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THE EFFECT OF SODIUM CHLORIDE AND TREHALOSE SUGAR IN IONIC CONTENT OF DATE PALM *PHOENIX DACTYLIFERA* L. CV. UM - ALDEHINE *IN VITRO*

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Abstract

Date palm *Phoenix dactylifera* L. was cultivated since pre-historic and it is the most important fruit crop in Iraq. Salt stress is a major abiotic stress which has many effects on the productivity of date palm in the world and Iraq. This study was conducted to test the effect of four concentrations of sodium chloride (0, 25, 50 and 75 mM) and four concentrations of trehalose sugar (0, 5, 10 and 15 g. L^{-1}) in ionic content of somatic embryos of date palm c.v Um-Aldehine in vitro. The aim of this study is to improve date palm salt tolerance to salt stress.

The results showed the following: The increase in sodium chloride concentration in culture media causes a significant increase of the concentrations of sodium and chloride ions. In addition, the treatment of sodium chloride (75 mM) achieved the highest averages and also it has a significant decrease in potassium, potassium to sodium ratio and nitrogen concentrations. The effects of increasing trehalose sugar concentration in culture media has a significant decrease of sodium and chloride ions concentrations, however, the treatment of (15 g. L^{-1}) had achieved the lowest averages, besides, the treatment itself has a significant increase in potassium, potassium to sodium ratio and nitrogen concentrations. The results showed a significant effects of interaction between the treatments of sodium chloride concentrations and trehalose sugar concentrations in the whole studied characteristics.

Key words *:* Ionic content, Trehalose sugar, Somatic embryos, Nitrogen, *In vitro*.

Introduction

Date palm *Phoenix dactylifera* L. is an important horticultural crop, often cultivated in arid and semi-arid regions of the world, also Iraq has a great salt affected soils, thus, the cultivated date palm is known to be tolerant to salinity (Franco *et al.,* 1997). The salinity in nature imposes two primary harmful effects on plants: one is osmotic stress and the other ionic toxicity. Due to presence of high salt, salinity content increases the osmotic pressure in the soil solution over the osmotic pressure in plant cells. As a result, plant loses its ability for uptake of water and minerals, especially the uptake of K^+ and Ca^{+2} (Mahajan and Tuteja, 2005; Munns *et al.,* 2006; Hasanuzzaman *et al.,* 2012). Plant adaptation to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulated Na+ or Cl^-. (Munns and Tester, 2008). Some date palm varieties can tolerance salinity levels up to 22000 ppm (Ec 34 $ds.m^{-1}$). However, their growth and yield productivity were affected (Erskine *et al.,* 2004). There are many strategies and methods followed to improve salt tolerance for plant, one of this methods, tissue culture was used in improving or increasing salt tolerance of plant, as the exposure of cells or tissues to different levels of salt stress may be useful in selecting tolerant cells to saline stress from sensitive cells to saline stress (Zekri, 2004; Munns, 2005). On the other hand, second of this methods, the accumulation of organic solute materials, including trehalose, is a necessary to osmotic regulation of the plant when had exposed to saline stress (Nounjan *et al.,* 2012). The exogenous addition of trehalose sugar to culture media causes increasing plant tolerance to salt stress due to reducing the accumulation of toxic ions and lipids oxidation (Zaid, 2009). Also, the addition of low concentrations of trehalose sugar had contributed to the reduction of the accumulation of sodium

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chloride ions in plant cells and improved the growth of the plant under salt stress (Garcia *et al.,* 1997). Trehalose is considered a source of energy and osmoprotectant of each of proteins and membranes structure (Iordachescu and Imai, 2008). This study was conducted to known the effect of sodium chloride and trehalose sugar and their interactions in ionic content of somatic embryos of date palm c.v. Um-Aldehine in vitro. Besides, to improve date palm tolerance to salt stress.

Materials and Methods

The present study was carried out at the plant tissue culture laboratories, Date palm research center, Basra University, Iraq. This study was implemented by using somatic embryos which formed of embryogenic callus with three months old for date palm um Al-dehine growing on nutrient medium (Murashig and skoog, 1962). The nutrient media was known as MS salts which support by the following materials on basic $g.L^{-1}$ (30 Sucrose, 0.200 Sodium hydrogen orthro phosphates, 0.0005 Thiamine – HCl, 0.040 adenine Sulphates, 0.030 NAA, 0.003 Isopentenyl adenine (2-ip) and 7 Agar). For execution this study nutrient medium (MS) and the above materials were used except the addition of activated charcoal. The treatments were four concentrations of each of sodium chloride (0, 25, 50, 75 mM) and trehalose sugar (0, 5, 10, 15 $g.L^{-1}$). After adding the components of the nutrient medium, the acidity regulated on PH = 5.7 and after that the agar was added and then heated the medium by magnetic starrier on 950, the nutria medium is distributed by 25 ml in measuring tubes 2.5 ×20 cm and blocked the spouts of the tubes by cotton and roles by Aluminum foils, and then sterilized the tubes and cultures tools evaporation or steam sterilization by auto clave on temperature 121°C under steam pressure 1.05 $Kg.cm^2$ for twenty minutes. After finished from sterilization take out the tubes and culture tools and the tubes were shook for the purpose of harmonizing the nutrient medium and left to cool and kept in the refrigerator until the culture. Used 10 replicates for each treatment and cultured 50 mg from somatic embryos, rarely in each tube and the cultivated tubes were placed at 27±1 co and light intensity 3000 Lux and then reculture was implemented one time every month. The period of the experiment was 8 months. The experimental measurements were studied as follow:

Ionic content

Sodium and potassium ions: Sodium and potassium ions were measured according to Cresser and parsons (1979) by using Flame photometer.

Chloride ion: Estimation of chloride ion according to the method described by (Kalra, 1998).

Nitrogen: The micro Kjeldhal was used to measure nitrogen by steam distillation method (Page *et al.,* 1982).

Statistical analysis of the results was done for a factorial experiment with two factors in completely randomized design (Snedicor and Cochran, 1980). The means scores were tested according to the least Significant difference LSD with probability level (0.05) and the GenStat 12 was used to analyze the results

Result and Discussion

Sodium ion: Results presented in table 1. appear the effect of sodium chloride and terhalose sugar in sodium content of somatic embryos for date palm c.v. Um-Aldehine *in vitro*. The increase in Sodium chloride Concentration in media had a significant increase in the sodium concentration, In addition, the treatment 75 mM of sodium chloride achieved the highest averages, (20.78 $mg.g^{-1}$), while the sodium chloride treatment 25 mM achieved the least averages (12.43 mg.g-1).

Table 1: Effect of sodium chloride and trehalose sugar and interaction between them on sodium concentration ($mg.g^{-1}$) of somatic embryos of date palm Um-Aldehine *in vitro*.

NaCl mM	Trehalose sugar ($g.L^{-1}$)				NaCl Average
	0	5	10	15	
0	7.64	18.93	16.86	15.25	14.67
25	15.35	13.25	11.15	9.97	12.43
50	18.98	17.27	15.43	14.21	16.47
75	23.46	21.32	20.01	18.33	20.78
Trehalose average	16.38	17.69	15.86	14.44	
$L.S.D_{0.05}$	NaCl	Trehalose	Interaction		
	0.32	0.32	0.65		

The effect of trehalose sugar was significant in sodium concentration, however the treatment of 5 $g.L^{-1}$ achieved the highest average (17.69 $mg.g^{-1}$) while, the treatment trehalose sugar 15 g. L^{-1} achieved the least averages (14.44 $mg.g^{-1}$). The results in the table 1. showed a significant effects of interaction between treatments above in sodium concentration, the interaction between Sodium chloride 75 mM and trehalose sugar 0 $g.L^{-1}$ achieved the highest averages (23.46 $mg.L^{-1}$), while the interaction between sodium chloride (0 mM) and trehalose sugar (0 g. L^{-1}) achieved the least averages (7.64 $mg.L^{-1}$).

Chloride ion: Results in the table 2. show the effect of sodium chloride and trehalose sugar in chloride ion concentration of somatic embryos. Results indicated that an increase in sodium chloride concentration in culture media causes a significant increase in chloride

concentration of somatic embryos especially, the treatment of sodium chloride (75mM) achieved the highest averages which was (16.85mg.g^{-1}) compared to control treatment achieved the least averages (4.81mg.g^{-1}). The effects of increasing trehalose sugar concentration in culture media has a significant decrease of chloride, the treatment of (15g.L^{-1}) was represented the lowest averages (10.12 mg.L^{-1}) compared to control treatment achieved the highest averages (14.15mg.g^{-1}).

The interaction treatment between sodium chloride (75mM) and trehalose (0g.L^{-1}) achieved the highest averages (19.33mg.g^{-1}), while, the interaction treatment between sodium chloride (0 mM) and trehalose (15g.L^{-1}) achieved the least averages (3.25mg.g^{-1}).

Table 2: Effect of sodium chloride and trehalose sugar and interaction between them on chloride concentration (mg.g^{-1}) of somatic embryos of date palm Um-Aldehine *in vitro*.

NaCl mM	Trehalose sugar (g.L^{-1})				NaCl Average
	0	5	10	15	
0	3.37	4.94	3.65	3.25	4.81
25	13.65	12.35	11.12	10.15	11.82
50	16.23	14.22	13.25	12.14	13.96
75	19.33	16.82	16.31	14.92	16.85
Trehalose average	14.15	12.09	11.08	10.12	
L.S.D$_{0.05}$	NaCl	Trehalose	Interaction		
	0.57	0.75	1.13		

Potassium ion: Results in table 3. indicated that the treatment (75 mM) of sodium chloride achieved the least averages significantly (17.96mg.g^{-1}) compared to control treatment which was achieved the highest averages (22.20mg.g^{-1}). The effects of increase trehalose sugar concentration in culture media has a significant increase of potassium concentration, however the treatment (15 gm.L^{-1}) causes the highest averages (21.45mg.g^{-1}) compared to control treatment achieved the least average (17.57mg.g^{-1}).

The results showed that the interaction treatment of sodium chloride (0mM) and trehalose sugar (15g.L^{-1}) has led to a significant increase in potassium concentration and achieved the highest averages (23.26mg.g^{-1}), while the interaction treatment of sodium chloride (75mM) and trehalose sugar (0g.L^{-1}) achieved the lowest averages (14.81mg.g^{-1}).

Potassium to sodium ratio: The results in table 4. appear the increase sodium chloride concentration in culture media has led to decrease in potassium to sodium ratio except sodium chloride treatment (25 mM) achieved significantly highest average (1.73) and non – significant

Table 3: Effect of sodium chloride and trehalose sugar and interaction between them on potassium concentration (mg.g^{-1}) of somatic embryos of date palm Um- Aldehine *in vitro*.

NaCl mM	Trehalose sugar (g.L^{-1})				NaCl Average
	0	5	10	15	
0	20.78	21.83	22.94	23.26	22.20
25	18.57	20.89	21.36	21.94	20.69
50	16.13	19.28	20.78	20.42	19.15
75	14.81	17.33	19.56	20.16	17.96
Trehalose average	17.57	19.83	21.16	21.45	
L.S.D$_{0.05}$	NaCl	Trehalose	Interaction		
	0.42	0.21	0.21		

difference with control treatment which achieved average (1.69), while sodium chloride treatment 75 mM achieved the lowest average (0.88). The increase of trehalose sugar concentration in culture media achieved a significant increase of potassium to sodium ratio, in addition the treatment of trehalose sugar (15g.L^{-1}) achieved highest average (1.57), while the treatment (5g.L^{-1}) causes least average (1.17). The interaction treatments affected significantly in potassium to sodium ratio, the control treatment achieved highest average (2.72), while the interaction treatment of sodium chloride (75 Mm) and trehalose (0g.L^{-1}) achieved lowest average (0.63).

Table 4: Effect of sodium chloride and trehalose sugar and interaction between them on potassium to sodium ratio of somatic embryos of date palm Um- Aldehine *in vitro*.

NaCl mM	Trehalose sugar (g.L^{-1})				NaCl Average
	0	5	10	15	
0	2.72	1.15	1.36	1.52	1.69
25	1.21	1.58	1.92	2.20	1.73
50	0.85	1.12	1.35	1.44	1.19
75	0.63	0.81	0.98	1.10	0.88
Trehalose average	1.35	1.17	1.40	1.57	
L.S.D$_{0.05}$	NaCl	Trehalose	Interaction		
	0.05	0.05	0.11		

The results in (Tables 1, 2, 3 and 4) appeared that the increase in sodium and chloride ions concentrations of somatic embryos under salt stress might be related to the increase in concentration of sodium and chloride ions in culture media as a result of increasing in sodium chloride concentration in media, and thus increase their absorption by exposed cells salt stress (Tester and Darenport, 2003; Queiros *et al.,* 2007; Karimi *et al.,* 2009). In addition, the sodium ion also follows another entering pathway into the cell through potassium channels because the proteins in the membrane which are formed

Table 5: Effect of sodium chloride and trehalose sugar and interaction between them on nitrogen concentration of somatic embryos of date palm Um- Aldehine *in vitro*.

NaCl mM	Trehalose sugar (g.L^{-1})				NaCl Average
	0	5	10	15	
0	14.13	15.34	17.14	17.87	16.12
25	12.45	13.46	15.83	15.91	14.41
50	11.11	12.22	13.76	14.13	12.80
75	9.73	10.57	12.38	13.35	11.51
Trehalose average	11.85	12.90	14.78	15.32	
L.S.D$_{0.05}$	NaCl	Trehalose	Interaction		
	0.50	0.50	0.99		

in shape of channels that allow the entry of sodium ion in to the cells because the membranes are semi-selectivity, which leads to sodium accumulation at high concentrations compared to potassium ion (Munns, 2002). The effects of trehalose sugar and its interaction with sodium chloride have led to a significant decrease in sodium and chloride ions concentrations, and a significant increase in potassium ion concentration and potassium to sodium ratio, the reason may be due to the trehalose sugar considered as osmoprotectant for proteins and cellular membrane structures, thus contributing effectively of increasing cell tolerance of salt stress (Grow *et al.,* 1984; Muller *et al.,* 1995). One of the most important indicators of cells tolerance to salt stress is decreasing the sodium and chloride ions concentration and increasing the potassium ion concentration and increasing potassium to sodium ratio (Iordachescu and Imai, 2008), or perhaps the trehalose sugar contributed to improve the water potential of salt-exposed cells by inhibiting the accumulation of toxic ions and lipid oxidation (Ashraf *et al.,* 2008; zeid, 2009).

Nitrogen: Results in the table 5 showed that the increase in sodium chloride concentration in culture media decrease nitrogen concentration significantly. In addition, the treatment (75mM) of sodium chloride achieved the lowest averages (11.51mg.g^{-1}) compared to control treatment (16.12mg g^{-1}). While, the increasing of trehalose sugar concentration in culture media has increased significantly the nitrogen concentration, and the treatment (15g.L^{-1}) resulted the highest averages (15.32mg.g^{-1}) compared to control treatment (11.85mg.g^{-1}). Interaction results showed a significant effects in nitrogen concentration and the treatment of (0mM) with trehalose sugar (15g.L^{-1}) achieved the highest averages (17.87mg.g^{-1}), while the interaction between sodium chloride (75mM) and trehalose sugar (0g.L^{-1}) achieved the lowest averages (9.73mg.g^{-1}). The decrease in nitrogen concentration of somatic embryos under salt stress might be related to the competition between chloride and nitrate ions on ionic transport locations in cells, in addition to effects the plasma membrane permeability to sodium ions, thus it has been found that sodium ions inhibit the absorption of nitrate ion (Cram, 1983). The reason for the increased concentration of nitrogen by increasing the concentration of trehalose sugar in the culture media and its interactions with sodium chloride may be attributed to its role as an osmoprotectant for each of proteins and membranes structures (Crowe *et al.,* 1984; Muller *et al.,* 1995).

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