The response of different date palm (*Phoenix dactylifera* L.) cultivars to callus induction and development by *in vitro* culture under salt stress

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Abstract

The study aimed to investigate the effect of the cultivar type and orchard location (Safwan, Abu Al-Khaseeb and Shat Al-Arab locations) in the Basra governorate/Iraq on the response to callus induction (from culturing the shoot tips of the two cultivars [Hillawi and Sayer date palm trees]) on MS medium supplied with different concentrations of sodium chloride (0, 50, 100, 150, 200 and 250 mM). Results revealed the significant superiority of the Sayer cultivar in terms of the percentage of shoot tip response and the required period for callus induction. Additionally, the Safwan location was significantly superior to the other locations in the response and required period for callus induction. Bilateral interaction between the Sayer cultivar and the Safwan location recorded the highest response and required period for callus induction and a significant difference from other interactions. The results showed a decrease in the fresh and dry weights of the embryogenic callus and the number of somatic embryos, with an increase in the concentration of sodium chloride salt that was added to the MS medium. The callus cultured in the MS medium with 200 and 250 mM sodium chloride salt led to growth failure and browning tissues. The Sayer cultivar recorded an increase in the fresh and dry weights of the embryogenic callus and the number of somatic embryos in all saline concentrations compared with the Hillawi cultivar. Samples from the Safwan location also recorded high values in these characteristics in all saline concentrations compared with those from the Abu Al-Khaseeb and Shatt Al-Arab locations.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees in the Middle East due to its economic value in the region (El-Khairy, 2007) and plays a major role in providing food, preserving the ecosystem and the agricultural economy (Sedra, 2015). In vitro cultivation of date palm tissues have become an effective method for expanding and restoring date palm farms (El Khairy and Nike, 2017). Plant tissue culture is defined as the growth and multiplication of plant cells, tissues and organs grown in media under controlled conditions (Ahloowalia et al., 2004). The callus is defined as a mass of undifferentiated parenchyma cells that have the greatest power to continue cell division, growth and development. The callus arises from meristematic tissues in the cut or wound surface of explants and is formed either naturally or by plant tissue culture. Callus induction depends on the type of medium, the addition of growth regulators and incubation conditions involved (Al-Khayri and Ibraheem, 2014). Date palm trees differ in their response to in vitro culture and callus induction in different cultivars because there is high-, medium- and low-response cultivars (Jasim, 1999; Al-Khateeb et al., 2002). Plant cells and tissue culture can be used to examine plant responses to salinity by exposing plant cells (or tissues) to different levels of salt stress. Such a method may be useful in selecting salinity-tolerant cells from cells that are essentially sensitive to salinity. Thus, genetic information is necessary for the growth of these sensitive plant cells in saline environments, but it has not been expressed genetically. Moreover, the selection of salinity-tolerant cells with tissue culture techniques will increase the knowledge of salinity-tolerant cells (Munns, 2005). Salinity is one of the most important problems facing agriculture on a large scale and reduces crop productivity, especially in arid and semi-arid regions, including Iraq (Batanony, 1996; Abass, 2016; Ibrahim 2017).The use of tissue culture in this field allows the examination and separation of cells carrying the desired traits between thousands of cells in a short period and a limited area. Such work facilitates evaluations on plants in different stages of growth and development, as well as studies on their ability to inherit a desired trait through the produced generation.

Saline-tolerant cells can be selected by culturing them either as a separate or a mass of callus cells on a medium that contains different concentrations of essential elements that the plant cells need to continue dividing and growing. This medium adds different levels of sodium chloride to determine the concentration in which cell division and cell growth stop. The cells that grow in this concentration and slightly above it are salt-tolerant cells (Samad et al., 2001). Plant tissue culture causes variations in plants produced from the cell or callus proliferation called somaclonal variation. These variations can be considered a source of new genetically improved varieties and result in plants tolerant to environmental stress in a relatively short period by in vitro culture. Salinity influences the bioprocesses that occur within the plant cell, such as respiration. The salt-tolerant cells are distinguished by their ability to maintain the rates of bioprocesses necessary for growth and development when exposed to high salinity levels (El Hadrami et al., 2011; Jain, 2012). Plants growing in saline environments have developed many mechanisms for salt tolerance compared with saline-sensitive plants, such as the ability to accumulate ions of mineral elements in vacuoles, increased production of amino acids such as proline and glutamine and enhanced activity of some antioxidant enzymes (AL-Khayri, 2002). Given the importance of date palm and the desire to propagate it by tissue culture technology, this study was conducted to examine the effect of the location and interaction of the cultivar on the response to in vitro culture and callus induction from the shoot tip culture of date palm in MS medium supplied with different concentrations of sodium chloride salt**.**

Material and Methods

The preparation of culture medium

The culture medium was prepared from the addition of formula MS salts (Murashige and Skoog, 1962) that obtained from Zist Arman Sabz Company (ZAS) at a concentration of 4.33 g L^{-1}, sucrose at 40 g L^{-1}, sodium orthophosphate at 170 mg L^{-1}, myo-inositol at 100 mg L^{-1}, vitamin group at 1 mg L^{-1}, adenine sulfate at 40 mg L^{-1}, activated charcoal at 2 g L^{-1}, 2,4-D at 30 mg L^{-1}, NAA at 5 mg L^{-1} and 2iP at 1mg L^{-1}.The pH of the MS medium was then

adjusted to 5.7 with a solution of NaOH and HCl at 0.1 Normality for each of them. Subsequently, the agar was added at 6 g L^{-1} to MS medium, and this medium was heated to 90 °C by placing it on a heat source provided with a magnetic stirrer in order to dissolve the agar and homogeneity in the medium. The MS medium was distributed in the Pyrex glass test tubes at 20 ml per tube. The culture tubes were sterilized in the Autoclave instrument at 121 °C and 1.05 Kg $cm^{-2}$for 20 minutes. The study lasted 27 months, including 24 months for callus induction and three months to the experiment of the growth of cultures under salt stress.

The preparation of explants

The explants were taken from two cultivars of date palm trees, Sayer and Hillawi, from orchards at three locations from the Basrah city, Southern Iraq, namely, Safwan, Abu Al-Khaseeb and Shat Al-Arab.The data in Table 1 showed the values of pH and the level of electrical conductivity (EC) of irrigation water and soil for the three locations under study. These explants were represented by the shoot tips obtained from the anatomy of three offshoots of each cultivar in each location (Figure 1, A and B).The explants were then placed in an antioxidant solution of 100 mg L^{-1} citric acid and 150 mg L^{-1} ascorbic acid for 60 minutes to avoid phenolic compounds exudation during explants culturing. These explants were then rinsed with sterile distilled water for 3 times and surface sterilized with 20% commercial Chlorax solution containing 1.05% sodium hypochlorite, and a drop of tween 20 for 15minutes. The explants were rinsed in sterile distilled water for 3 times. The cultures were grown in a growth room at 26 ±2 °C and dark condition for 4-6 months. The cultures were transferred to the similar stimulating medium for the callus induction, five times between each time and the next is eight weeks.

A. Callus induction experiment

The following data has been recorded:

1. The percentage of response to callus induction.
2. The required period length to the callus induction (Days).
3. The fresh weight of callus mass per culture tube.

4. The dry weight of callus mass per culture tube.

B. Salt stress experiment

After induction and multiplication of callus, the embryogenic callus of the two cultivars (Sayer and Hillawi) were taken from the three locations (Safwan, Abu Al-Khaseeb and Shat Al-Arab) under study was obtained by *in vitro* culture during a period of 24 months. Then the callus was transferred to a new media containing the same components adding different concentrations of sodium chloride salt (0, 50, 100, 150,200 and 250 mM).The following data has been recorded:

1. The fresh weight of callus mass per culture tube.
2. The dry weight of callus mass per culture tube.
3. Number of somatic embryos per culture tube.

Table 1: The pH and EC levels of irrigation water and soil at three locations under study.

Location	pH of water irrigation	pH of soil	EC of water irrigation (dS m^{-1})	EC of soil (dS m^{-1})
Safwan	8.26	8.32	13.26	9.48
Abu Al-Khaseeb	8.45	7.35	9.48	5.39
Shat Al-Arab	8.47	7.23	8.47	5.13

Experimental design and statistical analysis

The experiment was designed using Randomized complete block design (RCBD). The type of experiment for the callus induction is two factors and salt stress is factorial with three factors. Each treatment repeated ten times. The data were statistically analyzed using variance analysis. The mean of the treatments was compared with the revised-least significant difference, depending on Al-Rawi and Khalafallah (2000).

Results and Discussion

The results showed in Table (2 and 3) proved that the Sayer cultivar was a significant differed in the percentage of shoot tip response and the required period to callus production, which reached 74.00% and 31.78 days, respectively,

compared to Hillawi cultivar after six months of culturing. But the Hillawi cultivar reported the lowest percentage of response and longest period to callus induction, which were67.11% and 35.22 days, respectively (Figure 1, C). The Safwan location was significantly effecting in the percentage of response and the required period to the callus induction, which were to 78.67% and 30.17 days, respectively, compared to the other two locations. Regarding Shat Al-Arab location, results showed the lowest percentage of response and longest period to callus induction, which reached 64.33% and 35.50 days, respectively(Figure 1, D). The interaction between the Sayer cultivar and Safwan location was significantly superior in the percentage of response and the required period to callus induction reached 82.00% and 29.67 days, respectively, compared to other interactions. While the interaction between the Hillawi cultivar and Shat Al-Arab location showed the lowest percentage of response and the longest period to callus induction reached 60.00% and 38.00 days, respectively (Table 2 and 3).

The results of Tables 4 and 5 indicated that there were no significant differences between the Sayer and Hillawi cultivars in the fresh weight of the callus after six months of culturing. However, the Sayer cultivar was significantly superior in the dry weight of the callus reached 0.0919 g compared to the Hillawi cultivar which was0.0864 g. The Safwan location was significantly superior to the other two locations, Abu Al-Khaseeb, and Shatt Al-Arab, in the fresh and dry weight of the callus, which recorded1.180 and 0.0990 g, respectively. The results of the Shat Al-Arab location recorded the lowest averages in the fresh and dry weight of the callus reached 0.920 and 0.0797 g, respectively. The interaction between Sayer or Hillawi cultivar and Safwan location showed a significant increase in the fresh and dry weight recorded 1.223, 0.1033 and 1.137, 0.0947, respectively. While the interaction between Hillawi cultivar and Shat Al-Arab location reported the lowest averages in fresh and dry weight of the callus which were 0.890 and 0.0797 g, respectively.

The results in Tables 6, 7 and 8 showed that there was a significant decrease in fresh and dry weight and the number of somatic embryos with the increase of sodium chloride concentrations (Figure 1, E). The control treatment (without

NaCl) recorded the highest values in these examined traits, which were1.0972 g, 0.2060 g and 6.28 embryos, respectively. While the concentration of 150 mM sodium chloride salt recorded the lowest values reached0.1539 g, 0.0106 g and 0.110 embryos, respectively. The date palm callus when was cultured on the MS medium supplied with sodium chloride salt at 200 and 250 mM led to complete growth failure, browning tissue and cell death. The interactions between the Sayer cultivar and all salt concentrations were recorded the highest averages of fresh and dry weight, as well as, the number of somatic embryos when compared with the Hillawi cultivar (Figure 1, F). Hence, the interactions between the Safwan location and all the salt concentrations recorded the highest averages of the fresh and dry weight of the callus and the number of somatic embryos compared to the other two locations, Abu Al-Khaseeb and Shatt Al-Arab. The triple interaction between Sayer cultivar, Safwan location and 0 mM sodium chloride showed the highest values in fresh and dry weight and a number of somatic embryos which were 1.3100 g, 0.2460 g and 8.67 embryos, respectively. While these traits in other triple interactions were decreased with the increase of sodium chloride concentration (Table 6, 7 and 8).

The different patterns in the response of both Hillawi and Sayer cultivars to the induction of callus, fresh and dry weight of primary and embryogenic callus, the number of somatic embryos under normal conditions or salt stress could be explained by the genetic variations between them (Ibrahim et al., 2017). The results of the current study agreed with the study of Al-Khateeb et al. (2002) about the study of tolerance of different date palm cultivars to salinity.

Regarding the differences in response between the shoot tips of the offshoots of Hillawi and Sayer cultivars that were collected from Safwan, Abu Al-Khaseeb and Shat Al-Arab locations in callus induction and development and the number of somatic embryos by *in vitro* culture under normal conditions and salt stress could be attributed to the difference in the environment of each location. The data in Table 1 showed that the averages of electrical conductivity of soil and irrigation water were high in the Safwan location which were 9.48 and 13.26 dS m^{-1}, respectively. While these EC values of the soil and irrigation water were low

in Abu Al-Khaseeb and Shat Al-Arab locations and reached 5.39 and 9.48 dS $m^{-1}$and 5.13 and8.47dS m^{-1}, respectively. This means that the offshoots of the two date palm cultivars, Hillawi and Sayer, have grown in an environment with a high level of salinity in the Safwan location, compared to the regions of Abu Al-Khaseeb and Shatt Al-Arab, which resulted in decreasing cell division rate and tissue growth. Compared to these explants which collected from the offshoots that grew in the Safwan location were cultured in an optimum and natural environment, this resulted in a high response to the shoot tips in stimulating and increasing the rate of cell division, induction and development of callus, fresh and dry weight and the embryogenesis during a short period compared to the explants of the date palm of Abu Al-Khaseeb and Shat Al-Arab (Table 2, 3, 4, 5 and 6).

The different patterns in the response of both Hillawi and Sayer cultivars to the induction of callus, fresh and dry weights of primary and embryogenic callus and the number of somatic embryos under normal conditions or salt stress could be explained by their genetic variations (Ibrahim et al., 2017). The results of the current study agreed with the study of Al-Khateeb et al. (2002), who reported the tolerance of different date palm cultivars to salinity.

The differences in response between the shoot tips of the offshoots of the Hillawi and Sayer cultivars from the Safwan, Abu Al-Khaseeb and Shat Al-Arab locations in callus induction and development and the number of somatic embryos by *in vitro* culture under normal conditions and salt stress could be attributed to the variations in the environment of each location. The data in Table 1 show that the averages of electrical conductivity of soil and irrigation water were high at 9.48 and 13.26 dS m^{-1}, respectively, in the Safwan location. Meanwhile, the EC values of the soil and irrigation water were low in the Abu Al-Khaseeb and Shat Al-Arab locations and reached 5.39 and 9.48 dS $m^{-1}$and 5.13 and8.47dS m^{-1}, respectively. Thus, the offshoots of the two date palm cultivars, Hillawi and Sayer, grew in an environment with a high level of salinity in the Safwan location compared with the regions of Abu Al-Khaseeb and Shatt Al-Arab, which resulted in decreasing cell division rate and tissue growth. The

explants collected from the offshoots that grew in the Safwan location and were cultured in an optimum and natural environment demonstrated a high response to the shoot tips in stimulating and increasing the rate of cell division, induction and development of callus, fresh and dry weights and embryogenesis during a short period compared with the explants of the date palm of Abu Al-Khaseeb and Shat Al-Arab (Tables 2, 3, 4, 5 and 6). The low response, fresh and dry weight of callus and a number of somatic embryos with increased concentrations of sodium chloride were due to the high concentrations of sodium and chloride ions and osmotic pressure. These changes led to a decrease in water and nutrient absorption by the explants from the medium. The results of the current study agreed with the results of Al-Khayri and Ibraheem (2014), who studied callus induction and embryogenesis of date palm by in vitro culture under salt stress. They cited genetic differences as the reason for the increased response in callus induction, fresh and dry weights of callus and the number of somatic embryos in the Sayer cultivar at all concentrations of sodium chloride compared with the Hillawi cultivar (Al-Khateeb et al., 2002). The high saline level of NaCl severely affects the cultivated callus, thereby causing cellular death and brown staining of the medium. These phenomena lead to a decrease in the lean weight of the callus due to the loss of the osmotic balance of the cells and loss of their fullness, which leads to the death of most cells and other adverse effects. These effects occur at the physiological and biochemical levels due to a decrease in the readiness of nutrients and inhibition of growth. The ionic disturbance inside the cell caused by the high level of salinity will push the cell to expend a large part of its energy allocated to metabolic activities to adapt to the conditions of salt tension (Mansour, 2000). The presented results clearly demonstrated that reduction in tensile salt growth is one of the most important methods used by cells growing in salt media to conserve energy for the continuation of vital processes in the cell (Venkataiah et al., 2014). The salt tolerance of date palm varieties is often tested ex vivo to determine the tolerance of the cultivars to stress conditions. Environmental saline and aqueous stress using NaCl and PEG have been previously investigated (El-Rabey et al., 2015; Jasim et al., 2016; Al Kharusi et

al., 2017). Sodium and chlorine are the two most destructive elements to plant tissues, causing tissue toxicity (Flowers et al., 2014). The presence of NaCl in plant growth medium induces secondary stresses (Al Kharusi et al., 2017).

The difference in date palm cultivars in their ability to tolerate salt may be due to the ability of some cultivars to restrict the movement of chloride ions; thus, these ions become less harmful in photosynthesis activity (Jasim et al. 2016). Al-Debiani et al. (2018) found a difference in the ability of cultivars to maintain the potassium ion in cells, and the recalcitrant cultivar has a greater ability to maintain the level of potassium ion in cells than the Berhi cultivar. Anschütz et al. (2014) highlighted the importance of potassium ion in maintaining the osmotic pressure of cells and cell membranes from damage.

The initial treatment of date palm callus with sodium chloride is an efficient way to identify the extent to which the varieties tolerate salt tension at an early date and determine the salinity-tolerant varieties. The difference between the date palm varieties to withstand salt stress may be due to genetic causes in terms of the cells' ability to accumulate the amino acid proline in tissues. Proline is one of the combinatorial solvents important in maintaining the osmotic pressure of cells exposed to salinity and drought conditions (Aldhebiani et al., 2018).

The direct effect of sodium chloride salt on the osmosis of cells causes a decrease in the water stress in cells, which leads to an obstruction to the absorption of water by the plan. The obstruction of water absorption causes decreased melting pressure, water stress and disturbance in the ionic balance. As the sodium ion rapidly approaches the cell, the negatively charged inner membrane voltage reaches 120–200 millivolts. The sodium ion accumulates inside the cell at a concentration ranging from 100 to 1000 times more than its concentration in the cell walls. The sodium ion competes with the potassium ion in the cellular sites, causing damage to the cell. Therefore, the salts decrease in the cell.

Conclusion

Sayer cultivar showed a remarkable superiority in its response to callus induction and somatic embryogenesis by in vitro culture under normal conditions as well as under stress of sodium chloride salt compared to the Hillawi variety, and the buds

extracted from the desert site in Safwan showed a clear response to tissue culture compared to the sites of Abi Al-Khasib and Shatt. Arabs and under normal conditions and sodium chloride stress, the response to callus induction and somatic embryogenesis decreased with increasing NaCl concentrations.

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Table 2: The effect of cultivar, location and their interactions on the percentage of response to callus induction after six months from culture.

Cultivar	Location			Cultivar effect
	Safwan	Abu Al-Khaseeb	Shat Al-Arab	
Hillawi	75.33%	66.00%	60.00%	67.11%
Sayer	82.00%	71.33%	68.67%	74.00%
Location effect	78.67%	68.67%	64.33%	
R-LSD p≥0.05	Cultivar	Location	Interaction	
	2.476	3.032	4.288	

Table 3: The effect of cultivar, location and their interactions on the period length (days) to callus induction after six months from culture.

Cultivar	Location			Cultivar effect
	Safwan	Abu Al-Khaseeb	Shat Al-Arab	
Hillawi	30.67	37.00	38.00	35.22
Sayer	29.67	32.67	33.00	31.78
Location effect	30.17	34.83	35.50	
R-LSD p≥0.05	Cultivar	Location	Interaction	
	1.819	2.228	3.151	

Table 4: The effect of cultivar, location and their interaction on the fresh weight (g) of callus after six months from culture.

Cultivar	Location			Cultivar effect
	Safwan	Abu Al-Khaseeb	Shat Al-Arab	
Hillawi	1.137	0.947	0.890	0.991
Sayer	1.223	0.953	0.950	1.042
Location effect	1.180	0.950	0.920	
R-LSD p≥0.05	Cultivar	Location	Interaction	
	0.0719	0.0880	0.1245	

Table 5: The effect of cultivar, location and their interactions on the dry weight (g) of callus after six months from culture.

Cultivar	Location			Cultivar effect
	Safwan	Abu Al-Khaseeb	Shat Al-Arab	
Hillawi	0.0947	0.0847	0.0797	0.0864
Sayer	0.1033	0.0863	0.0860	0.0919
Location effect	0.0990	0.0855	0.0797	
R-LSD p≥0.05	Cultivar	Location	Interaction	
	0.00818	0.01002	0.01416	

Table 6: The effect of cultivar type, location, Sodium chloride concentration and their interactions on the fresh weight (g) of embryogenic callus.

	Cultivar		Location			NaCl concentration (mM)			
	Hillawi	Sayer	Safwan	Abu Al-Khaseeb	Shat Al-Arab	0	50	100	150
	0.5342	0.6267	0.6592	0.5421	0.5400	1.0972	0.6950	0.3756	0.1539

Location	Cultivar		NaCl conc. (mM)	Cultivar		NaCl conc. (mM)	Location		
	Hillawi	Sayer		Hillawi	Sayer		Safwan	Abu Al-Khaseeb	Shat Al-Arab
Safwan	0.5550	0.7633	0	1.0689	1.1256	0	1.1967	1.0467	1.0483
Abu Al-Khaseeb	0.4575	0.6267	50	0.6311	0.7589	50	0.7567	0.6233	0.7050
Shat Al-Arab	0.5900	0.4900	100	0.2989	0.4522	100	0.4950	0.3567	0.2750
			150	0.1378	0.1700	150	0.1883	0.1417	0.1317

Cultivar	Location	NaClconcentration (mM)			
		0	50	100	150
Hillawi	Safwan	1.0833	0.6533	0.3333	0.1500
	Abu Al-Khaseeb	1.0267	0.4667	0.2100	0.1267
	Shat Al-Arab	1.0967	0.7733	0.3533	0.1367
Sayer	Safwan	1.3100	0.8600	0.6567	0.6567
	Abu Al-Khaseeb	1.0667	0.7800	0.5033	0.1567
	Shat Al-Arab	1.0000	0.6367	0.1967	0.1267

LSD (p≥0.05)	Cultivar (C)	Location (L)	NaCl conc. (N)	C × L interaction	C × N interaction	L × N interaction	C × L × N interaction
	0.03236	0.03964	0.04577	0.05606	0.06473	0.07927	0.11211

Table 7: The effect of cultivar type, location, Sodium chloride concentration and their interactions on the dry weight (g) of embryogenic callus.

	Cultivar		Location			NaCl concentration (mM)			
	Hillawi	Sayer	Safwan	Abu Al-Khaseeb	Shat Al-Arab	0	50	100	150
	0.0767	0.1142	0.1040	0.0988	0.0826	0.2060	0.1188	0.0464	0.0106

Location	Cultivar		NaCl conc. (mM)	Cultivar		NaCl conc. (mM)	Location		
	Hillawi	Sayer		Hillawi	Sayer		Safwan	Abu Al-Khaseeb	Shat Al-Arab
Safwan	0.0756	0.1324	0	0.1835	0.2283	0	0.2278	0.2218	0.1683
Abu Al-Khaseeb	0.0829	0.1167	50	0.0950	0.1427	50	0.1258	0.1117	0.1190
Shat Al-Arab	0.0716	0.0936	100	0.0182	0.0746	100	0.0518	0.0545	0.0328
			150	0.100	0.0112	150	0.0105	0.0112	0.0102

Cultivar	Location	NaCl concentration (mM)			
		0	50	100	150
Hillawi	Safwan	0.2097	0.0717	0.0120	0.0090
	Abu Al-Khaseeb	0.2053	0.0933	0.0223	0.0107
	Shat Al-Arab	0.1356	0.1200	0.0203	0.0103
Sayer	Safwan	0.2460	0.1800	0.0917	0.0120
	Abu Al-Khaseeb	0.2383	0.1300	0.0867	0.0117
	Shat Al-Arab	0.2010	0.1180	0.0453	0.0100

LSD ($p \geq 0.05$)	Cultivar (C)	Location (L)	NaCl conc. (N)	C × L interaction	C × N interaction	L × N interaction	C × L × N interaction
	0.01146	0.01404	0.01621	0.01986	0.02293	0.02808	0.03971

Table 8: The effect of cultivar type, location, Sodium chloride concentration and their interactions on the number of somatic embryos.

	Cultivar		Location			NaCl concentration (mM)			
	Hillawi	Sayer	Safwan	Abu Al-Khaseeb	Shat Al-Arab	0	50	100	150
	2.44	2.78	3.58	2.29	1.96	6.28	3.22	0.83	0.11

Location	Cultivar		NaCl conc. (mM)	Cultivar		NaCl conc. (mM)	Location		
	Hillawi	Sayer		Hillawi	Sayer		Safwan	Abu Al-Khaseeb	Shat Al-Arab
Safwan	3.08	4.08	0	6.33	6.22	0	7.83	5.67	5.33
Abu Al-Khaseeb	1.58	3.00	50	2.78	3.67	50	4.83	2.67	2.17
Shat Al-Arab	2.67	1.25	100	0.67	1.00	100	1.33	0.83	0.33
			150	0.00	0.22	150	0.33	0.00	0.00

Cultivar	Location	NaCl concentration (mM)			
		0	50	100	150
Hillawi	Safwan	7.00	4.00	1.33	0.00
	Abu Al-Khaseeb	5.00	1.33	0.00	0.00
	Shat Al-Arab	7.00	3.00	0.67	0.00
Sayer	Safwan	8.67	5.67	1.33	0.67
	Abu Al-Khaseeb	6.33	4.00	1.67	0.00
	Shat Al-Arab	3.67	1.33	0.00	0.00

LSD (p≥0.05)	Cultivar (C)	Location (L)	NaCl conc. (N)	C × L interaction	C × N interaction	L × N interaction	C × L × N interaction
	0.497	0.609	0.703	0.861	0.994	1.217	1.722

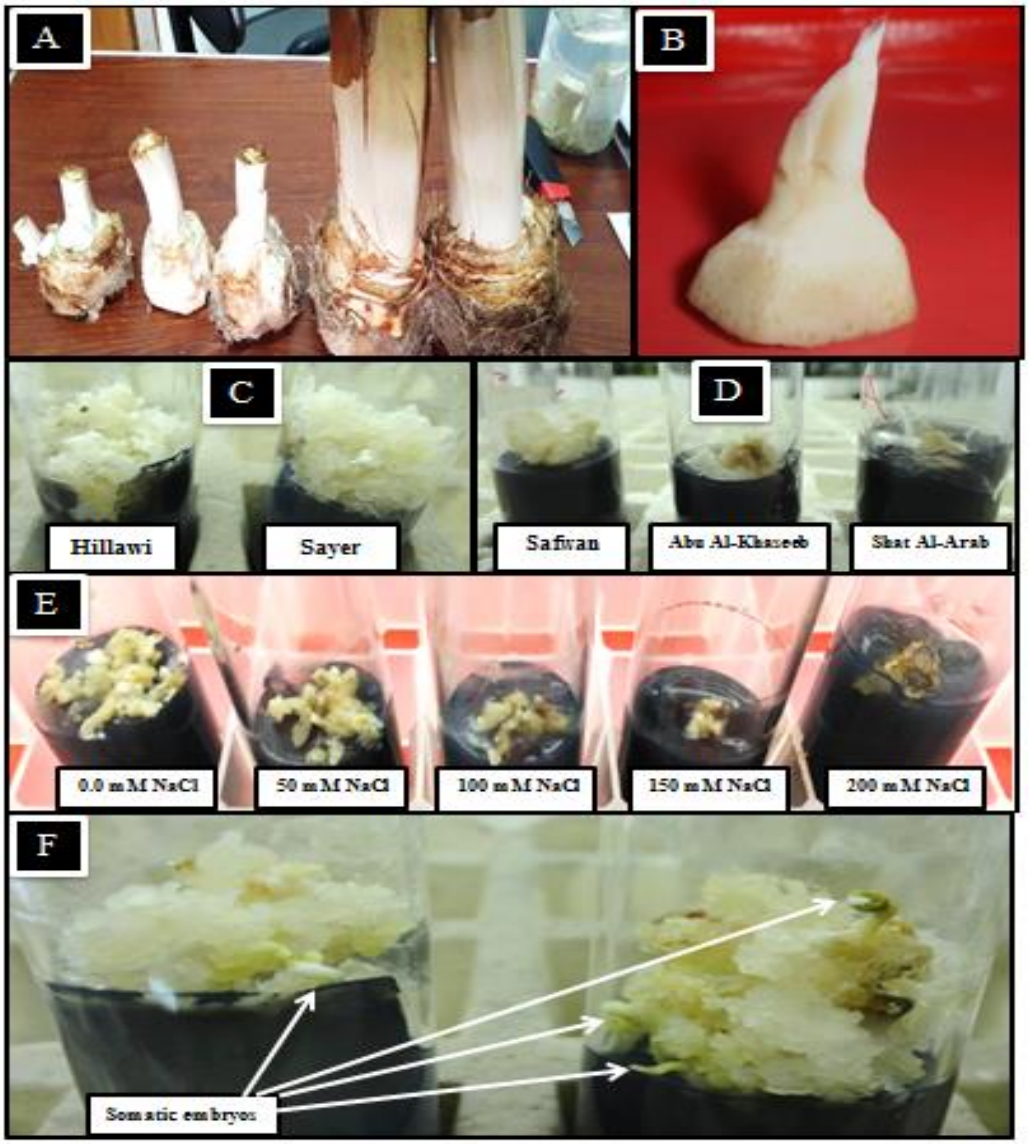

Figure 1: Microprapagation of date palm (*Phoenix dactylifera* L.) by *in vitro* culture technique.

A. Anatomy stages of date palm offshoots; B. Shoot tip of date palm cv. Sayer; C. Primary callus of Hillawi and Sayer cultivars; D. Primary callus of Sayer cultivar at three locations, Safwan, Abu Al-Khaseeb and Shat Al-Arab; E. Effect of different sodium chloride concentrations on growth of embryogenic callus; F. Embryogenesis from embryogenic callus of Sayer cultivar.