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The use of electrolyte leakage procedure in assessing heat and salt tolerance of Ruzaiz date palm (*Phoenix dactylifera* L.) cultivar regenerated by tissue culture and offshoots and treatments to alleviate the stressful injury

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Salt and heat stresses are one of the greatest constraints facing agricultural production worldwide, particularly in arid and semi-arid countries where scarcity of water and high temperatures prevail. Assessing tolerance level of date palm trees regenerated by tissue culture against heat and salt stresses is a prerequisite. This investigation aims to determine heat and NaCl tolerance of date palm (cv. Ruzaiz) produced by tissue culture and offshoots as well as to study the possibility of increasing tolerance to heat stress alone or in the presence of NaCl stress by using calcium, potassium or oleic acid by using electrolyte leakage method which based on sigmoidal curves at 50%. Tissue culture plants used in this investigation were at 4 and 10 months old *in vitro* plants namely; VP2 and VP3, respectively as well as offshoots from the same variety attached to mother plant in the field. Electrolyte leakage method was used to determine thermo-tolerance of leaf tissues. Leaf segments of five-centimeter length from VP2, VP3 and offshoots were assayed in the laboratory for tolerance to treatments (heat, heat plus NaCl, heat plus KCl, heat plus CaCl₂, heat plus oleic acid, heat plus NaCl and oleic acid, heat plus NaCl and KCl and heat plus NaCl and CaCl₂). The concentrations of the test compounds were: NaCl at 1% w/v, KCl or CaCl₂ (0.2 M) or oleic acid (0.1 M). A completely randomized design was used with three replications. The results revealed that thermo-tolerance values were 53, 53.5, and 58.5°C for VP2, VP3, and offshoot leaflets, respectively. Also there is a potential to increase the thermo-tolerance of VP2, VP3, or offshoots that could increase their survival under field conditions by pretreatment with KCl, CaCl₂ (0.2 M) or oleic acid (100 ppm).

Key words: Electrolyte leakage, thermo-tolerance, membrane stability, salinity, lethal temperature, heat regime, semipermeability.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious long-lived monocotyledon tree. It is a major crop in the Arabian Peninsula (Youssef and Wad, 2008). Offshoots are a

dominant multiplication method for date palm trees. However, it does not meet high demand due to a limited number of offshoots produced from each tree. Plant

tissue culture technique has been used on large scale to reproduce homogenous date palm plants that are true to type and free from diseases (Zaidabd de Wet, 1999; Awad et al., 2006). However, there is lack of information about the tolerance of regenerated *in vitro* plants to heat and salt stresses. These stresses are usually associated with slow growth rate and frequent death of plants after transplanting (Awad et al., 2006; Hammouda et al., 1998).

The agronomic importance of date palm is linked to its high tolerance to environmental stresses, such as salinity, drought and high temperature for limited levels as compared to many other fruit trees (FAO, 1982; Ramoliya and Pandey, 2003). To our best knowledge, till now there is no study so far that identified the exact tolerance level or the lethal temperature of heat and salt stresses to date palm offshoots or *in vitro* plants for Ruzaiz cultivar. Electrolyte leakage procedure has been used effectively to measure cell membrane stability due to environmental stresses such as water, drought, heat and cold stresses in date palm crop (Youssef and Wad, 2008; Awadet al., 2006; Farag and El-Konaissi, 2002) and other crops (KalimUllah et al., 2014; Adil et al., 2014; Anbu and Sivasankaramoorthy, 2014; Cha-um et al., 2013; Asemoto and Conaire, 2010; Cha-Um et al., 2010). Youssef and Wad (2008) found that the electrolyte leakage of date palm seedlings positively correlated with increasing salinity and they considered that as indications of the degree of impairment to membrane integrity.

In nature the plant is exposed to multiple stresses which interact to limit growth and productivity (Turner and Kramer, 1980). Al-Mulla et al. (2013) found that Khalas date palm cultivar tolerates water salinity to 20 dS/m and 'Nabusaiif' was the least tolerant cultivar. Similarly, Al-Abdoulhad 2011 found Saudi Arabia's premier date cultivar (Khalas) was the most salt tolerant cultivar. Alrasbi et al. (2010) concluded from their experiment that date palm seedlings of varieties 'Khalas', 'Khunaizy' and 'can be irrigated with saline water during vegetative growth till 9 dS m⁻¹, however water with E_c 18 dSm⁻¹ caused a significant decline in growth will reach up to 50%

There are many reports of a protective effect of Ca²⁺, K⁺ and unsaturated fatty acids such as oleic acid against heat or salt injury. Gary-boo (1970) reported that calcium might serve to bind the polar head groups of phospholipids together and thus limit membrane permeability. This was reflected on reduced electrolyte leakage. Poovaiah and Leopold (1976) also reported that the leakage of solutes from plant tissues induced by (NH₄)₂SO₄ could be relieved by the addition of CaCl₂. Similarly, Toprover and Glinka (1976) found that Ca²⁺ inhibited the reversible; heat-induced efflux of betacyanin

from beetroot exposed to 45°C for 90 min. Richard and Gray (1984) reported that CaCl₂ could protect leaf membranes against the leakiness induced by NaCl. Similarly, Ben-Hayyim et al. (1987) found that K⁺ application could reduce the deleterious effect of salinity on plant development. Darwesh (2013) found that application of amino acids had significantly ameliorated the harmful effects of salinity on date palm plantlets cv. Bartomouda. However, efficiency of Ca²⁺, K⁺ and oleic acid in alleviating or increasing heat or salt injury for Ruzaiz date palm has not studied yet.

The objectives of this study were to accurately identify the heat and salt tolerance of date palm plants (Ruzaiz) cultivar reproduced by tissue culture and offshoot as well as to study the possibility of increasing tolerance to heat stress alone or in the presence of sodium chloride stress by calcium and potassium, as well oleic acid.

MATERIALS AND METHODS

This study was conducted in the Horticulture laboratory at the Department of Plant Production, Faculty of Food Systems, United Arab Emirates University in 2002. Six and ten months old date palm *in vitro* plants (VP2 and VP3, respectively) regenerated by organogenesis tissue culture technique and 4 years old offshoots attached with mother plant of Ruzaiz date palm cultivar were used in this study. For more hardening, selected tissue culture plants were maintained for three weeks in controlled greenhouse (23 ± 1°C, 40-50% relative humidity) at Date Palm Research and Development Unit at Al-Oha Research Station. Offshoots Leaves were detached and directly used. Electrolyte leakage procedure was used to measure cell membrane thermo-stability after exposure to a heat regime. This regime followed the treatment with NaCl (1% w/v) or CaCl₂, KCl, or oleic acid. To determine the thermo-tolerance of plants, uniformed thirty-three fully expanded leaves of 30 cm length (3 leaves/plant) were collected. Leaves were washed with tap water and rinsed in deionized water to remove the dusts and electrolytes adhering to the surfaces and lightly cleaned with tissue papers. Leaf segments of five-centimeter length were cut from the middle of each leaflet and placed in each test tube. In each tube, 1 ml of deionized water was placed to prevent tissue desiccation and loosely covered with aluminum foil. All test tubes were placed in a water bath shaker (Thermostat) and tissues were exposed to a heat regime ranging from 30-75°C at 5°C increments for 30 min. Three tubes per plant stage remained at 22°C as controls. At the end of the 30 min exposure, leaflet segments were removed from the water bath, cut into about 1 mm strips to allow uniform diffusion of electrolytes, returned to the tubes along with 40 ml of deionized water, and incubated in refrigerator at 7°C overnight to allow more diffusion of electrolytes from treated damaged tissues before electrical conductivity of each solution was determined. In the next day leaf segments were taken out from refrigerator, warmed up to room temperature (22 ± 2°C) and placed in a shaker for 1 h to diffuse electrolytes, then electrolyte leakage, before killing, was measured with electrical conductivity meter. Leaf segments were then killed by autoclaving (121°C) for 10 min, after that were left on the shaker for 1 h to diffuse electrolytes, then the total electrolyte leakage reading were taken by using the same conductivity meter.

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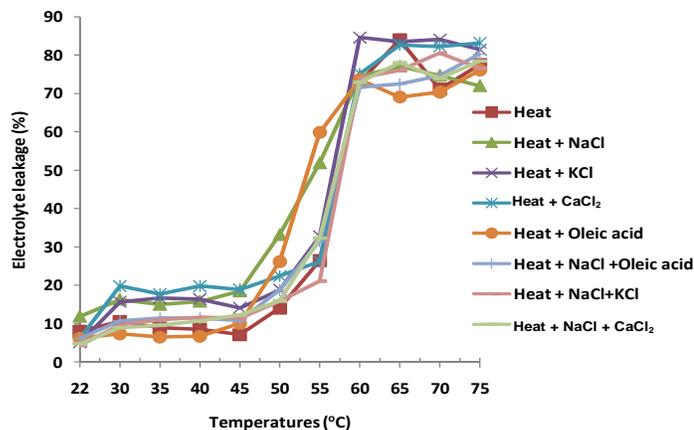


Figure 1. Lethal temperatures for VP2 plants of Ruzaiz date palm cultivar as influenced by various treatments.

Percentage of electrolyte leakage before killing to after killing was calculated according to Farag and El-Konaissi (2002) and Awad et al. (2006). The three replications of the control tissue went through the same procedure to determine percentage of electrolyte leakage. The experiment consists of 8 treatments, each treatment replicated 3 times, and each leaflet represents one replication. A completely randomized design was used.

To assess the thermo-tolerance of leaflets in the presence of high salt concentration, the same procedures as in heat test were followed, except leaflet segments of 5-centimeter length were cut into two pieces to enhance penetration of salts and immersed in 1% (10,000 ppm) of NaCl solution for hour. Segments were plotted dry with tissue papers then placed in test tubes with 1 ml of deionized water in each tube to prevent tissue desiccation. With regard to assessment of thermotolerance after certain treatments, leaf segments were taken and dipped for 1 h in either KCl, or CaCl₂ (0.2 M), or Oleic acid (0.1 M). Furthermore, 100 ppm of oleic acid was dissolved in 50 ml of ethanol. These segments were removed from the solutions and gently plotted dry with tissue paper. One segment was then placed in a test tube that already contained 1 ml of deionized water. Tubes of various treatments were exposed to the heat regime (from 30 - 75°C with 5° increments). At each temperature, three leaf segments of each treatment were removed from the water bath shaker. Measurements of electrolyte leakage and calculations of the percentage of this leakage before and after killing were determined according to Farag and El-Konaissi (2002). Three replications were used with each treatment in a completely randomized design. All data were analyzed using MSTATC program and the least significant difference (LSD) at 0.05 level was used to compare the means.

RESULTS

Generating the sigmoidal curves

As shown in Figures 1 to 3 the shape of the sigmoidal curve was consistent in all treatments. The trend of results was so clear that there was no need to fit a curve but connected the actual points. The lethal temperature was determined at 50% electrolyte leakage. Figures 1 to 3 for VP2, VP3 and offshoots plantlets, respectively represent the results of heat tolerance alone or after

treatments with sodium chloride, potassium chloride, calcium chloride, oleic acid, or in other combinations. The sigmoidal curve started with slow increase in electrolyte leakage then at a temperature that varies from one treatment to another it increases varies rapidly. After exceeding 50% electrolyte leakage, there is a leveling off with very minor changes in this leakage with increase in the temperature. Thus, the figures clearly show inflection of the line.

Identification of leaflets thermotolerance

VP2 plants

Differences in lethal temperatures after different treatments of VP2 plants are presented in Figure 1. From the inflection point at 50% electrolyte leakage, it was evident that the lethal temperature for VP2 was at 53°C. Furthermore, when these leaflet segments of plantlets were immersed in NaCl (1 %w/v) for hour before the exposure to the heat regime, the lethal temperature was 53.5°C. At this stage of plant age, tissues were not sensitive to used salt concentration However, when the tissues were treated with either potassium chloride or calcium chloride, heat tolerance of VP2 stage was increased as indicated by the lethal temperatures (57 and 57.5°C, respectively) (Figure 1), as compared with heat treatment alone. Thus, increasing tissues content of calcium or potassium could have a direct effect on increasing heat tolerance of date palm plantlets at this stage of acclimatization. Heat tolerance of oleic acid-treated tissues was slightly increased (the lethal temperature was 53.4°C). However, a marked change of lethal temperature for VP2 stage was obtained when the leaflet segments were pretreated with the salt plus oleic acid or KCL and CaCl₂ before the exposure to the heat regimes. The lethal temperature for heat plus salt and oleic acid was 57.1°C, while for heat plus salt and KCl and CaCl₂ were 58 and 57.1°C, respectively.

VP3 plants

Figure 2 shows the lethal temperature of leaflet segments of VP3 plantlets whether directly exposed to the heat regime or after treatments with NaCl, KCl, CaCl₂ or oleic acid. The heat tolerance of tissue did not significantly vary from those tissues exposed to heat in the presence of NaCl (1% w/v) (Figure 2). Each of KCl and CaCl₂ treatments increased the thermotolerance of leaflets tissues at this stage than non- treated. The lethal temperatures for heat plus KCl or plus CaCl₂ treatments were 56.5 and 57°C, respectively. A direct positive effect on the thermotolerance was also obtained when VP3 leaflets tissues were pretreated with oleic acid (100 ppm) before the exposure to the heat regime. The lethal

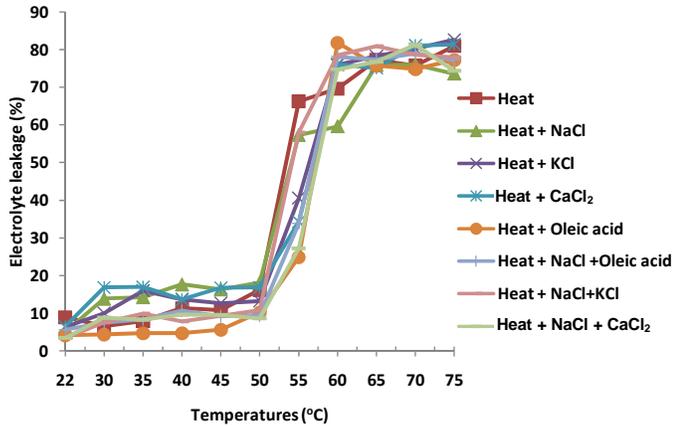


Figure 2. Lethal temperatures for VP3 plants of Ruzai date palm cultivar as influenced by various treatments.

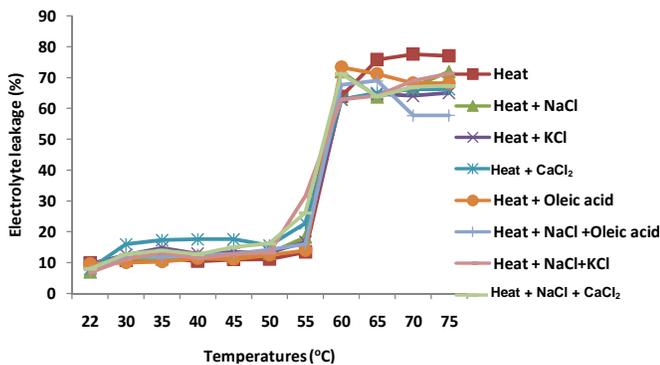


Figure 3. Lethal temperatures for offshoots plants of Ruzai date palm cultivar as influenced by various treatments.

temperature of heat plus oleic acid was 57.1°C. At this stage the response of the tissue to presence of oleic acid was greater than that obtained with VP2 stage. The lethal temperature of heat plus NaCl and oleic acid (Figure 2) was 57°C and not differ from that obtained with heat plus oleic acid since this salt concentration had no adverse effect on the thermo-tolerance of tissues. Furthermore, when the segments were pretreated with NaCl and KCl, their lethal temperature was 54°C. Calcium treated tissues even in the presence of salt maintained their improved thermo-tolerance where the lethal temperature was 57.4°C.

Offshoots

Lethal heat temperature of leaflets taken from Ruzai offshoots (58.5°C) presented in Figure 3 was much higher than that of VP2 and VP3 leaflets (Figures 1 and 2). These offshoots were still attached to mother plant under harsh conditions in the field. The offshoots went through a hardening process that was reflected on their

thermo-tolerance. In a similar way NaCl at the used concentration did not have an adverse effect on the leaflets (Pinnae) thermo-tolerance, as it was 58°C (Figure 3). There was no added advantage on the heat tolerance of offshoots leaflets when they pretreated with KCl or CaCl₂. Even oleic acid did not improve the tolerance to heat stress which indicates again that 58.5°C could be the maximum potential of these tissues to tolerate heat stress.

The interaction between the heat regime and VP2, VP3, and offshoots

The data in Table 1 indicated that leakage of electrolytes was significantly higher at VP2 stage than that obtained with VP3 stage even at relatively low temperatures such as 30, 35, 40, 45 and 50°C. However, at 55°C, both VP2 and VP3 leaflets had similar electrolyte leakage. Similar results were obtained at the high temperatures 60, 65, 70 and 75°C. These results are in agreement with that found in Figure 1 and 2 where the lethal temperatures for VP2, and VP3 stage were 53 and 53.5°C, respectively. Although these lethal temperatures were not very different from each other but the injury to VP2 leaflets started earlier than that occurred to VP3 even though it was not lethal prior to 50°C. If electrolyte leakage of VP2 leaflets was compared with that of offshoots, the data in Table 1 showed a similar trend to that found above between VP2 and VP3. Again, electrolyte leakage of VP2 leaflet segments was significantly higher than that of offshoots at the temperatures 30, 35, and 40°C and even at the sublethal temperature 50°C. Although leakage of electrolytes of VP2 at 55°C reached to the lethal value for VP2 tissues (64.1%), it was found that, at this temperature, electrolyte leakage was only 13.4% for offshoot leaflets. Even after exceeding the lethal temperature, electrolyte leakage of VP2 was still significantly higher than that obtained with offshoots at 75°C. When electrolyte leakage of the relatively more advanced stage in acclimatization (VP3) was compared with the field-hardened offshoots, we found that this leakage was not statistically different (P<0.05) at 30, 35, 40, 45 and 50°C. However, at 55°C, electrolyte leakage of VP3 leaflets was significantly higher than that of offshoots (66.2 and 13.4%, respectively). This was supported by the finding in Figure 3 and 2 where the lethal temperature for offshoot leaflets was 58.5°C and for VP3 leaflet was 53.0°C. After exceeding the lethal temperatures for both VP3 and offshoots, electrolyte leakage was very high (over 70 %) and similar for both. The data in Table 1 also indicates that as the VP2 leaflets lost 18.3% electrolytes at 35°C, while the offshoot leaflets lost only 13.4% at 55°C. Although this difference was not statistically significant (P<0.05), but the leakage was achieved at much higher temperature with the offshoot leaflets. This proves the importance of the duration factor in heat stress of date palm plantlets that could be

Table 1. The effect of interaction between the heat regime and the stages of acclimatization and hardening of Ruzaiz date palm plants on electrolyte leakage (%).

Temperature regimes (°C)	VP2*%	VP3*%	Offshoot*%	Mean
Control (22)	11.4 **ijkl	8.9 ^{kl}	10 ^{kl}	10.1
30	18.5 ^{gh}	6.5 ^l	10.7 ^{ijkl}	11.9
35	18.3 ^{gh}	7.9 ^{kl}	11.6 ^{ijkl}	12.6
40	19.4 ^g	11.3 ^{ijkl}	10.5 ^{ijkl}	13.7
45	16.6 ^{ghi}	10.6 ^{ijkl}	10.9 ^{ijkl}	12.7
50	36.3 ^f	16.1 ^{ghij}	11.3 ^{ijkl}	21.2
55	64.1 ^e	66.2 ^e	13.4 ^{hijk}	47.9
60	73.2 ^{cd}	69.5 ^{de}	63.8 ^e	68.8
65	76.4 ^{abc}	77.2 ^{abc}	76.3 ^{abc}	76.6
70	75.0 ^{bcd}	75.7 ^{bc}	77.5 ^{abc}	76.1
75	81.8 ^a	80.9 ^{ab}	74.3 ^{cd}	79.0
Mean	44.6	39.2	33.7	39.2

L.S.D. (0.05) = 5.925; C.V. % = 9.27. Values are means of three replications (each replication represents date palm plantlet or offshoot). **means within columns or rows followed by same letter do not differ significantly at 0.05 level (least significant difference test).

Table 2. The effect of interaction between treatments and the stages of acclimatization and hardening of Ruzaiz date palm plants on electrolyte leakage (%).

Treatments	VP2 *%	VP3*%	Offshoot *%	Mean
Heat	44.6 **a	39.2 ^{bcd}	33.7 ^{ghij}	39.2
Heat and NaCl	42.2 ^{ab}	38.9 ^{bcd}	32.7 ^{hij}	37.9
Heat and KCl	41.1 ^{abc}	39.0 ^{bcd}	32.2 ^j	37.4
Heat and CaCl ₂	42.2 ^{ab}	39.3 ^{bcd}	34.5 ^{fghij}	38.7
Heat and oleic acid	37.5 ^{cdefg}	33.4 ^{ghij}	32.7 ^{hij}	34.5
Heat and salt and oleic acid	36.2 ^{defghi}	36.0 ^{defghi}	30.7 ^j	34.3
Heat and salt and KCl	35.7 ^{defghi}	38.4 ^{bcd}	33.3 ^{hij}	35.8
Heat and salt and CaCl ₂	36.4 ^{defgh}	34.8 ^{efghij}	34.0 ^{ghij}	35.1
Mean	39.5	37.4	33.0	36.6

L.S.D. (0.05) = 4.14; C.V. % = 7.05; values are means of three replications (each replication represents date palm plantlet or offshoot); means within columns or rows followed by same letter do not differ significantly at 0.05 level (least significant difference test).

addressed in the further studies.

The interaction between treatments and VP2, VP3, and offshoots

Differences in electrolyte leakage of the two acclimatization stages VP2, VP3 and the field-hardened offshoots in relative to heat treatments are shown in Table 2. The interaction between various treatments and the two acclimatization stages (VP2 and VP3) did not show a significant difference in electrolyte leakage between treatments except with the heat treatment alone for VP2 and VP3. The data also indicated that VP2 leaflet lost significantly more electrolytes than VP3 leaflet (Table 2). When the leakage of VP2 leaflets was compared with that obtained with offshoot leaflets, it was

found that this leakage was higher in VP2 leaflets than that of the offshoot leaflets whether with heat alone or when pretreated with salt, potassium, calcium, oleic acid, or salt plus oleic acid. Similarly, VP3 leaflets had higher electrolyte leakage than offshoot leaflets with all treatments except with heat plus oleic acid treatment and heat plus salt and calcium (Table 2).

DISCUSSION

The sigmoidal pattern of electrolyte leakage obtained in this study, for heat stress alone or for pretreated tissues with NaCl, KCl, CaCl₂, or oleic acid then exposed to heat stress agrees with Farag and El-Konaissi (2002) on Barhi and Khalas date palm cultivar and other studies (Nilsen

and Orcutt, 1996; Agarie et al., 1995; Cha-Um et al., 2010; Anbu and Sivasankaramoorthy, 2014). Similar pattern was also obtained when the leaflet segments were exposed to NaCl plus each of oleic acid, or KCl, or CaCl₂, and then experienced heat stress. Similarly, Anbu and Sivasankaramoorthy (2014) found that supplementary of calcium chloride (10 and 15 mM CaCl₂) ameliorated electrolyte leakage of rice leaves as a result of salt stress.

Electrolyte leakage is an indicator to the injury occurred to plasma membrane after exposure to stresses. It has been recognized as a valid, reproducible, simple, and quantitative test for assessing cell viability after heat, salt water, or even cold stresses (Adil et al., 2014; KalimUllah et al., 2014; Cha-Um et al., 2010).

Under heat stress, proteins of the plasma membrane denature or aggregate according to the severity of stress and/or membrane lipids becomes hyperfluid. These changes result in increased leakage of electrolytes from the membrane (Levitt, 1980). The current study provided experimental evidences for the differences in heat tolerance between *in vitro* date palm plants and the offshoots grown along with the mother plant under natural conditions. It has been known for long time that date palm plants are tolerant to heat stress (Hammouda et al., 1998). However, no accurate test has been reported to show the exact tolerance level especially for those plantlets reproduced by tissue culture. As shown in results, the thermotolerance was 53.0, 53.5 and 58.5°C for VP2, VP3, and offshoots leaflets, respectively. This information has very important implications since many tissue culture plants could be die if air temperature during the day reached 50°C. Furthermore, we must take in consideration the heat absorption factor where in hot climate heat absorption is higher than heat dissipation. As a result of this, tissue temperature is usually higher than air temperature by at least 10 to 12°C (Levit, 1988). These results should guide date palm growers to the suitable time of the year before transplanting date palm plantlets especially those produced by tissue culture.

Differences between *in vitro* plants and offshoots of date palm are expected since tissue culture plants are produced under delicate microenvironment, while offshoots went through sufficient hardening conditions in the field. The other factor that must be considered that VP2 and VP3 plants can not reflect as much heat as that reflected by offshoots because the tissue culture plants do not have a thick cuticle or similar epicuticular waxes as that found on offshoot leaflets. Thus, the avoidance mechanism of tissue culture plants after these two stages of plants age is less efficient than the hardened offshoots.

With regard to heat stress tolerance in the presence of high salt concentration, the used NaCl in this study did not have an adverse effect on the thermotolerance of VP2, VP3 or offshoot leaflets. This could be due to the exposure to heat stress after salt stress, so there might not be a direct effect of the pretreatment with salt. In

other words, NaCl treatment might need more time to exhibit an injury by heat after that. The other possibility is that following the pretreatment with NaCl, the tissue might need more duration of heat stress. The differences in result were attributed to the amount taken by the tissues. If the tissues permitted more penetration or diffusion of salts, the thermotolerance will be lowered. Like this result was noticed by Husein et al. (1993) who found that growth of date palm seedlings was unaffected by low salinities. Borochoy et al. (1991) finding revealed that an excess of NaCl caused an increase in electrolyte leakage. Similarly, Youssef and Wad (2008) considered that the high electrolyte leakage from of date palm seedlings exposed to high salinity a strong indication of the degree of impairment to membrane integrity. The increase in thermotolerance of calcium- treated tissues, in this study, of VP2 and VP3 leaflets is supported by the findings many other researchers (Gary, 1970; Poovaiah and Leopold, 1987; Leopold et al., 1984; Richard and Gary, 1984). Calcium was reported to play a very vital role in maintaining the plasma membrane integrity. Thus, calcium could protect leaf membrane against leakiness induced by heat stress. The polar head groups of the membrane phospholipids are bound together by calcium that limits membrane permeability and reduce electrolyte leakage (Gary, 1970).

Similar role for potassium was reported. Ben-hayyim et al. (1987) found that potassium application could reduce the deleterious effect of salinity on plant development. Potassium can also bind to the plasma membrane and maintain its integrity that results in reducing electrolyte leakage. Results shown in Figures 2 and 3 agree with this explanation. There was no added advantage on the thermotolerance of offshoots if the leaflets were pretreated with potassium, calcium, or oleic acid when compared with heat treatment alone. Since the thermo-tolerance of offshoot leaflets was high (58.5°C), this might be the maximum tolerance for such hardened tissues in the field. Hassan and El Samnoudi (1998) found amount of potassium uptakes by date palm seedlings increased with increasing salinity within a moderate range.

Regarding the role of unsaturated fatty acid in increasing the thermotolerance of plants, it was reported by Harwood et al. (1994) that these acids protect the membrane from hyperfluidity that means keeping its integrity. Similarly, Nilsen and Orcutt (1996) reported that membrane thermo-stability at high temperature could be modified by changes in fatty acid unsaturation, the position of fatty acids on the glycerol backbone, the composition of fatty acids, and the abundance and compositional of sterols. Thus, the change in membrane fluidity affects the properties of embedded proteins (enzymes) that make the membrane leakier to electrolytes. In this study, the pretreatment with oleic acid caused an increase in the thermo-tolerance of VP2 and VP3 plants (Figures 1 and 2). These results agree

with what was reported on the positive effects of unsaturated fatty acids on the plasma membrane (Harwood et al., 1994; Nilson and Orcutt, 1996). Effect of the duration factor has been reported in stress studies (Levitt, 1980; Nilson and Orcutt, 1996). Results of this study also propose that. Although the thermo-tolerance between VP2 and VP3 leaflets did not vary much, but electrolyte leakage of VP2 leaflets was significantly higher than that of VP3 leaflets at sublethal temperatures (30-50°C). These results suggested the importance of studying the thermotolerance of this stage at various durations. In general, there is a great potential to increase the thermotolerance of the tissue culture regenerated plants and offshoots by pretreatment them with safe chemicals such as potassium chloride, calcium chloride, or oleic acid.

CONCLUSIONS AND RECOMMENDATIONS

This study showed for the first time a quantitative determination of the thermo-tolerance for tissue culture regenerated plants (VP2 and VP3) and for hardened offshoot of the same Ruzaiz cultivar. The results of heat stress regime alone or pretreatment of leaflets with NaCl, KCl, CaCl₂, or oleic acid then the heat regime resulted in consistently generating a sigmoidal curve as shown in other studies. With regard to multiple stresses heat tolerance of leaflets tissues after the exposure to salt (NaCl 1% w/v) did not vary from heat tolerance without the salt. Low concentration of salt could have a protective role against heat stress while high salt concentration lowers the thermo-tolerance. In our study these results presented also the response of VP2 and VP3 to pretreatment with KCl, CaCl₂, or oleic acid, in increase the thermotolerance. The highest value of thermo-tolerance was obtained with offshoot leaflets. It could be concluded that 58.5°C might be the maximum heat tolerance due to the sufficient hardening conditions in the field and available of thick cuticle layer. From the above results, this investigation provided for the first time an accurate determination for heat tolerance of the tissue culture regenerated plants cv. Ruzaiz that are distributed to date palm growers. There is a potential to increase the thermo-tolerance of VP2, VP3, or offshoots that could increase their survival under field conditions by spraying plants leaves with, KCl, CaCl₂ (0.2 M) or oleic acid (0.1 M). The results suggested the importance of studying the thermotolerance of VP2 plants at various durations. Also, there is a need to verify a protective role of low concentration of NaCl against heat stress.

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