Studies on Salt and Heat Stresses Tolerance of Date Palm Plants Regenerated by Tissue Culture

Khair Tuwair Said Al-busaidi

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STUDIES ON SALT AND HEAT STRESSES TOLERANCE OF DATE PALM PLANTS REGENERATED BY TISSUE CULTURE

By

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A thesis submitted to the Deanship of Graduate Studies United Arab Emirates University in partial fulfillment of the requirements for the Degree of Master of Science in Environmental Sciences

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ABSTRACT

This research was conducted during the period of 2001/2002 to determine the thermotolerance of date palm leaflets reproduced by tissue culture and compared that with the tolerance of offshoot for the same cultivar Rzaiz. Heat tolerance of these leaflets in the presence of high salt concentration was also determined. Tissue culture plantlets used in this investigation were at two stages of acclimatization, namely VP2 and VP3 (vitro plants under acclimation program for about 4 to 6 months and 10 months, respectively). Rzaiz offshoots attached to the mother plants were about 4 years old. The electrolyte leakage procedure was used to generate sigmodial curves, and from each curve the thermotolerance was determined at 50 % electrolyte leakage. Leaflets of the three stages of growth and development (VP2, VP3 and offshoot) were treated in the laboratory with each of heat, heat plus NaCl, heat plus KCl, Heat plus CaCl₂, heat plus oleic acid, heat plus salt and oleic acid, heat plus salt and KCl and heat plus salt and CaCl₂. Concentrations were: NaCl at 1 % w/v (mentioned as salt), KCl or CaCl₂ (0.2 M) or oleic acid at 100
ppm. Treatments by various chemicals was done by dipping leaflet segments for 1 hr before going through the heat regime (series of temperatures ranged between 30 to 75 °C with 5 °C incrementes).

A completely randomized design was used with three replications. Thermotolerance values were 53 °C, 53.5 °C, and 58.5 °C for VP2, VP3, and offshoot leaflets, respectively.

Pretreatment with potassium chloride, calcium chloride, or oleic acid markedly increased the thermotolerance of leaflets. The used NaCl concentration was not effective in lowering the leaflet thermotolerance. However, heat plus salt treatment resulted in significantly higher electrolyte leakage than heat plus salt in the presence of oleic acid, KCl, or CaCl₂ at sublethal temperatures. Moreover, VP2 leaflets had higher electrolytes leakage than VP3 leaflets at sublethal temperatures, even though their lethal temperature did not greatly vary.

Investigations in this thesis provided for the first time an accurate determination for heat tolerance of the two
acclimatization stages that are distributed to date palm growers. Results also revealed that there is a potential to increase the thermotolerance of VP2, VP3, or offshoots that could increase their survival under field conditions.
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CHAPTER I

INTRODUCTION
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INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious long-lived monocotyledon crop. It is a main part of the heritage in the gulf region. Along the history, this crop has been providing people with food, fuel, shelter and fiber. Traditionally, seeds are not appropriate for commercial propagation because of dioeciously nature and high genetic variation propagate date palm. Vegetatively, offshoots reproduce it. However, each tree could produce a limited number of offshoots. This has been a major problem for the multiplication of healthy plants (Tisserat *et al.* 1982).

Plant tissue culture technique has been playing a vital role in quick clonal multiplication of superior cultivars and elimination of diseases transmission. Thus, tissue culture of date palm can meet the increasing demand of growers. However, date palm producers lack accurate information about the tolerance of these *vitro* plants to heat and salt
stresses. These stresses are usually associated with slow growth rate and frequent death of plants (Hammouda et al., 1998).

Many people believe that the agronomic importance of date palm is linked to its high tolerance to environmental stresses, such as salinity, drought and high temperature. However, to the best of my knowledge there is no study that identified the exact tolerance level or the lethal temperature to date palm offshoots or in vitro plants. Tolerance of plants to high temperature varies with genotype (Ingram and Buchanan, 1984; Lester, 1985; Wallner et al., 1982). The ability of crop plants to adapt to heat stress is potentially an important component of tolerance to heat stress under field conditions (Chen et al., 1982). As it well known heat stress is a major factor limiting the productivity and adaptation of many crops, especially when temperature extremes coincide with critical stages of plant development. However, it is not accurate to generalize what is known about the offshoots tolerance and try to compare it to plants produced in a very delicate microenvironment through tissue culture.
Electrolyte leakage has been utilized as an indicator to the thermostability of the plasma membrane (Ahrens et al., 1988). Exposing plant tissue to a temperature regime and measuring electrolyte leakage generate a sigmoidal curve. The inflection point of sigmoid curve at 50% leakage detects the lethal temperature for many tissues. The direct-heat injury could be also detected by measuring electrolyte leakage (Ingram and Buchanan, 1981; Sullivan, 1972). Since membrane function is one of the physiological processes that are greatly disturbed by high temperature stress and cell membrane was the target of various stresses (Levitt, 1980 and Blum, 1988), consequently, changing the integrity of the plasma membrane due to the heat stress results in losing its semipermeability. Thus, the membrane becomes leaky and more electrolytes could easily leak out.

To the best of our knowledge, heat and salt tolerance of date palm plants reproduced by tissue culture has not been defined yet. Acclimatization is usually used to increase the tolerance of such plants to environmental conditions.
Researchers have been concerned about increasing the survival rate of transplanted plants. Zaid and Hughes (1986) used polyethylene glycol and silicon rubber in order to reduce water loss and consequently, increase success after transplantation.

By identifying the tolerance level of each acclimatization stage, we can reach to a beneficial recommendation to date palm plantlets growers in term of the suitable temperature range in the field during transplantation.

In nature, the plant is exposed to multiple stresses which interact to limit growth and productivity (Turner and Kramer, 1980). It has been recognized that the balance among multiple resource limitations affect competition and succession in natural ecosystem (Tillman, 1988). In the Gulf region, transplanted date palm could be exposed to heat stress in the presence of salt stress. Therefore, this study will take in consideration the effect of multiple stresses, namely heat and salt stresses (NaCl), on the tolerance of date palm plants reproduced by tissue culture as well as the offshoots produced
under field conditions. Aforementioned, the objectives of this thesis were:

1- To accurately identify the heat tolerance of date palm plants reproduced by tissue culture and subjected to two stages of acclimatization.

2- To assess the interactive effect between heat and salt stresses on tolerance of these plants.

3- To evaluate the possibility of increasing tolerance to heat stress alone or in the presence of salt stress by calcium, potassium, or oleic acid.

4- To alleviate the damage of high salt concentration when these plants were exposed to heat stress.
CHAPTER II

REVIEW OF LITERATURE
CHAPTER II

REVIEW OF LITERATURE

1. Determination of Heat Tolerance

Changes in the native structure of the plasma membrane components (lipids and proteins) due to heat stress were reported by many researches. These changes result in disruption and loss of its semi permeability. Thus the determination of cell electrolyte leakage has been employed to assess the tolerance level to heat stress. Consequently, the validity of this method has been approved by stress physiologists and was relevant to the tolerance level in the field or in situ (Marcum, 1998). Since various stresses target the plasma membrane of any plant, this review of literature was organized in such a way that shows use of the electrolyte procedure with woody and herbaceous plants to assess their tolerance to heat or multiple stresses.
1.1 Woody Plants

Electrolyte leakage procedure has been used with many plant parts to determine thermotolerance of their tissues. Ingram et al. (1981) measured direct heat injury to roots of three woody plants. About 50% electrolytes leakage was obtained from a 20-minute exposure of roots to 50.5 ± 0.5 °C, 48.5 ± 0.5 °C and 46.5 ± 0.5 °C for *Illicium anisatum* L., *Juniperus chinensis* cv. *Parsonii* and *Ilex cornuta* L. cv. *Burfordii*, respectively. At this leakage rate, the lethal temperatures of these tissues were determined.

Didden et al. (1982) determined the tolerance of *Opuntia bigelovii* Engelm. (Cactaceae) to high temperature by subjecting stems to temperatures ranging from 25°C to 65°C for a 1-hour period. The temperatures at which activities depending on membrane integrity decreased by 50% were 60 °C for electrolyte leakage, 52°C for staining by neutral red, and 51°C for plasmolysis for plants maintained at day/night air temperatures of 30°C /20°C. Nocturnal acid accumulation depends on stomatal opening and enzymatic reactions as well.
as membrane properties, was half-inactivated at a lower temperature (46°C). Visual observation indicated that 50% of the stems subjected to a heat treatment of 52°C became necrotic in 2 weeks. The heat tolerance of the cellular properties increased with increasing air temperature for a 10°C temperature increase, the half-activation temperature increased 2.9°C for electrolyte leakage, 3.0°C for staining, 3.8°C for stem survival, and fully 6.1°C for nocturnal acid accumulation. The relative order of these four properties with respect to heat tolerance did not change during the hardening. Nocturnal acid accumulation remaining the most heat sensitive. The upper temperature for 50% survival was 59°C for Opuntia bigelovii when acclimated to day/night temperatures of 50°C/40°C.

Wu and Wallner (1983) studied the response of suspension-cultured pear (cv. Bartlett (Williams' Bon Chretien) cells to heat stress by using 3 viability tests: regrowth (culture growth during 10 days after stress), triphenyltetrazolium chloride reduction; and electrolyte
leakage. Critical (50% injury) temperatures for a 20-minute exposure for three viability tests were 42°C, 52°C and 56°C, respectively. Heat stress inhibition of triphenyltetrazolium chloride reducing capacity was much greater if the viability test was conducted 3 days later, rather than immediately, after the stress treatment. Treatment with 3.6 mM cycloheximide and heat stress (20 minutes at 43 °C) affected culture regrowth similarly. It is concluded that measurements of direct response are not adequate substitutes for regrowth tests in assessing heat injury to cultured plant cells.

Moreover, Ingram et al. (1984) used electrolyte leakage technique to determine the temperatures causing direct heat injury to 3 citrus rootstocks; Sour orange, Carrizo citrange, and Swingle citrumelo which were exposed to various high temperatures for 20 minutes. Lethal temperatures for roots were 51.6 ± 0.5 °C, 52.5 ± 0.7 °C, and 53.5 ± 0.5°C for Carrizo citrange, Sour orange, and Swingle citrumelo, respectively.

Furthermore, Ingram et al. (1985) employed electrolyte leakage procedure to describe the interaction of treatment
temperature and exposure duration on *pittosporum tobia* root cell membrane thermostability. Roots of *pittosporum tobira thunb* were exposed to high temperature of 25 °C to 60 °C for 30 to 300 min. The results revealed that the critical temperature causing injury to root cell membranes decreased linearity as exposure duration increased exponentially.

Ingram (1986) also used electrolyte leakage technique to determine root cell membrane heat tolerance of two-dwarf hollies. They found that critical temperature decreased linearly as exposure duration increased exponentially, and predicted critical temperature for Helleri and Schellings to be 51 ± 0.8 °C and 52.6 ± 0.7 °C, respectively, for a 30-min exposure and 43.9 ± 0.8 °C and 46.7 ± 0.3 °C for a 300-min exposure which explains the significance of the duration factor in stress.

In another study for Ingram, *et al.* (1987) aimed at detecting the critical high root zone temperatures for Carrizo citrange (*Citrus sinensis X Poncirus trifoliata*) seedlings that were grown for 9 weeks at root-zone temperatures of 28 °C, 34
°C or 40 °C for 6 h daily. The shoot: root ratio was significantly increased at 40 °C.

Ahrens et al. (1988) also found when they employed electrolyte leakage procedure to determine the differences in tolerance to direct heat injury to leaves of some citrus cultivars that the lethal temperature for a 20 min. exposure was 54.3 ± 0.5 °C for ‘Glen’ citrange and 56.1 ± 0.4 °C for ‘Swingle’ citrumelo. This result indicates to the difference in heat tolerance of roots and leaves where Ingram et al. (1984) found that roots of “Swingle” citromelo died at 53 °C.

Xiao and Zhao (1990) conducted studies on physiological and biochemical indices of heat tolerance of citrus leaves by using electrolyte leakage procedure. Leaves of 5 citrus species were treated with warm water at temperature of 36 °C and 66 °C for 20 min. exposure time. Their results indicated that sour orange followed by lemon had the highest degree of heat tolerance.

Similar relation between heat stress and loss of cell compartmentalization was found in the tissues of grape hybrids
and the plasma membrane was irreversibly damaged at the killing temperature (Abass, 1991).

Martin (1991) exposed rooted cuttings of southern magnolia (*Magnolia grandiflora* cv. 'St. Mary') for 6 h daily to root-zone temperatures (RZT) of 28 °C, 35 °C or 42 °C for 8 weeks during the spring or fall of 1988. Length of survival for 8-month old rooted cuttings was shortened when roots were exposed during the spring to 42 °C or 35 °C compared to 28 °C. Electrolyte leakage from excised root tissue exposed for 30 min to temperatures ranging from 25 °C to 70 °C, was used to assess cellular injury of 13-month old rooted cuttings after RZT treatments. The critical killing temperatures (CT50) of root tissue pretreated at 35 °C or 42 °C RZT were 52.5 ± 0.9 °C and 54.0 ± 0.4 °C, respectively, and indicated differences in root membrane thermostability.

Ruter (1993) determined temperatures producing heat damage in leaves of *Ilex X meserveae* cv. 'Blue Prince' and *Ilex rugosa X cornuta Lindl.* and Paxt. 'Mesdob' (China Boy) using electrolyte leakage procedure. Whole leaves were exposed to
temperatures from 30 °C to 65 °C for 30 minutes. The heat killing temperature for 'Blue Prince' and 'Mesdob' was 52.4 ± 0.1 °C, and 53.8 ± 0.1 °C, respectively.

Similarly, Ruter (1993) also determined Membrane thermostability of 'Needlepoint' Chinese holly (Ilex cornuta Lindl. & Paxt.), 'Albo-marginata' English holly (Ilex aquifolium L.), and 'Nellie R. Stevens', an Ilex aquifolium X Ilex cornuta hybrid, by measuring electrolyte leakage in excised leaves and roots. His results showed that the critical midpoint heat-killing temperature (Tm) after a 30-min exposure was 54.4 ± 0.4 °C for 'Nellie R. Stevens' leaves was approximately 1 °C higher than that for Chinese (52.9 ± 0.3 °C) or English holly (52.9 ± 0.4 °C). The Tm for English holly roots (53.9 ± 1.5 °C) was higher than that for either 'Nellie R. Stevens' (51.7 ± 0.3 °C) or Chinese holly (50.1 ± 0.3 °C). Finally, he concluded the English holly and 'Nellie R. Stevens' leaves and roots can tolerate direct heat injury equal to or greater than that of Chinese holly.
Binder et al. (1995) found when they conducted a study on the effects of holding white spruce (Picea glauca (Moench.) Voss) seedlings in storage boxes at air temperatures of 5°, 10°, 20 °C, 30 °C and 40 °C for 12, 24, 48, 72 and 96 h before planting. Needle electrolyte leakage was indicative of visible needle damage 14 days after planting, whereas stem electrolyte leakage and root growth potential were more closely related to end of season plantation mortality.

Lurie et al. (1995) studied the effects of heat treatment on plasma membrane of apple fruits (cv. Golden Delicious), which hold at 20 °C or 38 °C for 4 days. The fruits hold at 38 °C were subsequently stored for 5 months at 0 °C. Membrane microviscosity and sterol content increased, phospholipid fatty acids became more saturated and there was increased electrolyte leakage from fruit discs during heat treatment at 38 °C, compared with treatment at 20 °C. In fruits held at 38 °C, ATPase activity increased during the first day of heating and then decreased. After 4 days ATPase activity was similar in fruits held at 38 °C and fruits held at 0 °C. ATPase activity
increased rapidly throughout the 4 days in fruits held at 20 °C. After 5 months of storage at 0 °C, microviscosity and leakage were lower, and increased more slowly during subsequent ripening at 20 °C, in fruits held at 38 °C than in control fruits. There was no significant difference in ATPase activity in fruits stored for 5 months at 38 °C in relative to the control fruits. Phospholipid content was higher in membranes from fruits held at 38 °C than in membranes from control fruits. The loss of unsaturated fatty acids during ripening occurred more rapidly in control fruits. Heated fruits recovered rapidly from heat stress and acclimatized more successfully to 0 °C than did control fruits.

To assess the role of heat shock proteins in increasing the thermotolerance of tissues, Morrell et al. (1995) studied thermotolerance of dormant and actively growing grape cultivar “Cabernet Sauvignon”. Plants were grown at 25 °C, for a 4-h heat shock at 40 °C, followed by a 4-h incubation period at 25 °C compared with untreated plants. Heat-shocked plants had less leaf damage, tissue water loss and electrolyte
leakage after exposure to 50 °C. Cuttings, removed from cold storage at 3 °C, were heat shocked for 30 min at either 45 °C or room temperature (23 °C), and then incubated for 4 h at room temperature. Untreated (control) cuttings were held continuously at 3 °C. Thermotolerance was tested by placing the cuttings in water baths at 54 °C, 56 °C, 58 °C or 60 °C for 30 min. Bud survival was high in all treatments at 54 and 56 °C. There was 100% bud death in all cuttings incubated at 60 °C. At 58 °C, cuttings that were heat shocked at room temperature were significantly more thermotolerant than control cuttings, and those that were heat shocked at 45 °C were significantly more tolerant than that heat shocked at room temperature. Both dormant and actively growing grape tissues responded to heat shock treatment by increasing their thermotolerance.

Ruter (1996) studied Membrane thermostability of Heritage river birch (*Betula nigra* L. Heritage) by electrolyte leakage from excised roots of plants grown in pot-in-pot (P1P) and conventional aboveground production systems (CPS). The predicted critical midpoint temperature (T_m) for a 30-min
exposure was 54.6 ± 0.2 °C for PIP and 56.2 ± 0.6 °C for CPS plants. Plants grown in PIP had a steeper slope through the predicted Tm, suggesting a decreased tolerance to high root-zone temperatures in relation to plants grown aboveground.

In a tropical fruit such as 'Hass' Avocados (*Persea americana* Mill.), Woolf (1997) treated fruits in water at 38 °C for 0 to 120 minutes, and then stored at 0.5 °C for up to 28 days. Electrolyte leakage of heated skin tissue increased approximately by 70% during storage, while for non-heated skin tissue increased approximately by 48% over the same period. In addition, a significant protection was conferred by hot water treatments against low temperature damage to avocados and these effects are reflected in the morphology and physiology of the skin tissue.

Electrolyte leakage procedure was also relied on to show the differences in thermostability of plants grown in different zones. Hardin *et al.* (1999) determined root membrane thermostability of flowering dogwood (*Citrus. florida*) seeds that collected from native trees in the USDA Hardiness Zones
6b (Rock Island, Tennessee), 7a (Lawrence County, Alabama) and 8a (Auburn, Alabama). Fine root tissues were subjected to temperatures ranging from 20 to 60 °C for 30 min and analyzed for cellular electrolyte leakage. Critical midpoint temperature (Tm) was greater for seedlings native to USDA Hardiness Zone 6b (AHS Heat-Zone 7), 52.4 ± 0.6 °C, than Tm for seedlings originating from USDA Zone 7a (AHS Zone 7), 51.2 ± 0.5 °C. However, seedlings from USDA Zone 8a (AHS Zone 8), at 51.5±0.4 °C, were similar to those collected in USDA Zones 6b (AHS Zone 7) and 7a (AHS Zone 7).

Peters et al. (1999) studied the extreme temperature on the quantum yield of fluorescence and membrane leakage of *Pinus canariensis* growing in 5 stands at different altitudes (550-1950 m) and orientation (north or south facing) in Tenerife (Canary Islands, Spain). Needles were collected and exposed for 30 min in plastic bags in a water bath at temperature between 24 °C and 56 °C. Different resistance to high temperature depending on the altitude and orientation was found. The temperature for incipient damage varied from
42 °C to 44 °C, detected with the fluorescence parameters but not with the leakage of electrolytes that was not found until 50°C.

Zhonghai et al. (1999) exposed tissues to temperatures regimes, 46 °C, 48 °C, 50 °C, 52 °C, 54 °C, 56 °C, 58 °C, or 60 °C and compared them with leaves treated at 35 °C (control) for 20 minutes by using electrolyte leakage procedure. They found that the plasma membrane was damaged and the rate of leakage increased by increasing temperature.

1.2 Herbaceous Plants:

Assessment of heat tolerance of different varieties was also used by electrolyte leakage procedure and other methods. Martineau et al. (1979) observed genetic differences in cell membrane thermostability with soybean (Glycine max. Merr.) by using electrolyte leakage procedure. Similarly, Sullivan and Ross (1979) used electrolyte leakage procedure to identify genetic variation in heat tolerance in grain sorghum (Sorghum bicolor L.). Garraway and Findley (1981) studied the effect of heat stress on electrolyte leakage from four corn inbred lines
leaves, which vary in susceptibility to northern corn leaf blight (*Setosphaeria turcica*). Leaves from different inbred lines, which varying in resistance to were immersed in distilled water and then subjected to alternating 12 h cycles of 40°C in the light and 30°C in the dark. Electrolyte leakage (EL) was moderate during the first 72 h, but increased significantly thereafter, and was least for the most resistant line, (Oh514), and greatest for the susceptible line (W64AN). Presence of the Htl gene caused a further decrease in electrolyte leakage.

Bennett and Mohammed (1982) studied the heat tolerance of ten peanut genotypes exposed to irrigated and rain-fed conditions in the field on four dates using an electrolyte leakage technique. Pearl Early Runner, X537B, Florunner and X487A were the most heat tolerant. Five of the ten genotypes were also studied during and after a period of naturally occurring water stress. Water stress resulted in increased heat tolerance among four of the five genotypes.

The tetrazolium chloride test has been used to assess the viability of plant tissue. This is why Chen *et al.* (1982)
compared the results of this test with the electrolyte leakage procedure. They measured heat tolerance of two genotypes for each of bean, potato, soybean and tomato known differences in heat sensitivity. Both methods gave similar results. The differences became dramatic after the plants were acclimated at temperature above 30 °C for 24 hours. The heat tolerance of leaves was lower at the earlier stages and reached a level similar to fully-grown plants after 30 days growth.

To evaluate the effect of heat acclimation on the thermotolerance, the electrolyte leakage procedure was also utilized. Shen and Li (1982) studied leaf heat tolerances of Saladette (heat tolerant) and "UC- 82 B" (Less heat tolerant) tomato (*Lycopersicon esculentum* L.) after heat acclimation. The results revealed that there was no difference in heat tolerance for both genotypes at temperature regime below 30 °C, but when they exposed to temperature of 35 °C, UC- 82 required 6 cycles of high temperature exposure till reached to level of heat tolerance, similar to Saladette, whereas Saaladette needed only a single cycle.
Schaff et al. (1982) conducted comparison trial of 2,3,5-triphenyltetrazolium chloride (TTC) and conductivity tests for evaluation of heat tolerance three greenhouse-grown plants each of one intolerant cultivar (Oregon 1604) and three tolerant cultivars (Provider, PI165616 and PI271998) were transferred to a growth chamber providing 16 h of light and day/night temperatures of 25/20°C with an acclimatization treatment of 2 h at 45 °C for four days. Leaf material of each cultivar was subjected to heat treatments of 44, 46, 48, 50 and 52°C for 1 h prior to assay with 2,3,5-triphenyltetrazolium chloride (TTC) and conductivity tests. Ranking by the temperature at which 50% TTC reduction occurred was (most to least tolerant): PI271998, PI165616, Oregon 1604 and Provider. The temperature at which 50% electrolyte leakage for conductivity occurred ranked the cultivars in the order: PI165616, PI271998, Provider and Oregon 1604.

Peck and Wallner (1982) conducted an experiment on heat resistance of aspen (Populus tremuloides) leaves taken from trees growing at altitudes of, 2195, and 2454 m. was
assessed by stressing leaf discs *in vitro* and measuring electrolyte leakage. Heat tolerance was greatest in leaf samples from trees growing at the lowest site. Trees propagated from these sites and grown at 1520 m for 2 yr showed some increase in heat tolerance, but apparent ecotypic differences persisted.

Wallner *et al.* (1982) applied electrolyte leakage to know the level of injury due to heat stress of turfgrass leaf segments. Species differences in heat tolerance were most apparent when injury was monitored over time at 50°C, using leaf segments that were obtained from heat-hardened plants and immersed in distilled water during the stress treatment. Quantitative differences in heat tolerance in *vitro* were consistent with qualitative desiccations of drought resistance for most of the species tested. Same procedure was followed by Wallner *et al.* (1982) who noted genotypic variation in membrane thermostability with various turfgrass species.

Lester (1986) studied melon leaf membrane thermo conditioning for 5 days at 15°C or 35°C in various day-temperature and heat-stressed for 15 minutes at 50°C. He
found that Leaves from seedlings held for 5 days at 15°C had a higher percentage of injury following heat stress than those held for 5 days at 35°C. Acclimatization for 2 to 4 h at 35°C was enough to reduce the percentage of injury on leaves from 15°C grown plants. Deacclimatization for 8 h at 15°C increased the percentage of injury to leaves from plants grown at 35°C.

Lester et al. (1988) conducted an experiment on muskmelon fruits of the Magnum cv. Harvested fruits were wrapped (or not) in shrink-film, and exposed to 45°C for 0, 1.5 or 3 h and then stored for up to 18 days. The results revealed that the wrapped fruits did not lose more than 0.8% of fruit weight after 18 days, whereas in non-wrapped fruits the weight loss ranged from 4.4 to 5.1% after 18 days. Electrolyte leakage of wrapped fruits increased after 1.5 h at 45°C but there was no difference in leakage rate between 1.5 and 3 h. In non-wrapped fruits, electrolyte leakage increased after all heat treatments.

Bhattacharjee et al. (1998) studied the effects of high temperature treatment on Amaranthus lividus seeds during the
early imbibitional phase. Transfer of seeds during the early imbibitional period from 25 °C to 45 °C for 4, 8, 12 and 16 hours resulted in the leakage of UV-absorbing substances and electrolytes up to 72 hours. High temperature treatment decreased ethylene formation in germinating seeds. It is suggested that high temperature caused greater membrane damage by membrane lipid peroxidation in germinating seeds.

Electrolyte leakage was also used to study high temperature injury on fruits. Inaba and Crandall (1988) also studied high temperature injury to harvested mature green tomatoes by using electrolyte leakage. They found that When fruit temperatures were > 50 °C, heat injury was obvious. Also when, mature green tomatoes were exposed to temperatures ranging from 25 °C to 65 °C for 30 to 180 minutes; the critical exposure times calculated for tomatoes were 34, 105 and 166 minutes for exposure to 55 °C, 50 °C and 45 °C, respectively. For practical field applications, this high-temperature stress may decrease the quality by causing the destruction of fruit cellular tissue and physiological disorders during storage.
Ingram and Ramcharan (1988) studied the effect of heat stress on banana plants. Excised roots of banana plants produced in vitro and of rooted cuttings of *Dracaena marginata* were subjected to temperatures of 30 °C to 60 °C for 30-300 min. The critical temperature for each plant decreased exponentially as electrolyte leakage increased. Predicted critical exposure times at 48 °C and 52 °C were >300 and 221 ± 51 min, respectively, for Dracaena, and 225 ± 36 min and 105 ± 14 min for banana, respectively.

White *et al.* (1988) measured injury to *Lolium perenne*, cultivars Prelude and Manhattan II, as a percentage of electrolyte leakage from leaf segments after stress to determine the influence of pre-stress growth temperature and post-stress temperature on heat tolerance. The temperature required to cause 50% cell solute efflux was 59.5°C for Prelude and 56.5°C for Manhattan II, when measured immediately after stress treatment. However, electrolyte leakage increased with time after termination of stress. When measurements were recorded 24 h after termination of stress, treatment at 52 °C caused 50%
cell solute efflux from leaf segments of both cultivars. Injury levels 44 h after treatment for 30 minutes at 50 °C were approximately 12 and 89% when incubated at post-stress temperatures of 7 and 35°C, respectively. The incubation temperature following a 55°C treatment did not affect electrolyte leakage rate in either cultivar. Severe injury occurred in both cultivars when grown at 25 °C than at 41 °C.

Garraway et al. (1989) exposed maize (Zea mays L.) leaves to high temperature stress, i.e., 42°C for 6 hr before inoculation with Bipolaris maydis race T followed by incubation in the dark at 28°C for 24 hr. They found a significant decrease in peroxidase activity in both resistant and susceptible isolines compared with the control. Also at 48 hr of incubation, high temperature stress before inoculation decreased peroxidase activity compared with the control in the resistant but not in the susceptible isolate. Moreover, the level of peroxidase activity in high temperature stress-treated and a control leaf was significantly lower in the susceptible than in the resistant isolate 48 hr after inoculation. Exposure to high
temperature stress resulted in a significant increase in electrolyte leakage as well as in sporulation in both isolines. Whereas increased sporulation on maize leaves in response to high temperature stress appeared to be related to increased electrolyte leakage.

Anderson *et al.* (1990) also determined the capacity of pepper leaves to acclimatize to high temperature by using electrolytes leakage procedure. Their research results showed an interaction between exposure temperature and duration, as well as lethal temperature decreasing linearly from 53 °C to 46 °C as exposure duration increased exponentially from 5 to 240 min. Plants grown at 22/20°C day/night cycles and held 24 hours at 38/30 °C had increased their heat tolerance by 35 °C, 51 °C to 54 °C.

Binelli and Mascarenhas (1990) studied the sensitivity of growth to high temperature and they found that an electrolyte leakage assay revealed that *Arabidopsis thaliana* seedlings were extremely sensitive to high temperature stress compared to the high temperature tolerant soyabean variety Tracy. Over 50%
ion leakage occurred in Arabidopsis leaves during a 15 min exposure to 50 °C, indicating a heat killing time of less than 15 min. In contrast, the heat killing time for soyabean at 50 °C was over 5 times longer. When soybean or Arabidopsis seedlings in culture plates were exposed to 37 °C for 2 h and then returned to 23 °C they suffered no apparent short-term or long-term damage. Soybean seedlings exposed to 42°C treatment for 2 h also showed no damage. Arabidopsis seedlings after a 42°C treatment for 2 h showed no apparent immediate damage, but 48 h after return to 23°C severe damage symptoms were visible and after 96 h all the seedlings were dead. Both soybean and Arabidopsis seedlings synthesize heat shock proteins when exposed to 42 °C for 2 h.

Chaisompongpan (1990) studied the responses of photosynthesis to heat stress in common bean (*Phaseolus vulgaris*) genotypes differing in acclimation potential. Without heat acclimation, heat stress at 42 °C decreased O₂ evolution in the six genotypes from 50 to more than 95%, compared with the controls, and heat stress at 45 °C almost totally inhibited O₂
evolution in all genotypes. Heat stress had less effect on peak fluorescence level than on $O_2$ evolution. Heat acclimation slightly reduced $O_2$ evolution, compared with nonacclimated controls. In heat-acclimated plants, heat stress at 42 °C had no effect on $O_2$ evolution, whereas stress at 45 °C significantly reduced $O_2$ evolution. Changes in levels of peak fluorescence under heat stress in heat-acclimated plants showed patterns similar to changes in $O_2$ evolution. Full recovery of $O_2$ evolution from heat injury at 42 °C for 5 min occurred within 4 h in GNU1 59, whereas $O_2$ evolution rates were still lower than the controls after 6 h in the other genotypes. The recovery of chlorophyll fluorescence was slower than that of $O_2$ evolution.

Saadalla et al. (1990) used the electrolyte leakage procedure to evaluate the effect of hardening on the thermotolerance of seven winter wheat ($Triticum aestivum$) cultivars. They exposed some of them to 0, 6, 12, 18, 24 h of hardening (34°C) and flag leaf material from the same cultivars exposed to 0, 48 and 120 h of hardening (34°C). Large differences in relative injury were observed among the seven
cultivars, ranging from 37 to 80 % for seedlings and from 31 to 69 % for anthesis. Similarly, Saadalla et al. (1990) conducted an experiment to determine the relationship between membrane thermostability (MT) and other agronomic traits of 144 genotypes of winter wheat (*Triticum aestivum*). Relative injury varied significantly among the 144 genotypes, with a range of 31 to 78 %. Based on relative injury, 27 genotypes were classified as heat tolerant, 71 as intermediate, and 46 as heat sensitive.

Shanahan et al. (1990) studied thermostability and heat tolerance of spring wheat. The electrolyte leakage or membrane thermostability (MT) test was conducted at anthesis on flag leaves of 8 field-grown genotypes. Based on MT values, 4 genotypes were grouped as heat-tolerant (HT) and 4 as heat-sensitive (HS). The HT and HS genotypes produced similar grain yields at the northern and central locations. However, the HT group of genotypes produced 21% more grain (4.28 t/ha) than the HS group (3.55 t/ha) at the southern site, which was considerably warmer during the grain-filling period than the
other 2 locations. Average temperatures during July were 19.5 °C, 21.7 °C and 23.4 °C at the northern, central and southern locations, respectively.

Research results of Prasil and Zamecnik (1990) on rape leaves, wheat crowns, onion bulbs that killed by a slow freeze-thaw cycle or immersion in liquid N showed a similar time course of electrolyte leakage and leakage was accelerated in treatments when the sample, immersed in water, and killed by heating for 5, 15 or 30 min in a boiling water bath. The longer the boiling, the sooner the maximum conductivity was obtained.

Nanaiah (1992) studied response of pepper (Capsicum annuum L. 'Early Calwonder') leaf disks to temperature stresses by using electrolyte leakage. His research results revealed midpoints of sigmoïdal response curves following freezing stress was -4.6, and 49.0°C following high temperature stress.

Kuo et al. (1993) assessed thermostability of local cultivars and improved lines of 20 species of vegetable crops
grown in the cool season by measuring electrolyte leakage of leaf tissue exposed to high temperatures (30 °C, 35 °C, 40 °C and 50 °C for 60 min). Distinct differences in leaf tissue high-temperature injury was found at incubation temperatures of 35 °C and higher. A subsequent experiment with 59 species/varieties of vegetable crops grown during 3 different seasons in the same year showed that monocotyledons and members of the Convolvulaceae were heat tolerant, while members of the Cruciferae [Brassicaceae] and Umbelliferae [Apiaceae] were heat sensitive. In most species, leaf tissue membranes were more injured in spring and winter than in summer.

Anderson (1994) found when exposed 'Early Calwonder' pepper (Capsicum annuum L) and 'Jubilee' corn (Zea mays L) leaf disks to high temperature stress produced ethylene, ethane, methanol, acetaldehyde, and ethanol based on comparison of retention times during chromatography to authentic standards that Corn leaf disks produced lower levels of ethylene, ethane, and methanol, but more acetaldehyde and
ethanol than pepper. Production of ethane, a by-product of lipid peroxidation, coincided with an increase in electrolyte leakage in pepper but not in corn.

Li et al. (1994) studied the relationship between changes of endogenous abscissic acid (ABA) level and resistance in hybrid rice Hybrid rice cv. Shanyou 63, Shanyou 287, Weiyou 63 and Weiyou 287 seedlings which subjected to 2 °C or 42 °C temperatures for 48 h. ABA levels and electrolyte leakage were highest at 2 °C. Abscisic acid concentration was not related to chilling resistance, while at 42 °C there was a close relationship between increased ABA levels and heat resistance. Weiyou 63 had the highest ABA content and the lowest electrolyte leakage.

Liu et al. (1996) used electrolyte leakage to determine Thermotolerance in Leaf discs of Capsicum annum cv. Early Calwonder through vacuum-infiltrated in distilled water, anisomycin, aurintricarboxylic acid, cycloheximide, ethionine, norvanine or puromycin to determine whether protein synthesis inhibitors blocked high-temperature acclimatization. Test tubes containing the discs were then placed in a water
bath at 50.5 °C for 0, 1, 5, 10, 15, 25, 35 or 50 min. They found that high-temperature acclimatization was blocked in all 6-protein synthesis-inhibitor treatments.

Loik and Harte (1996) compared leaf tolerance of high temperatures, as determined by electrolyte leakage and chlorophyll “a” fluorescence, for *Artemisia tridentata*, and *Potentilla gracilis*, specific differences in leaf temperature. Measurements were made for plants exposed to a climate change warming manipulation on a major ecosystem border in Colorado in July and August 1995. In July, daily maximum leaf temperatures were significantly higher for *P. gracilis* than for *A. tridentata*. Leaf temperatures were slightly lower in August than July for leaves of both species, on control and heated plots, despite the fact that daily maximum air temperatures were not significantly different for the 2 months. High-temperature tolerance was determined for leaves treated for 1 h at temperatures ranging from 15 to 65 °C. The LT50 was approximately 46 °C for both species on control plots, but
was 43 °C for leaves of both species from heated plots, contrary to the predictions of the hypothesis.

Another evaluation of germplasms using the electrolyte leakage procedure was done by Yang et al. (1996) who employed electrolyte leakage to determine membrane integrity in maize lines. The results revealed that when leaf segments were subjected to temperatures above 40 °C, BET1 corn lines exhibited less membrane injury than other lines.

Sethar et al. (1997) studied electrolyte leakage in leaves, roots and seeds of soybean cv. NARC-1 and cotton cv. Rehmani that subjected to temperatures of 30 °C, 34 °C, 38 °C, 42 °C, 46 °C and 50°C. Soybean leaves leaked more electrolytes (electrical conductivity of 313 μS/cm) than cotton leaves (143 μS/cm) at 50 °C. Below 42 °C much less ion efflux occurred as compared with 42 °C and above where severe damage to cell membranes occurred and more than 50% of the electrolytes leaked from leaves, seeds and roots of both crop plants.

Marcum (1998) also conducted an experiment to determine differences in cell membrane thermostability (CMT)
among cultivars of Kentucky bluegrass (*Poa pratensis* L.), in which leaf segments were subjected to heat shock exposure time, and also whole-plant to 41 °C day /34 °C night at 95 % relative humidity for 62 days and 47 days, in controlled environment chambers. The results revealed that CMT was negatively correlated with relative percentage leaf firing and positively with relative percentage short dry weight. It was found that BM-3 and Midnight cultivars were more heat tolerant than lavang, mugget and ryss.

Ismail *et al.* (1999) used electrolyte leakage technique to measure heat tolerance level of cowpea at flowering and pod set. The results revealed that genotypes with heat tolerance during flowering and pod set had less leaf susceptibility to heat during flowering and pod set or during early flowering.

Maheswari *et al.* (1999) also used electrolyte leakage percentage to study 4 cultivars of wheat differing in their sensitivity to high temperature stress with increasing temperature between 25 and 35 °C. A comparison of relative injury based on electrolyte leakage of these cultivars was done.
Cell permeability increased with increasing temperature in susceptible and tolerant cultivars.

Sibley et al. (1999) also conducted an experiment to study root thermostability of nine red maple (*Acer rubrum*) cultivars originating in different USDA hardiness zones that grown in containers. Electrolyte leakage from excised root tissue exposed for 30 min to temperatures ranging from 20 °C to 63 °C, was used to assess cellular injury of fine roots. Their result revealed that the critical killing temperatures of root tissue of evaluated cultivars ranged from 52.0 ± 0.8 °C to 53.3 ± 0.5 °C, indicating minimal differences in root membrane thermostability.

In another reported research by Talwar et al. (1999) who evaluated three-groundnut genotypes (ICG 1236, ICGS 44, and Chico) for their heat acclimation potential (HAP), and examined responses of the growth, yield, and photosynthetic of these genotypes to temperature was related to the HAP. They used electrolyte leakage to define HAP as the change in leaf heat tolerance based on plasmalemma thermostability at 40 to
60°C at 35/30°C day/night temperatures. All genotypes maintained greater vegetative growth and higher photosynthesis rates when grown under the higher temperature regime and genetic differences in photosynthesis rate were related to heat killing temperature. The higher temperature regime affected the reproductive growth adversely by increasing flower abortion and decreasing seed size, however. Differences in chlorophyll fluorescence and membrane thermostability between growth temperature were found only after incubating the leaf tissue at temperatures of 50 °C or higher. Genetic differences in HAP were small and unrelated to growth differences.

Ozalp et al. (2000) used photosystem II and cellular membrane stability to evaluate in wheat (*Triticum aestivum* L.) genotypes for NaCl tolerance. Wheat seedlings were grown hydroponically in environmental chambers and treated with a range of NaCl concentrations. Both methods clearly distinguished between salt-sensitive and salt-tolerant genotypes. Cellular membrane stability as measured by a
conductivity method and PS II activity values was affected adversely by NaCl concentration and duration of treatment.

2. Determination of Date palm Heat Tolerance

To the best of our knowledge, no assessment has been done to the tolerance of date palm plants regenerated by tissue culture to environmental stresses. No information available about the tolerance of these plants to heat stress alone or in the presence of high concentration of salts. Even for adult plants, very scant literature is available about their thermotolerance. It is well known that date palm plants tolerate high temperature stress that kills other evergreen plants. However, there are no mentioned methods that accurately determine the tolerance level. Hammouda et al. (1998) reported that adult date palm tree tolerates up to 68 °C and the ideal range for vegetative growth is 32 °C to 38 °C. The rise of temperature above range causes pinnae sunscald and scorch of young pinnae.
3. Tolerance of Plants to Multiple Stresses

Tal and Shannon (1983) studied the effect of dehydration and high temperature on the stability of leaf membrane of *lycopersicon esculentum, Solonium pennellii, lycopersicon peruvianum* and *lycopersicon cheesmanii*. Determining electrolyte leakage from leaf discs that exposed to heat and dehydration stress assessed stability. Membrane injury under stresses was greater in winter-grown than in summer-grown plants of all species. The membrane of the wild species appeared to be less stable than that of the cultivated one. In all species discs taken from salt-treated plants leaked more electrolytes under heat stress than did those taken from control plants.

Conner et al. (1991) compared between Flax cv. Andro, and McGregor, for heat and frost tolerance and for germination and emergence rate. The growth of excised shoots was compared with electrolyte leakage from excised leaves and with regrowth of intact plants. Excised shoots of Andro were more heat tolerant than McGregor shoots obtained from plants grown on salt gradient in the field. The excised-leaf test
overestimated heat tolerance in comparison to the excised-shoot test. Preconditioning plants grown at 15°C /5°C (light/dark) by slow heating (5 °C /h) dramatically increased their heat tolerance with Andro being superior in heat tolerance to McGregor. In field and controlled environment studies, both cultivars were similar in frost tolerance.

Kaku (1993) used water proton NMR relaxation times (T1), supercooling ability, and water content to measure sensitivity of azalea species; cv. Shounoshin, Lasiophyllum and Kumano-satsuki to freezing, dehydration, high temperature and salt stresses. A species with a large change in T1 is more stress-sensitive than species showing the opposite tendency. As well as the relative sensitivity to various stresses of each species appears to be related to the severity of conditions in its natural habitat.

Agarie et al. (1995) measured cell membrane thermostability by electrolyte leakage (EL) as a drought and heat tolerance test in rice (Oryza sativa L.). They found in desiccation test with polyethylene glycol (PEG), EL increased
from 10% to 80% with increasing from 20% to 60% of PEG concentration [PEG]. EL was increased with the time of PEG treatment; accordingly, 24 hours as a period of submerged leaf were selected. As well as they investigated an optimal temperature and treatment period, and around 42 °C is likely to be suitable for testing membrane thermostability. EL was increased almost linearly with the time of leaf tissues submerged in test temperature (42 °C) solution; EL of PEG test was greater in senescing leaf (the forth leaf from the youngest fully expanded leaf) than leaf in which senescence is less advanced (the youngest fully expanded leaf). EL of leaf tissue was also increased with water deficit, indicating physiological status of plant itself can be reflected by the technique.

Percival et al. (1998) studied the influence of sodium chloride and waterlogging stresses on Alnus cordata trees grown in freely drained or waterlogged compost substrates. Waterlogging using distilled water caused no significant stress compared with controls, apart from increasing root iron and manganese concentrations. Complete mortality was recorded
in trees watered with a sodium chloride solution 4.5% or waterlogged in sodium chloride solutions 2%. Watering or waterlogging with distilled water had no significant effect on mortality, while 66.6% of trees watered with a 2% sodium chloride solution died. Irrespective of whether trees were grown in freely drained or waterlogged compost, applications of sodium chloride to roots decreased leaf chlorophyll fluorescence and increased cell electrolyte leakage and leaf necrosis for up to 15 days after bud burst. Significant reductions in chlorophyll fluorescence were detected by day 3 following bud burst; however, significant increases in cell electrolyte leakage and leaf necrosis were not detected until day 6. Applications of sodium chloride significantly increased sodium and chloride concentrations in root, leaf, and shoots irrespective of drainage, with higher concentrations reflecting applications of stronger salt solutions. Calcium, phosphorus, magnesium, and potassium concentrations in cell tissues were unaffected by treatments. Depressed leaf and shoot copper,
zinc, and iron concentrations were recorded in trees waterlogged in sodium chloride solutions 4.5\%.

Yu et al. (1998) studied the effects of salt stress (200 mmol L\(^{-1}\)) and calcium (Ca\(^{2+}\)) on the lipid composition and function of plasma membrane and tonoplast vesicles isolated from the roots of barley seedlings (*Hordeum vulgare* L. Jian 4). Nine days of exposure to 200 mmol L\(^{-1}\) NaCl resulted in a significant increase in the electrolyte leakage and Na\(^+\)/K\(^+\) ratio in the roots, sheaths, and leaves of barley. The index of unsaturated fatty acids (IUF A) in plasma membrane (PM) and tonoplast (TP) vesicles, and the levels of galactolipid in TP increased. The results showed also that the alleviating effect of Ca\(^{2+}\) on NaCl induced injury in barley seedlings may be related to the changes of membrane lipid composition including phospholipids, galactolipids, and their fatty acids.

Akimova et al. (1999) studied the effect of shoot or root heating of leaf and root cells of winter (*Triticum aestivum*) and cucumber (*Cucumis sativus*) seedlings on heat, cold, and salt tolerance. Seedlings were heated at 40\(^{\circ}\)C and 30\(^{\circ}\)C,
respectively. The results revealed that leaf tolerance improved after heating both roots and shoots.

Tewary et al. (2000) conducted an experiment on five mulberry genotypes (G2, G3, G4, S34 and S13) to screen them to salt and osmotic stress. The data revealed that the genotype G4 ranked the highest in terms of sprouting percentage and shoot length, compared to the control variety (S34) at each concentration of NaCl up to 1.0 % and pH up to 9.5. In case of osmotic stress condition (media supplemented with 1-10 % PEG), S13 exhibited the highest sprouting percentage and shoot growth compared to the other test genotypes. The genotypes G4 have been screened as alt tolerant genotype.

According to Jiang et al. (2001) who conducted a comparative study on the effects of heat stress alone or in combination with drought on photosynthesis, water relations, and root growth of tall fescue (Festuca arundinacea L.) and perennial ryegrass (Lolium perenne L.). Grasses were exposed to heat (35 °C day/ 30 °C night) or heat and drought (induced by withholding irrigation), in growth chamber for 35 days. The
results revealed that declines in canopy net photosynthetic rate, leaf photochemical efficiency, and leaf relative water content and the increase in electrolyte leakage were much more severe and occurred earlier for ryegrass than fescue subjected to both heat and heat and drought. Also fescue had a higher evapotranspiration than ryegrass at 3 and 6 days for heat and heat plus drought, respectively.

4. Mitigation or Alleviation of Heat and Salt Stress Damage and the Possibility of Increase the Tolerance to Heat and Salt stresses.

There are many reports of a protective effect of Ca$^{2+}$, K$^-$ and unsaturated fatty acids such as oleic acid against heat or salt injury. Lange (1961) succeeded in hardening Commelina africana and Phoenix dactylifera by cultivating them at high but not injurious temperatures. They obtained a 4 °C increase in heat tolerance (from 47 to 51 °C) in the leaves of Commelina africana by growing the plants at 28 °C for 5 weeks compared to control plants grown for the same length of time at 20 °C.

Similarly, Yarwood (1967) succeeded to harden bean, cowpea, Corn, Corn, Cucumber, fig, soybean, sunflower and
tobacco by exposure to air temperature (water bath) of 45–55 °C. He found that the optimum exposure time was 20 sec. at 50 °C. Zavadskaya and De’ nko (1968) also investigated twelve species of plants and found that the heat tolerance was higher when they were subjected to a water deficiency than when growing in humid habitats.

Gary-boo (1970) reported that calcium might serve to bind the polar heat 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)2 SO4 could be relieved by the addition of CaCl2. This calcium maintains the membrane integrity. 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.

Leopold et al. (1984) reported in their experiments on soybean leaf tissue role of calcium chloride in protecting plant
tissue membrane from the NaCl damage. Whereas, 200 mM NaCl, with additional amounts of CaCl$_2$ ranging from 1 to 100 mM was reduced the relative leakage of solutes from the tissue. Consequently, they concluded that CaCl$_2$ could protect leaf membrane against the leakiness induced by NaCl.

Richard and Gary (1984) reported that CaCl$_2$ could protect leaf membranes against the leakiness induced by NaCl. That means the damage induced by sodium chloride could be alleviated by calcium treatment. Similarly, Ben-Hayyim et al. (1987) found that K$^+$ application could reduce the deleterious effect of salinity on plant development. Thus, potassium also can mitigate the damaging effect of sodium.

Borochov et al. (1991) studied the response of melon plants to salt. Their results revealed that an excess of NaCl in the growth medium of melon (cv. Galia) seedlings affected growth rate, morphology and contents of dry matter chlorophyll, K$^-$, Na$^+$ and chloride in the root and leaf tissues. In roots of seedlings grown in NaCl, the electrolyte leakage to iso-osmotic medium was more rapid. Fluorescence polarization
measurements revealed that root membranes isolated from these seedlings were significantly less fluid.

Harwood et al. (1994) reported that changes in fatty acid unsaturation are considered one of mechanisms that affect membrane integrity. Similarly, Nilsen and Orcutt (1996) reported that membrane thermostability 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.

Allakhverdiev et al. (1999) studied the role of unsaturated fatty acids in membrane lipids in the tolerance of the photosynthetic machinery to salt stress by comparing the desA-/desD- mutant of Synechocystis sp. PCC 6803, which contained monounsaturated fatty acids, with the wild-type strain, which contained a full complement of polyunsaturated fatty acids. In darkness, the loss of oxygen-evolving photosystem II activity in the presence of 0.5 M NaCl or 0.5 M LiCl was much more rapid in desA-/desD- cells than in wild-
type cells. Oxygen-evolving activity that had been lost during incubation with 0.5 M NaCl in darkness returned when cells were transferred to conditions that allowed photosynthesis or respiration. Recovery was much greater in wild type than in desA-/desD- cells, and it was prevented by lincomycin. Thus, the unsaturation of fatty acids is important in the tolerance of the photosynthetic machinery to salt stress. It appears also that the activity and synthesis of the Na\(^+\)/H\(^+\) antiporter system might be suppressed under high-salt conditions and that this effect can be reversed, in part, by the unsaturation of fatty acids in membrane lipids.

Zhou and Leul (1999) also treated seedlings of winter rape (Brassica napus L.) cv. 601 with 50 mg L\(^{-1}\) of foliar-applied uniconazole and then exposed to heat stress with a light/dark temperature regime of 43 °C /38 °C for 3 days at the stem elongation stage. Heat stressed plants contained lower endogenous gibberellic acid (GA\(_3\)), IAA and zeatin contents than the controls, while Abscisic acid (ABA) content and ethylene level were increased significantly. Uniconazole-treated
plants had lower endogenous GA₃ and IAA contents, and higher zeatin and ABA contents and ethylene levels. Leaf chlorophyll content and respiratory capacity of roots were reduced markedly after plants were subjected to heat stress, and foliar sprays of uniconazole retarded the degradation of chlorophyll and increased respiratory capacity of roots. Foliar applications of uniconazole reduced electrolyte leakage and malondialdehyde accumulation caused by heat stress. Foliar sprays of uniconazole increased the tolerance of rape plants to heat stress.

Aforementioned, electrolyte leakage procedure proved to be a valid test and was adapted by many researchers all over the world. As shown in the literature review, this procedure was used to investigate the following:

1- Thermotolerance of different cultivars and species.

2- Evaluation of germplasms or genotypes tolerance to heat stress.
3- Thermotolerance of plant tissues after exposure to another type of stress or multiple stresses (salt or drought stresses).

4- Assessing the effect of heat acclimation on the thermotolerance of fruits and leaves.

5- The effect of intermittent warming on heat tolerance of various plant tissues.

6- The effect of slow heating (preconditioning) on increasing heat tolerance of some plant species.
CHAPTER III

MATERIALS AND METHODS
CHAPTER III

MATERIALS AND METHODS

This research was conducted in the Horticulture laboratory at the Department of Plant Production, Faculty of Food Systems, United Arab Emirates University during 2001/2002. Two acclimatization stages VP2 and VP3 (VP means vitro plants) and offshoots (about 4 years old) of Rzaiz date palm cultivar were used in this research. The VP2 stage of acclimatization meant that these plantlets were under the acclimatization program for 4-6 months, while VP3 meant that these plantlets were under this program for 10 months. Plants were transferred from tissue culture laboratory located at Date Palm Research and Development Unit to the greenhouse at Al-Oha Research Station. Plants were maintained in a greenhouse under temperature ranged and were not exposed to water stress. Electrolyte leakage procedure was used to measure cell membrane thermostability after exposure to a heat regime. This regime followed the treatment with NaCl (1 % w/v) or CaCl₂, KCl, or oleic acid as will be mentioned in details.
Description of Work

I. Determination of Thermotolerance

Thirty-three fully expanded leaflets (3 leaflets/plant) of similar physiological age were collected from each stage. 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 (Memmert-Type WB14-Germany) provided with thermostat and tissues were exposed to a heat regime ranging from 30°C to 75°C (5°C increments). The exposure time to each temperature was 30 min. Three tubes per acclimatization stage remained at 22 °C as controls. At the end of the 30 minutes 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 before electrical conductivity of each solution was determined. In the next day Leaf samples were taken out from refrigerator, warmed up to room temperature (22 ± 2 °C) and placed in shaker device (Gesellschaft Fur Labortechnik mbh- model 3015-Germany) for one hour to diffuse electrolytes, then electrolytes leakage before killing was measured with electrical conductivity meter (Orion-model 150-USA). Leaf samples were then killed by autoclaving (JKA- J. 39 Autoclave- Japan) (121°C) for 10 minutes, after that were left on the shaker device for 1 hour, then the total electrolyte leakage reading were taken by using the same conductivity meter. Percentage of electrolyte leakage before killing to after killing was calculated. The three replications of the control tissue went through the same procedure to determine percentage of electrolyte leakage. The experiment consists of eight treatments, each treatment replicated 3 times, and each leaflet represents one replication. A completely randomized
design was used. Mstat Computer Software was used for obtaining the analysis of variance. The least significant difference (LSD) at 0.05 level was used to compare the means.

II. Assessment of Thermotolerance in the presence of High Salt Concentration

The same procedures as in heat test were followed, except leaflet segments of five-centimeter length were cut into two pieces to enhance more penetration of salts (NaCl) and immersed in 1% (10,000 ppm) of sodium chloride solution for 1 hr. 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. A procedure similar to that followed with the determination of thermotolerance was also used in order to assess heat tolerance of tissues in the presence of high salt concentration. Statistical design and analyses were also the same as in previous experiments.
III. Assessment of Thermotolerance Directly After Certain Treatments.

Pinnae segments were taken as mentioned before (under the determination of heat tolerance section). Segments were dipped for 1 hr in either calcium chloride (0.2 M), or potassium chloride (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 from the surface. 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 to 75 °C with 5 degrees increments). At each temperature, the three replications 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 measured. Three replications were used with each treatment in a completely randomized design. The analysis of variance and the least significant differences (at 0.05 level) were also obtained by Mstat Computer Software.
CHAPTER IV

RESULTS AND DATA PRESENTATION
CHAPTER IV

RESULTS AND DATA PRESENTATIONS

1. Generating the sigmoidal curves:

As shown in figures (1-24) the shape of the sigmoidal curve was consistent in all heat 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 from 1 to 8 for VP2 plantlets represent the results of heat tolerance alone or after treatments with sodium chloride, potassium chloride, calcium chloride, oleic acid, or in other combinations. Similarly, figures from 9 to 16 for VP3 plantlets, and finally figures from 17 to 24 for the offshoots of the same cultivar. 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.

II. Identification of Leaflets Thermotolerance:

II. A. VP2 Acclimatization Stage:

Differences in lethal temperatures after different treatments of VP2 acclimatized plantlets are presented in Table 1. Small differences in heat tolerance are important in surviving heat stress under field conditions. 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 30 min. before the exposure to the heat regime, the lethal temperature was 53.5°C. At this stage of acclimatization, tissues were not sensitive to used salt concentration (Table 1). 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°C and 57.5°C, respectively), 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 before the exposure to the heat regime. Table (1) shows that the lethal temperature for heat plus salt and oleic acid was 57.1 °C. Similar result was obtained when VP2 tissues were pretreated with salt plus KCl or NaCl plus CaCl₂ before the exposure to the heat regime. The lethal temperature for heat plus salt and KCl was 58 °C, while for heat plus salt and CaCl₂ was 57.1 °C.

II. B. VP3 Acclimatization Stage

The data in table (2) show that the lethal temperature of leaflet segments of VP3 acclimatized plantlets whether directly exposed to the heat regime or after treatments with NaCl, KCl,
CaCl$_2$ 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). Each of KCl and CaCl$_2$ treatments increased the thermotolerance of leaflets tissues at this stage than non-treated. The lethal temperatures for heat plus KCl or plus CaCl$_2$ treatments were 56.5°C 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. 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 (Table 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 thermotolerance 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 thermotolerance where the lethal temperature was 57.4 °C.
II. C. Rzaiz offshoots

Lethal heat temperature of leaflets taken from Rzaiz offshoots (58.5 °C) was much higher than that of VP2 and VP3 leaflets. 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 thermotolerance. In a similar way NaCl at the used concentration did not have an adverse effect on the leaflets (Pinnae) thermotolerance, as it was 58 °C. 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 leaflet (pinnae) tissues heat lethal tolerance that were previously treated with NaCl did not vary from that regime exposed to the heat regime only (Table 3). Similarly, treatments with sodium plus either potassium or calcium before the heat regime did not have a significant effect on the lethal temperature of leaflets. In general, the thermotolerance
of treated pinnae was similar whether the tissues were exposed to heat only or to heat in addition to sodium, potassium, calcium, or oleic acid in various combinations. The thick cuticle developed on these offshoot pinnae should not have represented a problem for the uptake of chemicals since chemicals can diffuse through the cut surface of used segments.

III. The interaction between the Heat regime and the two Stages of Acclimatization VP2, VP3, and offshoots.

The data in table (4) 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 °C, 35 °C, 40 °C, 45 °C, 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 °C, 65 °C, 70 °C, and 75 °C. These results are in agreement with that found in table 1 and 2 where the lethal temperatures for VP2, and VP3 stage were 53 °C 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 4 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 °C, 35 °C, 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 °C, 35 °C, 40 °C, 45 °C, 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 Table 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 (4) 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 addressed in the further studies.

Similar comparison could be made between electrolyte leakage
of VP2 leaflets at 55 °C and that of offshoots at 60 °C. Values at
both temperatures were not statistically different (Table 4),
however this leakage was obtained earlier in VP2 leaflets.

Since heat stress that was preceded by oleic acid
treatment resulted in increasing heat tolerance especially in
VP3 leaflets, the interaction between heat plus oleic acid and the acclimatization stages VP2, VP3 and offshoot was analyzed (Table 5). Electrolyte leakage of VP3 leaflets exposed to the heat regime after oleic acid treatment was significantly less than found with VP2 leaflets at 45 °C, 50 °C and 55 °C. Similar trend was also found when comparing the leakage of VP3 leaflets with the offshoot leaflets. At 30 °C, 35 °C, 40 °C, and 45 °C, the leakage of VP3 leaflets was significantly lower than that of offshoot leaflets.

The data in Table 1 and 3 also showed that even though heat tolerance of oleic acid-treated tissues was 53.4 °C and 58.1 °C for VP2 and offshoot leaflets, respectively. Oleic acid treatment to VP2 leaflets, however, resulted in significantly lower electrolyte leakage than that obtained with the offshoot leaflets at the sublethal temperatures 35 °C and 40 °C.

The effectiveness of oleic acid in increasing heat tolerance was more pronounced in VP3 – treated leaflets than VP2 plantlets. The difference was not only in the lethal temperature but also in electrolyte leakage at sublethal
temperatures. For example, at 45 °C, 50 °C, and 55 °C, electrolyte leakage of VP2 leaflets was significantly higher than that of VP3 leaflets.

IV. The interaction between Treatments and the two Acclimatizing Stages 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 (6). 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 6). 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 6).

V. The interaction between various Treatments and Temperatures of Rzaiz date palm at the VP2, VP3 acclimatizing Stages and offshoot.

The data in Table 7 showed that when VP2 leaflets were pretreated with NaCl and exposed to heat regime, they had significantly higher electrolyte leakage than that obtained with heat following the treatment with NaCl plus oleic acid, KCl, or CaCl₂ even at the sublethal temperatures 45 °C and 55 °C. However, at 60 °C oleic acid, KCl, CaCl₂ were not able to reduce significantly electrolyte leakage, when each of them was combined with heat plus salt as compared with heat and NaCl treatment only. This proves again the significance of the duration factor in stress and the mitigation of salt effect by oleic acid, KCl, or CaCl₂. The data in Table 8 also confirm that heat plus salt treated tissues of VP3 started to leak out significantly higher amounts of electrolytes when exposed to
even sublethal temperatures such as 40 °C, 45 °C, and 50 °C as compared with heat plus salt but in the presence of either oleic acid or KCl or CaCl₂. This result confirms the role of these compounds in mitigating the salt effect. The lowest mean of electrolyte leakage (Table 8) was obtained with heat plus oleic acid. The leakage mean (33.4%) was significantly lower than that found with all other treatments except heat plus salt and CaCl₂. From the interactions in Table 9, it could be noticed at sublethal temperatures such as 40 °C, 45 °C, and 50 °C, electrolyte leakage of offshoot leaflets caused by heat plus salt was not significantly different from heat plus salt in the presence of either oleic acid, KCl, or CaCl₂. This was in agreement with our finding in Table 3 where various treatments had similar lethal temperature for offshoots. Even for treatment means (Table 9), there was no significant difference in electrolytes among all treatments. This provided further evidence for the unique response of offshoots to various heat treatments as compared with VP2 and VP3 acclimatization stages.
Figure 1. Heat tolerance of VP2 acclimatization stage of Rzaiz date palm Plantlets.

Figure 2. Heat tolerance of pretreated sodium chloride VP2 Acclimatization stage of Rzaiz date palm plantlets.
Figure 3. Heat tolerance of pretreated potassium chloride VP2 acclimatization stage of Rzaiz date palm plantlets.

Figure 4. Heat tolerance of pretreated calcium chloride of VP2 acclimatization stage of Rzaiz date palm plantlets
Figure 5. Heat tolerance of pretreated oleic acid of VP2 acclimatization stage of Rzaiz date palm plantlets.

Figure 6. Heat tolerance of pretreated sodium chloride and oleic acid of VP2 acclimatization stage of Rzaiz date palm plantlets.
Figure 7. Heat tolerance of pretreated sodium chloride and potassium chloride of VP2 acclimatization stage of Rzaiz date palm plantlets.

Figure 8. Heat tolerance of pretreated sodium chloride and calcium chloride of VP2 acclimatization stage of Rzaiz date palm plantlets.
Figure 9. Heat tolerance of VP3 acclimatization stage of Rzaiz date palm Plantlets.

Figure 10. Heat tolerance of pretreated sodium chloride of VP3 acclimatization stage of Rzaiz date palm plantlets.
Figure 11. Heat tolerance of pretreated potassium chloride of VP3 acclimatization stage of Rzaiz date palm plantlets.

Figure 12. Heat tolerance of pretreated calcium chloride of VP3 acclimatization stage of Rzaiz date palm plantlets.
Figure 13. Heat tolerance of pretreated oleic acid of VP3 acclimatization stage of Rzaiz date palm plantlets.

Figure 14. Heat tolerance of pretreated sodium chloride and oleic acid of VP3 acclimatization stage of Rzaiz date palm plantlets.
Figure 15. Heat tolerance of pretreated sodium chloride and potassium chloride of VP3 acclimatization stage of Rzaiz date palm plantlets.

Figure 16. Heat tolerance of pretreated sodium chloride and calcium chloride of VP3 acclimatization stage of Rzaiz date palm plantlets.
Figure 17. Heat tolerance of offshoot Rzaiz date palm plants.

Figure 18. Heat tolerance of pretreated sodium chloride of offshoot Rzaiz date palm plants.
Figure 19. Heat tolerance of pretreated potassium chloride of offshoot Rzaiz date palm plants.

Figure 20. Heat tolerance of pretreated calcium chloride of offshoot Rzaiz date palm plants.
Figure 21. Heat tolerance of pretreated oleic acid of offshoot Rzaiz date palm plants.

Figure 22. Heat tolerance of pretreated sodium chloride and oleic acid of offshoot Rzaiz date palm plants.
Figure 23. Heat tolerance of pretreated sodium chloride and potassium chloride of offshoot Rzaiz date plants.

Figure 24. Heat tolerance of pretreated sodium chloride and calcium chloride of offshoot Rzaiz date palm plants.
Table 1. Lethal temperature of Rzaiz date palm leaflets at the VP2 acclimatization stage as influenced by various treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lethal temperatures (°c)</th>
</tr>
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<tbody>
<tr>
<td>Heat</td>
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<tr>
<td>Heat and Salt</td>
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<td>Heat and KCl</td>
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<td>Heat and CaCl₂</td>
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<td>Heat and Salt and KCl</td>
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<td>Heat and Salt and CaCl₂</td>
<td>57.1</td>
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</table>

Table 2. Lethal temperature of Rzaiz date palm leaflets at the VP3 acclimatization stage as influenced by various treatments.

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<thead>
<tr>
<th>Treatments</th>
<th>Lethal temperatures (°c)</th>
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<tbody>
<tr>
<td>Heat</td>
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Table 3. Lethal temperature of Rzaiz date palm leaflets from offshoots as influenced by various treatments.

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<th>Treatments</th>
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<tr>
<td>Heat</td>
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<td>Heat and KCl</td>
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<td>Heat and Salt and CaCl₂</td>
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</table>
Table 4. The interaction between the heat regime and the stages of acclimatization and hardening of Rzaiz date palm plants on electrolyte leakage (%).

<table>
<thead>
<tr>
<th>Temperature regimes (°C)</th>
<th>VP2 * %</th>
<th>VP3 * %</th>
<th>Offshoot * %</th>
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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 5. The interaction between heat plus oleic acid treatment and the stages of acclimatization and hardening of Rzaiz date palm plants on electrolyte leakage (%).

<table>
<thead>
<tr>
<th>Temperature regimes (°C)</th>
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<th>VP3 *</th>
<th>Offshoot *</th>
<th>Mean</th>
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<tr>
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L.S.D. (0.05) = 3.61
C.V. % = 6.39

* 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 6. The interaction between treatments and the stages of acclimatization and hardening of Rzaiz date palm plants on electrolyte leakage (%).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>VP2 * %</th>
<th>VP3 * %</th>
<th>Offshoot * %</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>44.6 **a</td>
<td>39.2 bed</td>
<td>33.7 ghij</td>
<td>39.2</td>
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<tr>
<td>Heat and NaCl</td>
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<td>Heat and KCl</td>
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<td>Heat and CaCl₂</td>
<td>42.2 ab</td>
<td>39.3 bed</td>
<td>34.5 fghij</td>
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</tr>
<tr>
<td>Heat and oleic acid</td>
<td>37.5 cdefg</td>
<td>33.4 ghij</td>
<td>32.7 hij</td>
<td>34.5</td>
</tr>
<tr>
<td>Heat and salt and oleic acid</td>
<td>36.2 defghi</td>
<td>36.0 defghi</td>
<td>30.7 j</td>
<td>34.3</td>
</tr>
<tr>
<td>Heat and salt and KCl</td>
<td>35.7 defghi</td>
<td>38.4 bcdef</td>
<td>33.3 hij</td>
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<tr>
<td>Heat and salt and CaCl₂</td>
<td>36.4 defghi</td>
<td>34.8 efghij</td>
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<td>39.5</td>
<td>37.4</td>
<td>33.0</td>
<td>36.6</td>
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</table>

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).
Table 7. The interaction between various treatments and temperatures of Rzaiz date palm plantlets on electrolyte leakage (%) at the VP2 acclimatization stage.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Temperature regimes (°C) *</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Heat</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11.4**</td>
</tr>
<tr>
<td>Heat and salt</td>
<td>11.9</td>
</tr>
<tr>
<td>Heat and KCl</td>
<td>5.1</td>
</tr>
<tr>
<td>Heat and CaCl₂</td>
<td>6.2</td>
</tr>
<tr>
<td>Heat and oleic acid</td>
<td>6.1</td>
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<tr>
<td>Heat and salt and oleic acid</td>
<td>6.2</td>
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<td>Heat and salt and CaCl₂</td>
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</tr>
<tr>
<td>Mean</td>
<td>7.0</td>
</tr>
</tbody>
</table>

L. S. D. (0.05) = 4.46  
C.V. % = 7.01

* 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 8. The interaction between various treatments and temperatures of Rzaiz date palm plantlets on electrolyte leakage (%) at the VP3 acclimatization stage.

<table>
<thead>
<tr>
<th>Treatment</th>
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<th></th>
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<td>30</td>
<td>35</td>
<td>40</td>
<td>45</td>
<td>50</td>
<td>55</td>
<td>60</td>
<td>65</td>
<td>70</td>
<td>75</td>
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<tr>
<td>Heat</td>
<td>8.9 **</td>
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<td>7.9</td>
<td>11.3</td>
<td>10.6</td>
<td>16.1</td>
<td>66.2</td>
<td>69.5</td>
<td>77.2</td>
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<td>80.9</td>
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<td>14.2</td>
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<td>18.1</td>
<td>57.3</td>
<td>59.6</td>
<td>75.9</td>
<td>76.0</td>
<td>73.6</td>
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<td>13.6</td>
<td>12.5</td>
<td>13.1</td>
<td>40.5</td>
<td>76.0</td>
<td>78.4</td>
<td>80.3</td>
<td>82.5</td>
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<tr>
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<td>11.1</td>
<td>16.9</td>
<td>16.7</td>
<td>16.8</td>
<td>34.4</td>
<td>76.1</td>
<td>75.0</td>
<td>81.1</td>
<td>81.3</td>
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<td>77.3</td>
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<td>9.1</td>
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<td>77.4</td>
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<td>77.2</td>
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<td>9.8</td>
<td>7.8</td>
<td>9.4</td>
<td>10.7</td>
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<td>80.8</td>
<td>78.3</td>
<td>77.8</td>
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<td>9.5</td>
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<td>76.7</td>
<td>81.2</td>
<td>74.2</td>
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<td>42.8</td>
<td>74.3</td>
<td>77.1</td>
<td>78.2</td>
<td>78.1</td>
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</table>

L. S. D. (0.05) = 4.48  
C.V. % = 7.44

* 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 9. The Interaction between various treatments and temperatures regimes of Rzaiz date palm offshoot plants on electrolyte leakage (%).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature regimes (°C )</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
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<td>75</td>
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<tr>
<td>Heat</td>
<td>10 **</td>
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</tr>
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<td>11.5</td>
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<td>13.0</td>
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<td>66.9</td>
<td>71.9</td>
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<td>70.4</td>
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<td>17.1</td>
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<td>68.2</td>
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<td>68.9</td>
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<td>13.2</td>
<td>31.5</td>
<td>62.9</td>
<td>64.2</td>
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<td>69.9</td>
</tr>
<tr>
<td>Heat and salt and CaCl₂</td>
<td>7.8</td>
<td>12.7</td>
<td>14</td>
<td>12.7</td>
<td>15</td>
<td>16.2</td>
<td>26.1</td>
<td>71.2</td>
<td>63.9</td>
<td>66.9</td>
<td>67.3</td>
</tr>
<tr>
<td>Mean</td>
<td>8.2</td>
<td>12.0</td>
<td>13.0</td>
<td>12.6</td>
<td>13.2</td>
<td>13.6</td>
<td>19.8</td>
<td>67.1</td>
<td>67.2</td>
<td>67.0</td>
<td>68.9</td>
</tr>
</tbody>
</table>

L. S. D. 0.05 = 3.41
C.V. % = 6.41

* 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).
CHAPTER V

DISCUSSION
CHAPTER V

DISCUSSION

The sigmoidal pattern of electrolyte leakage obtained in this study, for heat stress alone or for pretreated tissues with NaCl, KCl, CaCl$_2$, or oleic acid then exposed to heat stress agrees with previous studies (Nilsen and Orcutt, 1996). Similar pattern was also obtained when the leaflet segments were exposed to salt plus each of oleic acid, or KCl, or CaCl$_2$, and then experienced heat 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 (Levitt, 1980). It has been reported for many years that the conductivity method is a direct indicator for membrane integrity (Oleinikova, 1965). Thus, it has been used to estimate heat injury by measuring electrolyte leakage since the plasma membrane semipermeability is lost or damaged.
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 plantlets and the offshoots grown along with the mother plant under natural conditions. To the best of our knowledge, this is the first study that provided a quantitative determination for heat tolerance of date palm plants. 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 °C, 53.5 °C, 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 – 12 °C (Levitt, 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 plantlets are produced under delicate microenvironment, while offshoots went through sufficient hardening conditions in the field. The comparison between heat tolerance of tissue culture date palm plantlets and offshoots is justifiable since these are the two methods used now days for date palm propagation. The other factor that must be considered that VP2 and VP3 plantlets 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 acclimatization 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. This possibility is supported by the analysis of the interaction between treatments and the heat regime (Table 7 and 8) where salt plus heat-treated tissues had significantly higher electrolyte leakage than heat-treated tissues even at sublethal temperature. Since early years of stress physiology, the effect of salts on thermotolerance has been controversial. Several investigators suggested a protective role for some salts (De Vries, 1871) against heat injury. Others (Bogen, 1948) found
that salts lowered the heat killing temperature. The differences in result were attributed to the amount taken by the tissues. If the tissues permitted more penetration or diffusion of salts, the thermoderivation will be lowered. Borochov et al. (1991) finding revealed that an excess of NaCl caused an increase in electrolyte leakage. The increase in thermoderivation 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, 1976, Leopold et al., 1984, and 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-hayyinum 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 Tables 2 and 3 agree with this explanation. The data in Table 3 indicated that 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 thermotolerance of offshoot leaflets was high (58.5 °C), this might be the maximum potential of tolerance for such hardened tissues in the field.

Regarding the role of unsaturated fatty acid on increasing the thermotolerance of plants, it was reported by Hardwood et al. (1994) that these acids protect the membrane from hyperfluidity that means keeping its integrity. The change in membrane fluidity affects the properties of embedded proteins (enzymes) that make the membrane more leaky to electrolytes. In this research, the pretreatment with oleic acid caused an increase in the thermotolerance of VP2 and VP3 leaflets (Table 1 and 2). These results agree with what was reported on the positive effects of unsaturated fatty acids.
on the plasma membrane (Harood et al., 1994 and Nilsen 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 thermotolerance 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. These results suggested the importance of studying the thermotolerance of this stage at various durations.

In a similar way, electrolyte leakage of heat plus salt treated- tissues (Tables 7 and 8) was significantly higher than that leakage obtained with heat plus NaCl in the presence of oleic acid, KCl, or CaCl₂ even at sublethal temperatures for VP2 and VP3. These findings suggested again that the effect of heat plus salt at various durations on the tolerance level of VP2 and VP3 could be addressed in further coming studies.

In general, there is a great potential to increase the thermotolerance of the two acclimatized stages by
pretreatment them with safe chemicals such as potassium chloride, calcium chloride, or oleic acid. This thesis suggests important applied aspects that could rescue acclimatized date palm plantlets from heat stress alone or in the presence of high salt concentration.
CHAPTER VI

CONCLUSIONS
CHAPTER VI

CONCLUSIONS

Results presented in this thesis demonstrated the following:

1. Heat stress regime resulted in consistently generating a sigmoidal curve as shown in other studies. Furthermore, pretreatment of leaflets with NaCl, KCl, CaCl$_2$, or oleic acid then the heat regime also resulted in a similar sigmoidal pattern.

2. This study showed for the first time a quantitative determination of the thermotolerance for tissue culture plantlets at two stages of acclimatization (VP2 and VP3) and for hardened offshoot of the same Rzaiz cultivar. These are the two stages that are currently distributed to date palm growers. Offshoots thermotolerance was greater than that VP2 and VP3 leaflet.

3. 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 thermotolerance. The salt concentration and duration used to treat with salt did not achieve either of the two possibilities.

4. A marked increase in the thermotolerance of VP2 and VP3 leaflets occurred when tissues were pretreated with KCl, CaCl₂, or oleic acid when compared with heat stress alone.

5. The highest value of thermotolerance was obtained with offshoot leaflets. However, pretreated with other compounds such as KCl, CaCl₂, or oleic acid did not result in increased thermotolerance. It could be concluded that 58.5 °C might be the maximum potential for heat tolerance.

6. Although heat tolerance of VP2 and VP3 leaflets did not vary much, but the interaction between used temperatures and the three stages of growth showed that VP2 leaflets had significantly higher electrolyte leakage than VP3 leaflets at sublethal temperatures.
7. Even though the pretreatment with salt did not shift the tolerance of VP2 and VP3 leaflets, but the interaction between treatments and the heat regime showed that heat plus salt treatment resulted in significantly higher electrolyte than heat plus salt in the presence of KCl, or CaCl$_2$ or oleic acid at sublethal concentrations.

8. Conclusions made under the last two points indicate that the duration factor in stress is very important as mentioned in the literature.
CHAPTER VII

BIBLIOGRAPHY
CHAPTER VII

BIBLIOGRAPHY


Prašil, I. and Zamecnik, J. Time course of electrolyte leakage from various samples killed by frost, liquid nitrogen or boiling. *Biologia*


## APPENDIX

Analysis of variance of the interaction between the heat regime and the two stages of acclimatization and offshoots of Rzaiz date palm.

<table>
<thead>
<tr>
<th>K Value</th>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
</tr>
</thead>
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<td>1983.335</td>
<td>991.667</td>
<td>66.0058</td>
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<tr>
<td>-3</td>
<td>Error</td>
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<td>90.144</td>
<td>15.024</td>
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<td></td>
</tr>
<tr>
<td>4</td>
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<td>6</td>
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<td>Total</td>
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<td>91548.327</td>
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</table>

Coefficient of Variation: 9.27%

S_ for means group 2: 0.6747  
\(Y\)

S_ for means group 4: 1.2093  
\(Y\)

S_ for means group 6: 2.0946  
\(Y\)

* Factor A = Stages  
** Factor B = Heat regimes

Analysis of variance of the interaction between heat plus oleic acid treatment and the two stages of acclimatization and offshoots of Rzaiz date palm.

<table>
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<tr>
<th>K Value</th>
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<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
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<td>95056.423</td>
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</table>

Coefficient of Variation: 6.39%
\[ S \text{ for means group 2: 0.4227} \]
\[ S \text{ for means group 4: 0.7360} \]
\[ S \text{ for means group 6: 1.2747} \]

* Factor A = Stages
** Factor B = Heat plus oleic acid treatment

Analysis of variance of the Interaction between various treatments and the two stages of acclimatization and offshoots of Rzaiz date palm.

<table>
<thead>
<tr>
<th>K Value</th>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
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<td>4</td>
<td>Factor B **</td>
<td>7</td>
<td>2563.961</td>
<td>366.280</td>
<td>54.9681</td>
<td>0.0000</td>
</tr>
<tr>
<td>6</td>
<td>AB</td>
<td>14</td>
<td>1699.447</td>
<td>121.389</td>
<td>18.2170</td>
<td>0.0000</td>
</tr>
<tr>
<td>8</td>
<td>Factor C ***</td>
<td>10</td>
<td>659954.219</td>
<td>659954.422</td>
<td>9904.0023</td>
<td>0.0000</td>
</tr>
<tr>
<td>10</td>
<td>AC</td>
<td>20</td>
<td>9967.635</td>
<td>498.382</td>
<td>74.7927</td>
<td>0.0000</td>
</tr>
<tr>
<td>12</td>
<td>BC</td>
<td>70</td>
<td>6241.492</td>
<td>89.164</td>
<td>13.3810</td>
<td>0.0000</td>
</tr>
<tr>
<td>14</td>
<td>ABC</td>
<td>140</td>
<td>11999.069</td>
<td>85.708</td>
<td>12.8622</td>
<td>0.0000</td>
</tr>
<tr>
<td>-15</td>
<td>Error</td>
<td>528</td>
<td>3518.333</td>
<td>6.664</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>791</td>
<td>701787.831</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coefficient of Variation: 7.05%

\[ S \text{ for means group 2: 0.1589} \]
\[ S \text{ for means group 4: 0.2594} \]
\[ S \text{ for means group 6: 0.4494} \]
\[ S \text{ for means group 8: 0.3042} \]
\[ S \text{ for means group 10: 0.5269} \]
Factor A = Stages  
** Factor B = various treatments  
*** Factor C = Temperature regimes

Analysis of variance of the interaction between various treatments and temperatures of Rzaiz date palm at the VP2 acclimatization stage.

<table>
<thead>
<tr>
<th>K Value</th>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Factor A *</td>
<td>7</td>
<td>2697.038</td>
<td>385.291</td>
<td>47.2841</td>
<td>0.0000</td>
</tr>
<tr>
<td>-3</td>
<td>Error</td>
<td>16</td>
<td>130.375</td>
<td>8.148</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Factor B **</td>
<td>10</td>
<td>228593.700</td>
<td>22859.370</td>
<td>2984.9741</td>
<td>0.0000</td>
</tr>
<tr>
<td>6</td>
<td>AB</td>
<td>70</td>
<td>8115.224</td>
<td>115.932</td>
<td>15.1384</td>
<td>0.0000</td>
</tr>
<tr>
<td>-7</td>
<td>Error</td>
<td>160</td>
<td>1225.304</td>
<td>7.658</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>263</td>
<td>240761.640</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Coefficient of Variation: 7.01%

Analysis of variance of the interaction between various treatments and temperatures of Rzaiz date palm at the VP3 acclimatization stage.

<table>
<thead>
<tr>
<th>K Value</th>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Factor A *</td>
<td>7</td>
<td>1226.737</td>
<td>175.248</td>
<td>14.6459</td>
<td>0.0000</td>
</tr>
<tr>
<td>-3</td>
<td>Error</td>
<td>16</td>
<td>191.451</td>
<td>11.966</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Factor B *</td>
<td>10</td>
<td>259341.813</td>
<td>25934.181</td>
<td>3353.6073</td>
<td>0.0000</td>
</tr>
<tr>
<td>6</td>
<td>AB</td>
<td>70</td>
<td>7011.198</td>
<td>100.160</td>
<td>12.9519</td>
<td>0.0000</td>
</tr>
<tr>
<td>-7</td>
<td>Error</td>
<td>160</td>
<td>1237.315</td>
<td>7.733</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>263</td>
<td>269008.516</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Coefficient of Variation: 7.44%

\[ S_\text{Y} \text{ for means group 2: } 0.6022 \quad \text{Number of Observations: 33} \]

\[ S_\text{Y} \text{ for means group 4: } 0.5676 \quad \text{Number of Observations: 24} \]

\[ S_\text{Y} \text{ for means group 6: } 1.6055 \quad \text{Number of Observations: 3} \]

* Factor A = Treatments

** Factor B = Temperature regimes

Analysis of variance of the Interaction between various treatments and temperatures of Rzaiz offshoot.

<table>
<thead>
<tr>
<th>K</th>
<th>Value</th>
<th>Source</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Prob</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Factor A *</td>
<td>7</td>
<td>316.421</td>
<td>45.203</td>
<td>9.5485</td>
<td>0.0001</td>
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</tr>
<tr>
<td>-3</td>
<td>Error</td>
<td>16</td>
<td>75.745</td>
<td>4.734</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Factor B *</td>
<td>10</td>
<td>181962.296</td>
<td>18196.230</td>
<td>4072.1385</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>AB</td>
<td>70</td>
<td>3028.537</td>
<td>43.265</td>
<td>9.6822</td>
<td>0.0000</td>
<td></td>
</tr>
<tr>
<td>-7</td>
<td>Error</td>
<td>160</td>
<td>714.955</td>
<td>4.468</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total | 263 | 186097.954 |

Coefficient of Variation: 6.41%

\[ S_\text{Y} \text{ for means group 2: } 0.3788 \quad \text{Number of Observations: 33} \]

\[ S_\text{Y} \text{ for means group 4: } 0.4315 \quad \text{Number of Observations: 24} \]

\[ S_\text{Y} \text{ for means group 6: } 1.2204 \quad \text{Number of Observations: 3} \]

* Factor A = Treatments

** Factor B = Temperature regimes
## Conversion Table

<table>
<thead>
<tr>
<th>Used Units</th>
<th>Unit in ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2 M CaCl$_2$</td>
<td>22200</td>
</tr>
<tr>
<td>0.2 M KCl</td>
<td>14910</td>
</tr>
<tr>
<td>1 % NaCl</td>
<td>10000</td>
</tr>
</tbody>
</table>
بين الأنسجة المستخدمة، فكانت الحرارة القاتلة للوريقات هي 300 م في مراحل VP2 و VP3. 

في مراحل VP3، بالنسبة للفسائل الناتجة بالحقل.

كما نتج عن المعاملة الحرارية في وجود تركيز عالي من كلوريد الصوديوم زيادة في 

تسرب المواد الأليكتروتيتية وذلك بالمقارنة بتلك المعاملة في وجود الكالسيوم أو البوتاسيوم أو 

حمض الأوليك عند درجات الحرارة تحت القاتلة، كذلك كان تسرب المواد الأليكتروتيتية للمرحلة 

الأولى للتأقلم VP2، أكثر معنويًا من تلك الناتجة من المرحلة الثانية للتأقلم VP3 عند درجات 

الحرارة تحت القاتلة مما يدل على أهمية طول فترة التعرض للحرارة المرتفعة.

وتكمن الأهمية الخاصة لهذه الدراسة في أنها حددت ولأول مرة وبشكل دقيق مدى 

تحمل الإجهاد الحراري سواء لمرحلة الأقلمة VP1 أو للفسؤول الناتجة بالحقل من 

VP3. وبناءً على النتائج، كما أثبتت النتائج أنه يمكن زيادة تحمل تلك الشتلات إجهاد الحرارة المرتفعة عن 

صنيف رفيع، مما يعكس النتائج أنه يمكن زيادة تحمل تلك الشتلات إجهاد الحرارة المرتفعة عن 

طريق المعاملة بالكالسيوم أو البوتاسيوم أو حمض الأوليك، كما أنه يمكن التوصية بالمياد 

المناسب لنقل تلك الشتلات للحقل المستند من حيث درجات الحرارة المثالية حتى لا يحدث فقد 

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كبير منها.
الحراري لكل مرحلة من التآكل وكذلك للفسائل الناتجة بالحقل بجوار النبات الأم. وقد قيس التحمل الحراري مع معاملات الحرارة وحدها أو في وجود تركيز عالي من كلوريد الصوديوم (١٠٠٠ جزء في المليون).

وذلك تحمّل الحرارة المرتفعة في وجود الكالسيوم أو البوتاسيوم أو حمض الأوليك.

ومن ذلك يتضح أنه تم إجراء ثمان معاملات في هذا البحث وكتبت كالتالي:

الحرارة المرتفعة، الحرارة المرتفعة في وجود الملوحة، الحرارة المرتفعة في وجود الكالسيوم أو البوتاسيوم أو حمض الأوليك، إجهاد الحرارة المرتفعة والملوحة في وجود حمض الأوليك أو الكالسيوم أو البوتاسيوم وكان مصدر الأجهزة الملوحة هو كلوريد الصوديوم (١٠٠٠٠ جزء في المليون) أما الكالسيوم فاستخدمه على هيئة كلوريد كالسيوم (٢.٠ مولار) والبوتاسيوم على هيئة كلوريد بورتاسيوم (٢.٠ مولار) أما حمض الأوليك فاستخدم بتركيز ١٠٠ جزء في المليون.

وقد أدت معاملة قطاعات محددة من الورق (الخوص) بواسطة البوتاسيوم، الكالسيوم أو حمض الأوليك على زيادة التحمل للحرارة المرتفعة، ولكن لم يؤثر تركيز كلوريد الصوديوم المستخدم (الملوحة) في مدى التحمل الحراري كما وجدت اختلافات في تتحمل الأجهزة الحراري
الملخص العربي

أجري هذا البحث بمختبر البسمتين التابع لقسم زراعة الأراضي القاحلة بكلية نظم الأغذية، جامعة الإمارات العربية المتحدة خلال السنة الدراسية 2001/2002م لدراسة تحمل نباتات نخيل التمسيجة من صنف "رزي" لإجهاد الحرارة المرتفعة والملوحة، وكذلك دراسة إمكانية زيادة هذا التحمل عن طريق المعاملات (ب الكالسيوم أو البوتاسيوم أو حمض الأوليك. وقد كانت النباتات التمسيجة المستخدمة في مرحلتي الأقلية VP3, VP2 حسب تسمية معدل زراعة الأنسجة بالعوجة، كما تم مقارنة نتائج تلك المرحلة بنتائج نفس المعاملات على فسائل حقلية من نفس الصنف عمرها 4 سنوات.

وسعتم الأخبار المعتمد عالمياً في المراجع المختلفة والمسمى بطريقة تسرب المواد الأليكترولتيتية حيث أنه من العلامات الدقيقة على مدى حدوث الضرر بالغشاء البلوري للخلية الذي يتآثر بالإجهادات البيئية مثل إجهاد الحرارة المرتفعة والملوحة.

ومن نتائج تسرب المواد الأليكترولتيتية مع معاملات الحرارة المختلفة بدءاً من 30° إلى 75° أمكن الحصول بدقة على المنحنى الزيجومودي الذي يتعدد عن طريق درجة التحمل
دراسات على تحمل نباتات نخيل التمر النسيجية لإجهاد الحرارة المرتفعة والملوحة

رسالة مقدمة من الطالب

خير بن طوير بن سعيد البوسعيدي

استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم البيئية

تحت إشراف

أ. الدكتور/ كريم محمد فرج

مشارف مساعد

كلية نظام الأغذية
قسم زراعة الأراضي الفاحلة
جامعة الإمارات العربية المتحدة

مايو 2002