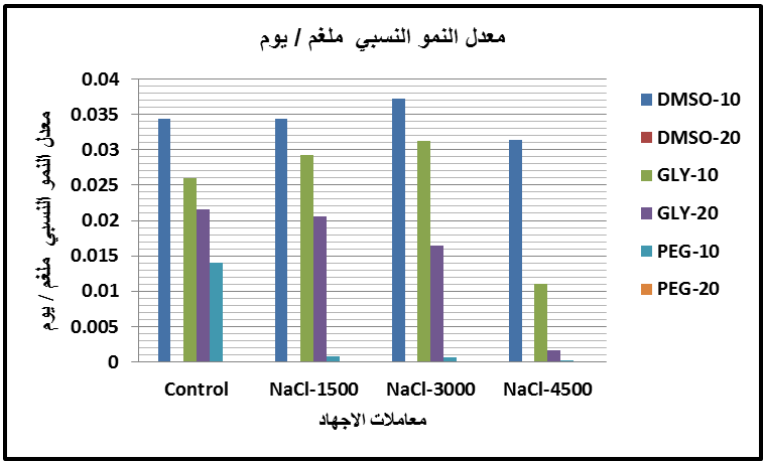

Figure 2: Effect of Sodium Chloride and Preservative Solution and its Concentrations and their Interaction in the Relative Growth Rate (mg.day^{-1}) for embryonic callus of Date Palm after 12 Weeks of Preservation.

Table (7) shows that the main effect of sodium chloride was significant. The sodium chloride 1500 ppm treatment recorded the highest average number of embryos was 8.17 embryo while the control treatment recorded the lowest average number of embryos of 4.27 embryo. The effect of preservative solutions, the treatment of DMSO and Glycerol and recorded the highest average number of embryos was 8.67 and 8.27 embryos, respectively, compared to the treatment of PEG, which recorded the lowest average number of embryos reached 1.1 embryo. A significant effect was observed for concentration of preservative solutions, the 10% concentration treatment recorded the highest average number of embryos was 9.8% while the 20% concentration treatment gave the lowest average number of embryos was 2.2. The effect of the bi-interaction between sodium chloride and preservative solutions was significant in the average number of embryos. The interaction between sodium chloride 1500 ppm, DMSO, and Glycerol was excelled by giving it the highest average number of embryos was 11.6 and 11.7 embryo, respectively, the interaction treatment between control and PEG gave the lowest average number of embryos of 0.9 embryo. The bi-interaction between sodium chloride

and concentrations of the preservative solutions was significant in the average number of embryos. The interaction between sodium chloride 1500 ppm and 10% concentration gave the highest average number of embryos was 13.07 embryo. The interaction treatment between sodium chloride 4500 ppm and 20% concentration recorded the lowest average number of embryos was 1.6 embryo. The effect of the bi-interaction between preservative solutions and their concentrations was significant in this trait. The interaction between DMSO and 10% concentration gave the highest average number of embryos was 17.35, while the interaction between PEG and 10% concentration gave the lowest average number of embryos was 2.2. The effect of triple interaction between sodium chloride and preservative solutions and its concentrations was significant in increasing the average number of embryos. The interaction treatment between sodium chloride 1500 ppm, DMSO and 10% concentration was excelled by giving it the highest average number of embryos 23.2 while the interaction treatment between

Table 6: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the average number of embryos (%) of embryonic callus for date palm after 6 weeks of preservative.

Stress treatments	**Preservative treatments**	**Concentrations**		**Stress treatments × Preservative treatments**
		10	**20**	
Control	**DMSO**	9.0	0.0	4.5
	GLY	7.4	5.8	6.6
	PEG	1.4	0.0	0.7
NaCl-1500	**DMSO**	11.0	0.0	5.5
	GLY	9.4	4.2	6.8
	PEG	0.0	0.0	0.0
NaCl-3000	**DMSO**	5.4	0.0	2.7
	GLY	3.8	3.4	3.6
	PEG	0.0	0.0	0.0
NaCl-4500	**DMSO**	2.4	0.0	1.2
	GLY	1.6	1.4	1.5
	PEG	0.0	0.0	0.0
LSD		**1.2**		**0.9**
Averages Concentration		4.3	1.2	
LSD		**0.4**		
Stress × Concentration				
		10	**20**	**Averages**
Control		5.933	1.933	3.93
NaCl-1500		6.8	1.4	4.10
NaCl-3000		3.067	1.133	2.10
NaCl-4500		1.333	0.467	0.90
LSD		**0.7096**		**0.5017**
		10	**20**	**Averages of preservative treatments**
DMSO		6.95	0	3.475
GLY		5.55	3.7	4.625
PEG		0.35	0	0.175
LSD		**0.6145**		**0.4345**

control, PEG and 10% concentration recorded the lowest average number of embryos was 1.8 embryos. Table (7) indicates an increase in the number of embryos at the concentration of 1500 ppm of sodium chloride. This may be due to the fact that the callus cells at the beginning contain small size gaps and spread in the dense cytoplasm. Then, these gaps begin to gradually increase in size as a result of the entry of the materials resulting from the vital functions of the cell that are necessary to withstand the cells to the effects of saline stress, so the cell attempts to limit saline ions inside the gaps and the cell sap serves as an Osmotic regulating unit within the cell, Thus increasing the cell's susceptibility to salt stress conditions and the occurrence of vital activities necessary for normal growth and agreed (Jeschke, 1984). The decrease in the number of embryos at the concentration of 4500 ppm sodium chloride may be due to the fact that salinity affects the synthesis of proteins necessary for cell growth, leading to a reduction in the RNA content necessary for the synthesis of protein as well as inhibit the work of some enzymes responsible for building proteins, especially the enzyme Nitrate Reductase (Munns, 2006).

Table 7: Effect of sodium chloride and preservative solutions, their concentrations and their interactions in the average number of embryos (%) of embryonic callus for date palm after 12 weeks of preservative.

Stress treatments	**Preservative treatments**	**Concentrations**		**Stress treatments × Preservative treatments**
		10	**20**	
Control	**DMSO**	9.6	0.0	4.8
	GLY	7.6	6.6	7.1
	PEG	1.8	0.0	0.9
NaCl-1500	**DMSO**	23.2	0.0	11.6
	GLY	13.6	9.8	11.7
	PEG	2.4	0.0	1.2
NaCl-3000	**DMSO**	20.4	0.0	10.2
	GLY	12.2	5.4	8.8
	PEG	2.6	0.0	1.3
NaCl-4500	**DMSO**	16.2	0.0	8.1
	GLY	6.2	4.8	5.5
	PEG	2.0	0.0	1.0
LSD		**1.9**		**1.4**
Averages Concentration		9.8	2.2	
LSD		**0.6**		
Stress × Concentration				
		10	**20**	**Averages**
Control		6.33	2.2	4.27
NaCl-1500		13.07	3.27	8.17
NaCl-3000		11.73	1.8	6.77
NaCl-4500		8.13	1.6	4.87
LSD		**1.11**		**0.785**
		10	**20**	**Averages of preservative treatments**
DMSO		17.35	0	8.67
GLY		9.9	6.65	8.27
PEG		2.2	0	1.1
LSD		**0.961**		**0.68**

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