EFFECT OF SORBITOL ON PERCENTAGE OF GERMINATION AND FRESH WEIGHT OF EMBRYOGENIC CALLUS FOR DATE PALM (BARHI CULTIVAR) AFTER CRYOPRESERVATION

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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 cryopreservation, The embryonic callus from the cultivating of the Shoot tips, planted on the Murashige and Skoog media, was subjected to immersion in preservation solution and cultivation on a sucrose-containing medium (342.3 $g.L^{-1}$) for seven days at 27ºC in the dark, save the embryonic callus in liquid nitrogen at -196ºC for 6 and 12 weeks, The callus was cultivated on the sorbitol container medias with 0, 0.1, 0.2 and 0.3 molar concentrations. The effect of sorbitol on the percentage of germination and fresh weight of embryonic callus was studied, complete randomize design was used. The interaction treatment between (0.1 molar) sorbitol, DMSO and 10% concentration was excelled, the highest average of fresh weight was 4.64 g, while the interaction treatment between (0.3 molar) sorbitol and PEG and 20% concentration recorded the lowest average weight of 0.04 g. The triple interaction between the sorbitol, the preservation solutions and their concentration was significant in the decrease in the percentage of germination. The interaction treatment between (0.2 molar) sorbitol, DMSO and (10%) concentration recorded the lowest percentage of germination of 16.7% Compared with the interaction treatment between control, DMSO and 10% concentration Which recorded the highest rate of percentage of germination reached 44.3%.

Keywords: *Phoenix dactylifera*, somatic embryogenesis, cryopreservation.
** Part of Ph.D. thesis of first author.

تاثير السوربيتول في النسبة المئوية للانبات والوزن الطري للكالس الجنيني لنخيل التمر صنف برحي بعد الحفظ بالتجميد الفائق

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المستخلص:

اجريت الدراسة الحالية للمدة من تشرين اول 2014 حتى تموز 2017 بهدف زيادة قابلية الكالس الجنيني لنخيل التمر *Phoenix dactylifera* L. على تحمل الاجهاد المائي 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 والزراعة على وسط حاوي على السكروز بتركيز (342.3 غم.لتر$^{-1}$) لمدة سبعة ايام وعلى درجة حرارة 27 ^{o} م في الظلام، وحفظ الكالس الجنيني في النيتروجين السائل على درجة -196 ^{o} م لمدة 6 و 12 اسبوعاً ، زرع الكالس على اوساط حاوية على السوربيتول بتراكيز 0 ، 0.1 ، 0.2 و 0.3 مولر، . دُرس تأثير السوربيتول في النسبة المئوية للانبات والوزن الطري للكالس الجنيني ,واستخدم التصميم العشوائي الكامل ، وقورنت الفروقات بين المتوسطات حسب اختبار اقل فرق معنوي LSD وعلى مستوى احتمالية 0.05 بالنسبة للوزن الطري كان تأثير التداخل بين السوربيتول ومحاليل الحفظ وتراكيزها معنوياً في معدل الوزن الطري وقد تفوقت معاملة التداخل بين السوربيتول(0.1 مولر) و DMSO والتركيز(10 %) اذ سجلت اعلى معدل للوزن الطري بلغ 4.64 غم ، بينما سجلت معاملة التداخل بين السوربيتول (0.3 مولر) وPEG و التركيز (20%) اقل معدل للوزن بلغ 0.04 غم. أما التداخل الثلاثي بين السوربيتول ومحاليل الحفظ وتراكيزها فقد كان معنوياً في انخفاض النسبة المئوية للانبات ، و سجلت معاملة التداخل بين السوربيتول(0.2 مولر)و DMSO و التركيز (10 %)اقل معدل للنسبة المئوية للانبات بلغ 16.7 % ، مقارنة مع معاملة التداخل بين القياس و اDMSO و التركيز 10% والتي سجلت اعلى معدل للنسبة المئوية للانبات بلغ 44.3 % .

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

1. INTRODUCTION

Due to the economic importance of date palms, researchers have sought to study the possibility of using tissue culture technology for rapid genealogical propagation [Hegazy et al., 2009]. Despite the progress made in cultivating plant tissues and obtaining good results through propagation by somatic embryos formed of embryonic callus, but many problems and difficulties are still facing the proliferation of palms in this way. Previous studies have shown that the germination rate of vegetative embryos consisting of callus tissue varies between 0-050% by type and treatment, which is a low rate that causes at least 50% loss in the best cases [Anjaran et al., 1995]. The emergence of somatic embryos of date palms in vitro is influenced by several interrelated factors, including the source of carbon and the material of control or adjusting osmosis [Litz, 1989; Ramarosandratana, 1999], Sucrose is the most commonly used in agriculture media compared to other sources of carbohydrates such as fructose, glucose, sorbitol and mannitol [Custers et al., 1988 and Chee, 1990], Te-chato and Hilae, (2007) show that among the many sources of carbon tested, the use of sorbitol 0.2 ml in agriculture media gave the best number of secondary somatic embryos (21.55 Embryo) of palm oil. Sanputawong and Te-chato (2011) reported that the addition of sorbitol at a concentration of 0.2 mol with 200 mg.L^{-1} ascorbic acid to agriculture media of palm oil gave the highest number of secondary somatic embryos, The results which Mona and Rania have reached (2012) indicate that the date palm response in vitro for the source of carbon is associated with genotypes and the stage of agriculture. The highest percentage to induce callus was 100% for Malakaby cultivar compared with 72 and 84% for both cultivars of Amry and Zaghlol palm, respectively at the concentration of 0.20 mol of sorbitol, The highest fresh weight of callus for Zaghlol was 5.50 g compared to Amry and Malakaby which reached 4.75 and 2.30 g respectively, at 0.1 mol concentration of sucrose and 0.05 mol of sorbitol. Therefore, the present study aimed at improving the ability of the date palm (Barhi cultivars) in order to withstand water stress by using plant tissue culture and treating with some of the materials used for cryopreservation.

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:

2.1.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).

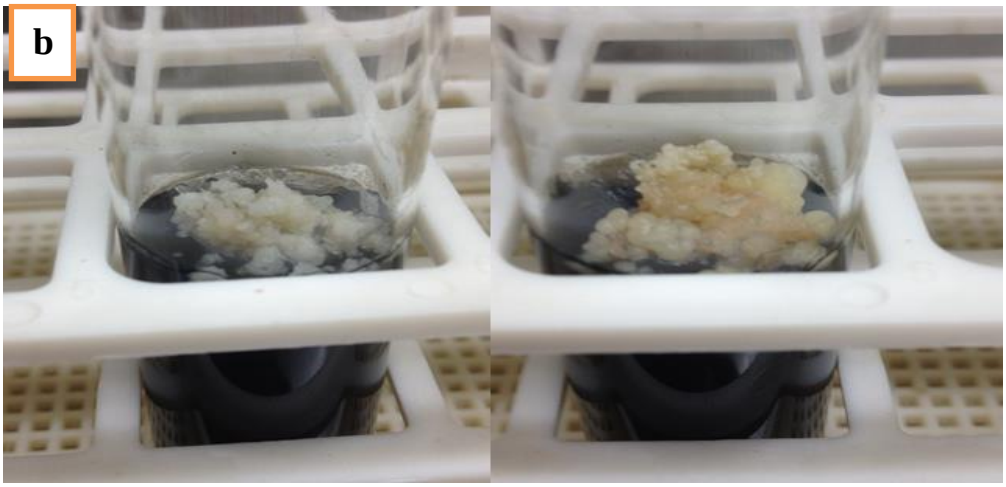

Figure 1: a) The growing apical meristem and plant parts b) the embryonic callus after six months of cultivation

1- Saving embryonic callus in the cryopreservation

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 preservation 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).

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

2- Effect of sorbitol

250 mg of embryonic callus was cultivated in MS that was provided with with 1 $mg.L^{-1}$ NAA and 0.1 $mg.L^{-1}$ BA with different concentrations of sorbitol (0, 0.1, 0.2, 0.3). 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.

3- 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.

4- Percentage of germination:

It is calculated as follows Percentage of germination (%) = $\frac{\text{Number of embryos grown}}{\text{Total number}} \times 100$

6- 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 [Sahuki and Wahib, 1990].

3. RESULTS AND DISCUSSION

1- Effect of sorbitol and preservatives and their concentrations in percentage of germination

The results shown in Table (2) and Figure (3) show that the effect of sorbitol treatment has reduced the percentage of germination, The sorbitol treatment 0.2 mol recorded the lowest average percentage of germination was 2.78% compared with the control treatment which recorded the highest average percentage of germination of 22.61%. As for the effect of preservation solutions, PEG treatment was recorded the lowest average of 2.08% compared to the DMSO treatment which recorded the highest percentage of 10.12%, The concentrations of the preservation solutions were significant and the concentration of 10% was excelled by giving it the highest percentage of germination 11.7% compared to the concentration treatment of 20%, which recorded the lowest average of percentage of germination was 2.7%. There was a significant effect of bi-interaction between sorbitol and preservation solutions in reduction percentage of germination while the interaction treatment between 0.2 M sorbitol and DMSO recorded the lowest average percentage of germination of 8.3%, The interaction between control and Glycerol treatment recorded the highest average of germination percentage of 37.3%. The interaction between sorbitol and concentration of preservative solutions had a significant effect on the decrease in the percentage of germination. The treatment between 0.2 M sorbitol and 10% concentration showed the lowest percentage of germination of 5.56%, while the interaction between control and 10% concentration recorded the highest percentage of germination was 34.44%. The effect of bi-interaction between preservation solutions and their concentrations was significant in the percentage of germination, The interaction between DMSO and 10% concentration recorded the highest percentage of 20.25% While The interaction between PEG and 10% concentration recorded the lowest average percentage of 4.17%. The triple interaction between sorbitol and preservation solutions and their concentrations was significant in reduction percentage of germination, the interaction treatment between 0.2 M sorbitol, DMSO and 10% concentration recorded the lowest average of percentage of germination was 16.7% Compared with the interaction treatment between control, DMSO and 10% concentration, which recorded the highest average of percentage of germination of 44.3%.

The results of Table (3) and Figure (4) showed that the sorbitol effect was significant in the decrease the percentage of germination. The sorbitol 0.2 molar recorded the lowest average percentage of germination of 3.89% compared to the control treatment which recorded the highest average percentage of 30.61%, The effect of preservation solutions concentrations was significant in the percentage of germination, the concentration of 10% was excelled by giving it the highest average percentage of 21.3% while the concentration of 20% recorded the lowest average of 5.6%. Glycerol treatment recorded the highest average of 21.38% compared to the PEG treatment, which recorded the lowest average of 2.92%. The results showed that the bi-interaction between sorbitol and preservation solutions had a significant effect on the decrease in the percentage of germination. The interaction between 0.3 molar sorbitol, DMSO and Glycerol showed the lowest average percentage of germination 8.3%. The bi-interaction between sorbitol and the concentrations of preservation solutions was significant in the decrease percentage of germination percentage. The interaction treatment, 0.1 molar sorbitol and 20% concentration recorded the lowest average for percentage of germination was 5.89%. The interaction between control treatment and 10% concentration recorded the highest average for percentage of germination, amounted to

44.95%. The effect of bi-interaction between preservation solutions and their concentrations was significant in the percentage of germination, The interaction between DMSO and 10% concentration was excelled by giving it the highest average for the percentage of germination of 32.17%, while the interaction between PEG and 10% concentration recorded the lowest average for the percentage of germination of 5.83%. The effect of triple interaction between sorbitol and preservation solutions and their concentrations was significant in the decrease the percentage of germination. The interaction treatment between 0.3 molar sorbitol, DMSO, Glycerol and 10% concentration recorded the lowest average for the percentage of germination of 16.7%, while the interaction, control, DMSO and 10% concentration recorded the highest average was 55.7%.

Table 2: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the percentage of germination the embryonic callus of date palm after 6 weeks of preservation.

Stress treatments	**Preservation treatments**	**Concentrations**		**Stress treatments × Preservation treatments**
		10	**20**	
Control	**DMSO**	44.3	0.0	22.2
	GLY	42.3	32.3	37.3
	PEG	16.7	20.0	8.3
SOR-0.1	**DMSO**	20.0	0.0	10.0
	GLY	0.0	0.0	0.0
	PEG	0.0	0.0	0.0
SOR-0.2	**DMSO**	16.7	0.0	8.3
	GLY	0.0	0.0	0.0
	PEG	0.0	0.0	0.0
SOR-0.3	**DMSO**	0.0	0.0	0.0
	GLY	0.0	0.0	0.0
	PEG	0.0	0.0	0.0
LSD		**3.6**		**2.5**
Averages Concentration		11.7	2.7	
LSD		**1.0**		
Stress × Concentration				
		10	**20**	**Averages**
Control		34.44	10.78	22.61
SOR-0.1		6.67	0	3.33
SOR-0.2		5.56	0	2.78
SOR-0.3		0	0	0.00
LSD		**2.081**		**1.471**
		10	**20**	**Averages Preservation treatments**
DMSO		20.25	0	10.12
GLY		10.58	8.08	9.33
PEG		4.17	0	2.08
LSD		**1.802**		**1.274**

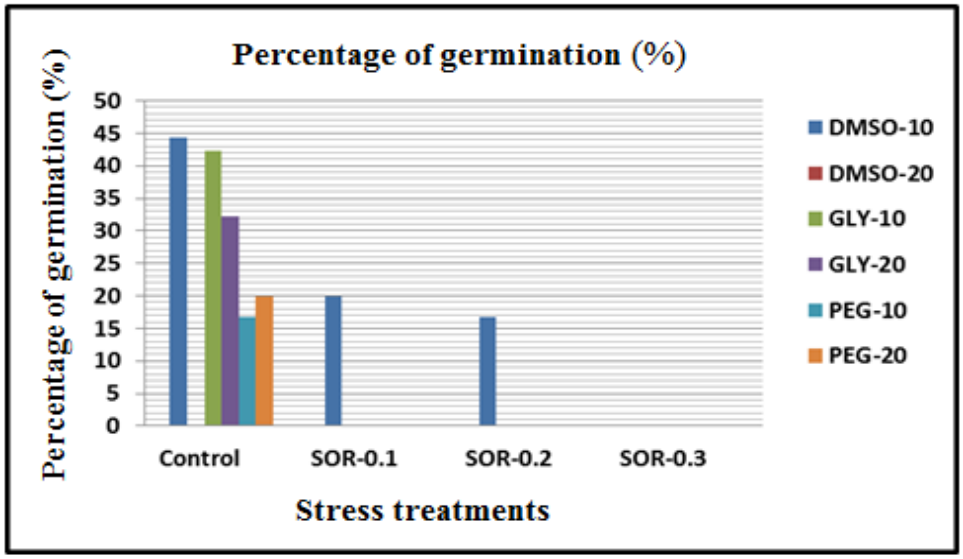

Figure 3: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the percentage of germination the embryonic callus of date palm after 6 weeks of preservation.

Table 3: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the percentage of germination the embryonic callus of date palm after 12 weeks of preservation.

Stress treatments	Preservation treatments	Concentrations		Stress treatments × Preservation treatments
		10	20	
Control	DMSO	55.7	0.0	27.8
	GLY	54.7	50.0	52.3
	PEG	23.3	20.0	11.7
SOR-0.1	DMSO	33.0	0.0	16.5
	GLY	32.0	17.7	24.8
	PEG	0.0	0.0	0.0
SOR-0.2	DMSO	23.3	0.0	11.7
	GLY	0.0	0.0	0.0
	PEG	0.0	0.0	0.0
SOR-0.3	DMSO	16.7	0.0	8.3
	GLY	16.7	0.0	8.3
	PEG	0.0	0.0	0.0
LSD		3.6		2.6
Averages Concentration		21.3	5.6	
LSD		1.1		
Stress × Concentration				
		10	20	Averages
Control		44.56	16.67	30.61
SOR-0.1		21.67	5.89	13.78
SOR-0.2		7.78	0	3.89
SOR-0.3		11.11	0	5.56
LSD		2.122		1.501
		10	20	Averages Preservation treatments
DMSO		32.17	0	16.08
GLY		25.83	16.92	21.38
PEG		5.83	0	2.92
LSD		1.838		1.3

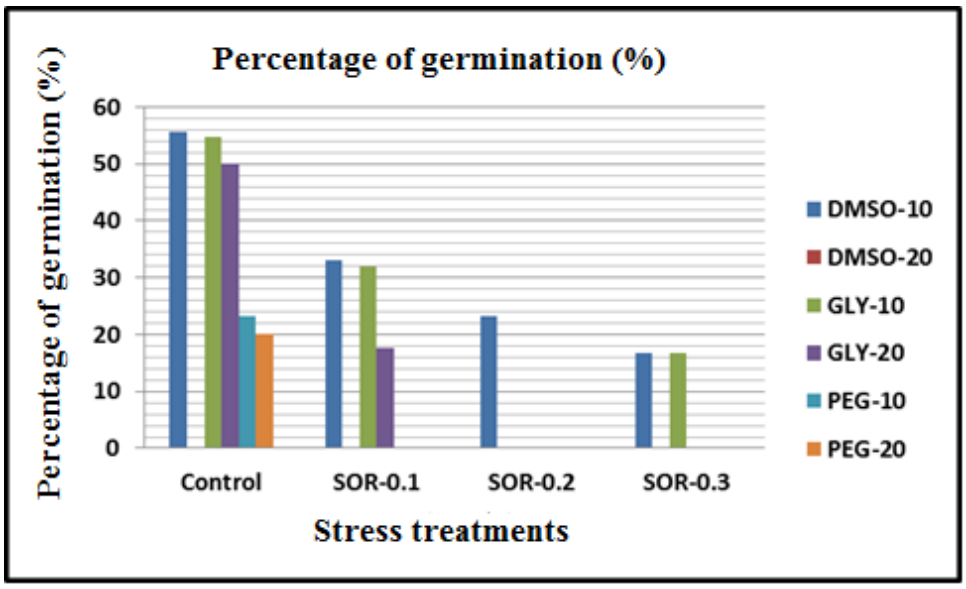

Figure 4: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the percentage of germination the embryonic callus of date palm after 12 weeks of preservation.

2- Effect of sorbitol and preservative solution and their concentrations in the fresh weight of embryonic callus

The results in Table (4) show that the effect of the treatment with 0.3 molar sorbitol was significant in lowering the average fresh weight, The lowest average weight recorded was 0.36 g compared with the control treatment which recorded the highest average fresh weight of 1.01 g, The effect of the preservation solutions was significant in the average fresh weight. The DMSO treatment was excelled by giving it the highest average fresh weight was 1.044 g while the PEG treatment recorded the lowest average of 0.197 g. A significant effect was observed for concentration of preservation solutions. The 10% concentration excelled by giving it the highest average fresh weight of 1.1 g while the 20% concentration recorded the lowest average weight 0.3 g. The bi-interaction between sorbitol and preservation solutions had a significant effect on the average fresh weight, The treatment of 0.1 molar sorbitol and DMSO was given the highest average weight of 1.42 g, While the interaction treatment between (0.2 and 0.3 molar) sorbitol and PEG recorded the lowest average weight of 0.06 g. The bi-interaction between sorbitol and the concentrations of the preservative solutions was significant in the low average of fresh weight, the interaction treatment between 0.3 molar Sorbitol and 20% Concentration recorded the lowest average fresh weight of 0.14 g compared with the control treatment which recorded the highest average weight of 1.5 g. The effect of the bi-interaction between preservation solutions and their concentrations was significant in this traits, The interaction treatment between DMSO and 10% concentration recorded the highest average fresh weight of 2.02 g While the interaction treatment between DMSO and 20% concentration recorded the lowest average fresh weight of 0.05 g. The interferon interaction between sorbitol and the preservative solution and their concentrations had a significant effect on the fresh weight. The interaction treatment between 0.1 molar sorbitol and DMSO was 10% concentration recorded the highest average of 2.78 g while the interaction treatment between 0.3 molar sorbitol, PEG and 20% concentration recorded the lowest weight is 0.04 g. Table (5) indicates that the effect of treatment with 0.1 molar sorbitol was significant in the fresh weight it recorded the highest average weight of 1.68 g compared with 0.3 molar sorbitol by giving it the lowest average weight of 0.54 g, The effect of the preservation solutions was significant in this trait, the Glycerol treatment recording it the highest average of fresh weight of 1.53 g,

while the PEG treatment gave the lowest average of fresh weight of 0.30 g. There was a significant increase in the concentration of preservation solutions where the 10% concentration excelled by giving it the highest average weight of 1.7 g compared to the 20% concentration which gave the lowest average weight of 0.5 g. The bi-interaction between sorbitol and preservation solutions had a significant effect in the average fresh weight, The interaction treatment between 0.1 molar sorbitol and Glycerol was given the highest moderate weight of 2.48 g while the interaction treatment between 0.3 molar sorbitol and PEG recorded the lowest average weight of 0.13 g. The interferon between sorbitol and concentrations of preservative solutions had a significant effect in the average of fresh weight. The interaction treatment between 0.1 molar sorbitol and 10% concentration showed the highest average weight of 2.71 g compared with the interaction treatment between 0.3 molar sorbitol and 20% concentration which gave the lowest average weight of 0.2 g.

Table 4: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the average of fresh weight for the embryonic callus of date palm after 6 weeks of preservation.

Stress treatments	Preservation treatments	Concentrations		Stress treatments × Preservation 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
SOR-0.1	**DMSO**	2.78	0.06	1.42
	GLY	1.07	0.70	0.89
	PEG	0.14	0.08	0.11
SOR-0.2	**DMSO**	2.25	0.06	1.16
	GLY	0.89	0.56	0.73
	PEG	0.08	0.05	0.06
SOR-0.3	**DMSO**	1.04	0.06	0.54
	GLY	0.63	0.35	0.49
	PEG	0.08	0.04	0.06
LSD		**0.224**		**0.158**
Averages Concentration		1.1	0.3	
LSD		**0.065**		
Stress × Concentration				
		10	**20**	**Averages**
Control		1.5	0.515	1.01
SOR-0.1		1.327	0.281	0.80
SOR-0.2		1.074	0.225	0.65
SOR-0.3		0.583	0.145	0.36
LSD		**0.1293**		**0.0914**
		10	**20**	**Averages Preservation treatments**
DMSO		2.029	0.059	1.044
GLY		1.006	0.75	0.878
PEG		0.328	0.065	0.197
LSD		**0.1119**		**0.0792**

The effect of bi-interaction between preservation solutions and their concentrations was significant in this trait, where the interaction treatment between DMSO and 10%

concentration recorded the highest average of weight was 2.57 g, while the interaction treatment between PEG and 20% concentration recorded the lowest average weight of 0.06 g. The effect of triple interaction between sorbitol and preservative solutions and their concentrations was significant in average fresh weight. The interaction treatment between 0.1 molar sorbitol, 10% concentration and DMSO were excelled by giving it the highest average of fresh weight 4.64 g, while the interaction treatment between 0.3 molar sorbitol, 20% concentration and PEG was observed the lowest average of fresh weight of 0.04 g.

Table 5: Effect of sorbitol and preservation solutions, their concentrations and their interactions in the average of fresh weight for the embryonic callus of date palm after 12 weeks of preservation.

Stress treatments	**Preservation treatments**	**Concentrations**		**Stress treatments × Preservation treatments**
		10	**20**	
Control	**DMSO**	2.34	0.16	1.25
	GLY	1.83	1.57	1.70
	PEG	1.12	0.09	0.61
SOR-0.1	**DMSO**	4.64	0.06	2.35
	GLY	3.14	1.82	2.48
	PEG	0.37	0.07	0.22
SOR-0.2	**DMSO**	2.20	0.05	1.12
	GLY	1.14	0.92	1.03
	PEG	0.48	0.06	0.27
SOR-0.3	**DMSO**	1.12	0.06	0.58
	GLY	1.28	0.54	0.91
	PEG	0.22	0.04	0.13
LSD		**0.499**		**0.353**
Averages Concentration		1.7	0.5	
LSD		**0.144**		
Stress × Concentration				
		10	**20**	**Averages**
Control		1.762	0.606	1.18
SOR-0.1		2.715	0.649	1.68
SOR-0.2		1.273	0.342	0.81
SOR-0.3		0.873	0.207	0.54
LSD		**0.288**		**0.2037**
		10	**20**	**Averages Preservation treatments**
DMSO		2.573	0.076	1.325
GLY		1.847	1.212	1.53
PEG		0.547	0.064	0.305
LSD		**0.2494**		**0.1764**

The results of Table (5) show the significant effect of the triple interaction between 0.1 molar sorbitol, DMSO and 10% concentration in the average of fresh weight. The reason may be due to the ability of date palm embryos to continue to grow and develop normally after cryopreservation, provided that they have been treated with a mixture of protective agents of freezing damage consisting of Glycerol, sucrose and dimethyl oxide (DMSO) after exposure to liquid nitrogen, Date palm seedlings were obtained from body tissues that had been frozen for several months in liquid nitrogen [MyCock et al., 1995; 1997]. It may

be due to the role of sorbitol as an Osmosis substance or source of energy as the role of sugars in plant tissue culture is divided into two parts, As a source of carbon / energy and as an Osmosis substance, Osmosis substances are those substances that reduce the availability of water for plant cells. It has been shown to be effective in reducing growth and thus prolonging the storage period in plant tissues of many plant species. According to the growth hypothesis resulting from cell expansion, The high levels of osmotic substances in the environment are counteracted by cell expansion pressure, which must occur Before the expansion of cells [Zimmermann, 1978]. These stress conditions inhibit the growth of both callus and vegetative branches [Brown et al., 1979]. In this regard, mannitol, sucrose and sorbitol have been recognized as optimal Osmosis substances to prolong the storage period of plant tissue cultures. High concentrations of sucrose can be used to reduce the growth rate of plants growing in the tubes. Manitol can also be used as an asymptomatic substance, an alcoholic sugar produced as a primary compound of photosynthesis by some plants. Sorbitol is another alcoholic sugar that inhibits vegetative growth in planting tubes. High concentrations of sucrose, sorbitol and mannitol were found that significantly reduce the growth of bitter almonds in the tubes and increase the regeneration interval to four months under room temperature [Shibli et al., 1999]. These results are consistent with [Bekheet, 2015, Al-Bahrany and Al-Khayri 2012].

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