Changes in cellular membrane tolerance due to heat stress during *Triticum sativum* L. seeds germination

Evan Ibrahim Al-Jebory
Dept. Of Biol., College of Sci., Univ. of Babylon

Abstract

Heat stress due to increased temperature is an agricultural problem in many areas in the world. The objective of this study was to examine changes in seed germination characters, cell membrane stability, lipid peroxidation level and electrolyte leakage in germinated seeds exposed to heat stress. Seed germination, co-efficient of germination and germination vigor index asignificant decreased (due to high temperature) is compared to control (untreated seeds). The MDA a signficant increased, whereas the protein content asignficant decreased due to high temprature. Heat stress a signficant increased proline content. Electrolytes level (as indicator on perturbation of membrane permeability) a signficant increased with exposure period increase to high temperature.

Introduction

A key adaptive mechanism in many plants grown under abiotic stresses, including salinity, water deficit and extreme temperatures, is accumulation of certain organic compounds of low molecular mass, generally referred to as compatible osmolytes (Hare *et al.*, 1998; Sakamoto and Murata, 2002). Under stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi, 2004). Accumulation of such solutes may contribute to enhanced stress tolerance of plants.Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. Heat stress is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature. Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001).

By definition, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994). Different environmental factors may determine seed germination, although the essentials are an appropriate combination of temperature, moisture and light (Bewley and Black, 1994; Baskin and Baskin, 1998).

High-temperature-induced modifications on plants may be direct on existing physiological processes or indirect in altering the pattern of development. These responses may differ from one phenological stage to another. High temperatures can cause considerable pre- and post-harvest damages, including scorching of leaves and twigs, sunburns on leaves and branches as well as stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield (Guilioni *et al*., 1997; Ismail and Hall, 1999; Vollenweider and Gunthardt- Goerg, 2005).Protoplasmatic resistance level results from the cellular membranes integrity conservation and cellular compartimentalization (El-Tohamy *et al*., 1999). Cellular membranes damage can be verified through different procedures, for example, directly by electronic microscopy (Sajo and Machado, 2001; Douliez, 2004) and indirectly by electrolytes leakage test (Ismail *et al.*, 2000; Thiaw and Hall, 2004). Lipids are also the structural elements responsible for the selective permeability membranes. Therefore, the maintenance of these molecules physical and

chemical properties renders the biological membranes functional (Liljenberg *et al.*, 1992). The level of lipid peroxidation has been used as an indicator of free radical damage to cell membrane s under stress conditions. MDA is a product of peroxidation of unsaturated fatty acids in phospholipids and is responsible for cell membrane damage (Halliwell and Gutteridge, 1989). A decrease in the unsaturation degree means a cellular membranes rigidity increase (Monteiro de Paula *et al*., 1993; Costa Oliveira, 2001).Electrolyte leakage is influenced by plant/tissue age, sampling organ, developmental stage, growing season, degree of hardening and plant species. The electrolytes leakage test can evaluate damages shown by the cellular membranes, where ions flow raise occurs in consequence of a larger cellular membranes permeability (Ismail *et al.,* 1997).

Materials and Methods
Seed germination and stress conditions

Seeds sterilized with hypochloride sodium (5%), then treated by boiling water for (0, 2, 5, 10, 15 and 20) second and germinated in growth chamber at 25±1 ºC and relative humidity of 60-70% in darkness for 7 days. Then all biochemical analyses were assayed.

Evaluation of seed germination, coefficient of germination and germination vigor index

Germination percentage of seeds was determined after 7 days. Seeds are considered germinated when the radicle emerged from the testa. The percentage of germination (%G) was equal to:

$$\%G = \frac{NSG}{NST} \times 100$$

NSG: Number of germinated seeds
NST: Total number of tested seeds

Co-efficcent of germination$=(\dfrac{A_1 + A_2 + ... + A_n}{A_1T_1 + A_2T_2 + ... + A_nT_n}) \times 100$

Vigor index$=\dfrac{A_1}{T_1} + \dfrac{A_2}{T_2} + ... + \dfrac{A_n}{T_n}$

A: Number of seeds germinated, **T:** Time (days)

Malondialdehyde content estimation

Lipid peroxidation level was determined in terms of malondialdehyde (MDA) content by the method of (Zacheo *et al*., 2000) as follow:
1. 1 gm was crushed in a mortar with 20 ml trichloroacetic acid (TCA 50 g/L) to pericipate proteins.
2. The extract centrifuged, then 1 ml of extract was taken and added 1ml of thiobarbituric acid (TBA 5 g/L).
3. The mixture was heated in a water bath at 95 C for 30 min., cooled to room temperature.
4. The absorbance at 532 nm was determined. The MDA content was calculated by the extinction coefficient of 153m mol/L/cm.

Protein content estimation

1 g fresh weight was crushed in a cold mortar with a pestle. Total proteins were extracted and estimated according to method of (Bishop *et al*., 1985). using albumin as the standard. The absorbance of protein samples was measured at 555 nm using a spectrophotometer.

Proline determination

Proline content was measured as described by Bates *et al.* (1973) as follows:-
1. Dried sample was crushed in a mortar with a pestle.
2. 10 ml sulfosalicylic acid solution was added to each tube containing 0.1 g of the dried sample.
3. 1 ml of the extract was reacted with 1 ml glacial acetic acid and 1 ml ninhydrin (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until resolved) in a water bath (100°C) for an hour.
4. The reaction was terminated in an ice bath to stabilize the purple color of the extract.
5. 0.2 ml toluene was added to each tube and the absorbance of top purple aqueous layer was measured at 520 nm in a spectrophotometer.
6. The concentration of proline samples was determined according to the standard curve plotted with known concentrations of L-proline.

Membrane Resistance Test

The cellular membranes integrity was measured through (1 gm) of seeds put in 20 ml distilled water in the test tube and kept in the darkness (to prevent electrolytes loss induced by the light). The seeds were washed three times with the intervals of 15 minutes in order to eliminate the electrolytes adsorbed on seeds surface. After the last wash, the seeds were left in repose for an hour at 25ºC in the darkness. The electric conductivity (EC) was measured during a period of 1 h by EC-meter.

Statistical analysis

Statistical analyses of the data was performed using the Completely Randomized Design (CRD). Least significant difference test (LSD) was used to test for the significance of the differences among means at $P < 0.05$ (Glantz, 2005).

Results and Discussion

The percentage of germination, co-efficient of germination and germination vigour index of *Triticum* seeds are presented in Table 1. Results showed that all the three germination characteristics were influenced by the high temperature. Results showed a signficant decrease in germination percentage of seeds treated with heat. The seed germination was (90, 58.33, 25, 5 and 5)% when seeds treated with high temperature for (2, 5, 10, 15 and 20) sec. respectively is compared to control (100)%. The coefficient showed no signficant decrease between different treatments (Table-1). It was (83, 72.3, 70.3, 66.6 and 44) % for seeds treated by heat for (2, 5, 10, 15 and 20) sec. respectively as compared to (90.3)% in untreated seeds (control). Whereas germination vigor index showed an decreasing trend as exposure period increase to temperature. It was (16.1, 6.8, 3.2, 0.6 and 0.7) for seeds treated by boiling water at (2, 5, 10, 15 and 20) seconds respectively as compared to control (19).

Table (1): Effect of heat stress on germination percentage (%), co-efficient of germination (%), germination vigor index of *Triticum sativum* L. seeds.

Parameters / Exposure period	Germination percentage (%)	co-efficient of germination (%),	germination vigor index
Control (untreated seeds)	100	90.3	19.0
2 second	90.0	83.0	16.1
5 second	58.3	72.3	6.8
10 second	25.0	70.3	3.2
15 seconds	5.0	66.6	0.6
20 seconds	5.0	44.0	0.7
LSD (0.05)	**4.572**	**N.S**	**0.291**

The effect of heat stress on malondialdehyde (a final product of lipid peroxidation) content is shown in Figure (1). The MDA content was signficantly higher in heat-stressed seeds than in control seeds. The MDA content was (2.8, 3.1, 3.6, 3.9, 4.7) µmol/gm.F.W in seeds treated for (2, 5, 10, 15 and 20) respectively sec by heating as compared to control (1.7) µmol/gm.F.W.
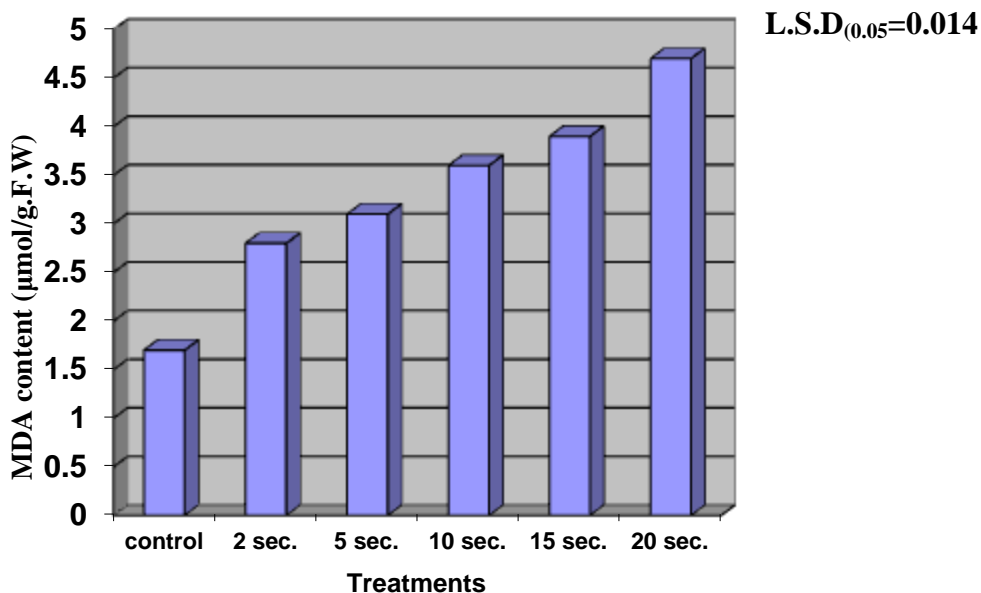

Figure (1): Effect of heat stress on MDA content (µmol/g.F.W) in *Triticum sativum* L. seeds

Figure (2) showed a signficant decrease in protein content of seeds treated with heat compared to control. The protein content progressively decreased with increase of exposure period to heat stress. The protein content was (13.6, 11.8, 11.9, 10.3 and 7.9) mg/g.F.W in seeds exposed for (2, 5, 10, 15 and 20) sec in boiling water respectively as compared to control (32.4) mg/g.F.W.
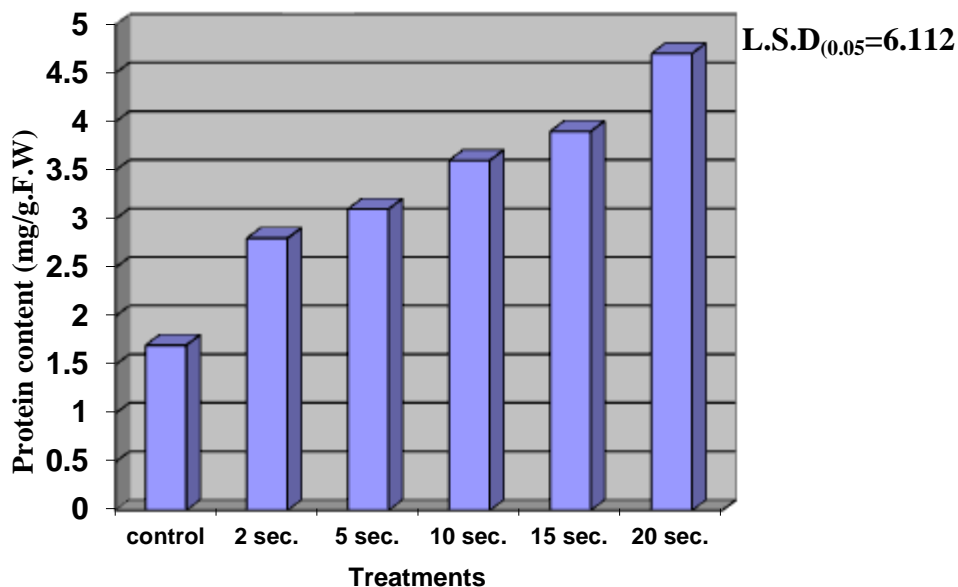
Figure (2): Effect of heat stress on protein content (mg/g.F.W) in *Triticum sativum* L. seeds

The proline content of seeds is shown in figure (3). Proline progressively increased significantly with exposure period to heat stress. Proline concentration was (5.4, 8.2, 8, 9.1 and 9) μg/g.D.W in seeds exposed for (2, 5, 10, 15 and 20) second respectively, as compared to control (4.6) μg/g.D.W.
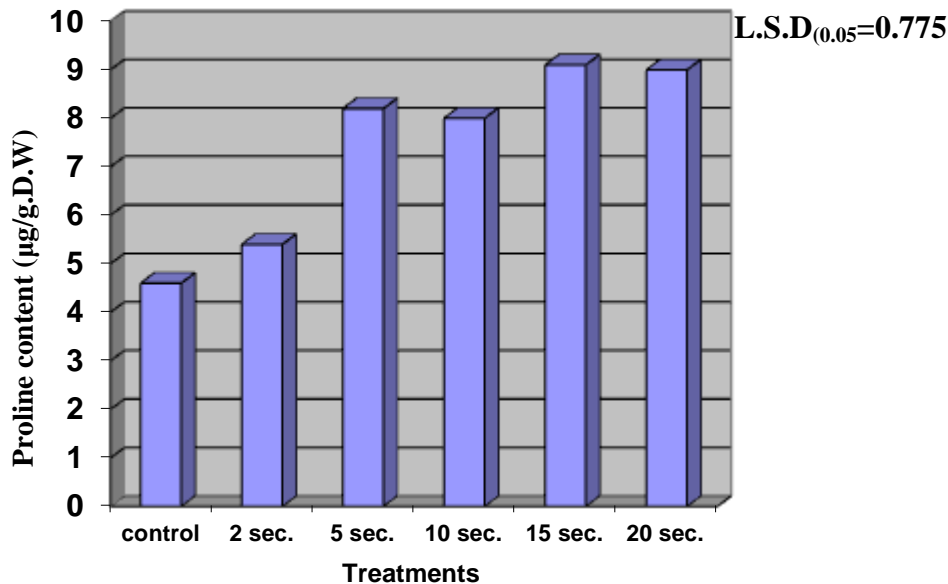

Figure (3): Effect of heat stress on proline content (μg/g.D.W) in *Triticum sativum* L. seeds

The electrolyte leakage (EL) of *Triticum* seeds increased under heat stress (as indicator on cellular membranes damage) (Figure-4). EC was (26.7, 28.5, 28.6, 30.4 and 33.9) μs/cm for seeds which treated with high temperature at (2, 5, 10, 15 and 20) seconds respectively as compared to control (17.9) μs/cm.

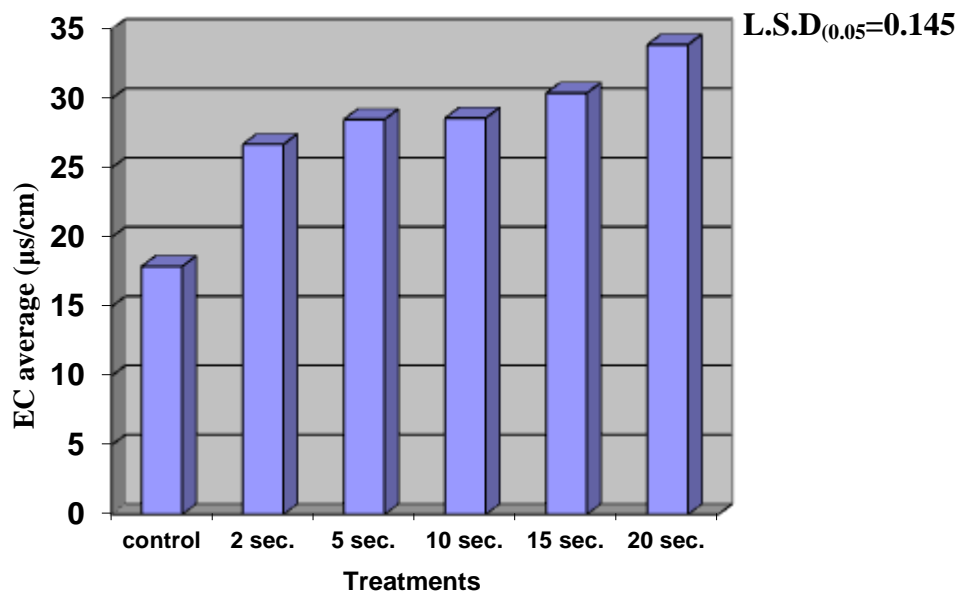Figure (3): Effect of heat stress on electrolye leakage (µs/cm) in *Triticum sativum* L. seeds

Discussion

Many physiological changes under high temperature stress may result in poor plant growth and productivity. Seed germination and co-efficient germination, germination vigor index declined because of the high heat (Table-1). Heat stress is a major factor affecting the rate of plant development (Hall, 1992; Marcum, 1998; Howarth, 2005), by its effect on the organization of microtubules by splitting and/or elongation of spindles, formation of microtubule asters in mitotic cells, and elongation of phragmoplast microtubules (Smertenko *et al*., 1997). These injuries eventually lead to starvation, inhibition of growth, reduced ion flux, production of toxic compounds and reactive oxygen species (ROS) (Schöffl *et al.,* 1999; Howarth, 2005), and affected by limiting carbohydrate accumulation (Howard and Watschke, 1991); and can damage cell membranes (Marcum, 1998), may be related to oxidative damage to cell membranes by active oxygen species (Bowler *et al.,* 1992; Zhang and Kirkham, 1994), leading to cell death (Abernethy *et al.,* 1989), and affecting on membrane resistance through loosening chemical bonds within molecules of biological membranes. Direct alterations due to high temperatures include protein denaturation and aggregation, therefore protein content declined because heat stress (Figure-2). Decline of protein content may be due to the inhibition of protein synthesis or protein degradation (Howarth, 2005). The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins. Such alterations enhance the permeability of membranes (Savchenko *et al*., 2002), as evident from increased loss of electrolytes (Figure-4). Zhang *et al.* (2005) pointed out that heat stress severely damaged the mesophyll cells and increased permeability of plasma membrane. Savchenko *et al*. (2002) mentioned that heat stress accelerates the kinetic energy and movement of molecules across membranes. This makes the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids, the lipids destroyed by lipid peroxidation process (MDA as a final product) (Figure-1). In *Arabidopsis* plants grown under high temperature, total lipid content in membranes decreased to about one-half and the ratio of unsaturated to saturated fatty acids decreased to one-third of the levels at normal temperatures (Somerville and

Browse, 1991).Proline progressively decreased with increase exposure period to heat (Figure-3). It is also known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kavi Kishore *et al.*, 2005). In assessing the functional significance of accumulation of compatible solutes, it is suggested that proline synthesis may buffer cellular redox potential under heat and other environmental stresses (Wahid and Close, 2007).

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التغيرات في تحمل الاغشية الخلوية بسبب الاجهاد الحراري خلال أنبات بذور الحنطة *Triticum sativum* L.

يعد الاجهاد الحراري بسبب زيادة درجة الحرارة مشكلة كبيرة في العديد من الاراضي في العالم. كان موضوع الدراسة فحص التغيرات في انبات البذور، ثباتية الاغشية، عملية أكسدة الدهون وفقدان الالكتروليتات في البذور النابتة والمعرضة للاجهاد الحراري. أنخفضت نسبة الانبات ومعامل سرعته معنوياً بسبب الحرارة العالية مقارنة بمجموعة السيطرة. أزداد محتوى المالونداي ألديهايد معنوياً، في حين أنخفض محتوى البروتين معنوياً. يسبب الاجهاد الحراري زيادة معنوية في محتوى البرولين. وأزداد مستوى الالكتروليتات (كمؤشر على الاضطراب الحاصل في نفاذية الاغشية الخلوية) بشكل معنوي مع زيادة فترة التعرض للحرارة العالية.