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Enhancing thermotolerance of tomato plants (*Lycopersieon esculentum* mill) by heat hardening of seeds

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Abstract

Background: High temperature is a crucial problem in growing good crops of high temperature-sensitive vegetables including tomato. Therefore, this study was carried out to investigate the effects of pre-sowing heat treatments of tomato seeds on germination, growth, and biochemical changes of the plants grown under high temperature stress.

Material and methods: The study included two experiments, experiment (I) dealt with the effect of pre-sowing heat hardening of tomato (*Lycopersiecon esculentum* Mill cv Marmand VF) for different periods of soaking at 25 °C for 5 min, 3 h and 6 h before exposing to 50 °C, 60 °C, and 70 °C for 0.5 h, 1 h, and 2 h.

Results: Best results represented by germination parameters were obtained by soaking the seeds for 5 min before exposure to 50 °C, 60 °C, and 70 °C for 0.5 h, 1 h, and 2 h. According to the germination data, these treatments were chosen to study their effect on vegetative growth as well as some biochemical parameters (Experiment II). The study showed that seed hardening increased growth criteria expressed by stem length, number of branches, number and area of leaves, and fresh and dry weight of shoots. The same treatments increased photosynthetic pigments, i.e., (chlorophylls "a" and "b") and carotenoids of tomato leaves as well as the studied chemical constituents of shoots (reducing sugars, sucrose, amino acids, proline and proteins, as well as nucleic acids and saturated fatty acids). Maximum response was attained by treatment with 60 °C for 1 h and 2 h.

Conclusion: Thus, these treatments can help the plant to cope with the adverse effects of high temperature prevailing during their growth stage.

Keywords: Tomato, Seed hardening, High temperature stress, Growth, Metabolism

Introduction

Tomato (*Lycopersicon esculentum* mill) is one of the widely grown vegetable crops. It is an important crop for human consumption.

It is usually cultivated in the open field in Saudi Arabia during September as the prevailing temperature is suitable for growth and development of the plant. Tomato plants grow rapidly so extending the season and increasing continuity of supply can be achieved by sowing the seeds during spring. However, the plants exhibited to high temperature during their growth and development.

High temperature is a crucial problem in growing good crops of high temperature-sensitive vegetables including tomato. It impairs different morphological criteria (Khalil and Moursy 1983 and Warrag 1999). High temperature affects wide spectrum of both biochemical and physiological response within the plant cell. These results are expected and described by many researchers especially in the case of growing organs, since all the reaction in the plant already take place rapidly and further rise in temperature might easily disturb the balance (Fisher 1980). Other investigators reported that extreme and variation of high temperature can damage the intermolecular interactions needed for growth (Bita and Gerats 2013).

Protection of plants from high temperature stress can be achieved by several mechanisms; one of these

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mechanisms is seed hardening; it is a physiological seed enhancement method (Taylor et al. 1998). Seed hardening can be achieved by pre-sowing treatments in which the seeds are soaked in water, an osmotic solution or growth regulators, as well as exposing the seeds to elevated temperature above the maximum temperature prevailing during their growth. These treatments allow the seeds to go to the first stages of germination but not permit radicle protrusion through the seed coat (Heydeker, 1977). Planting hardened seeds in the field giving the plant a better start than the non-hardened plants. Thus, hardened plants might survive adverse environmental stresses like high temperature more easily because of the advanced state of development.

The present study is an additional contribution for understanding the effects of pre-sowing heat treatments of tomato seeds on germination, growth, and biochemical changes of the plant grown under high temperature stress (30–40 °C).

Material and methods

The study comprised of two experiments; the planning of the second experiment depended on the results of the first one according to the following order.

Experiment I: germination tests

Uniform seeds of tomato (*Lycopersicon esculentum* Mill cv. Marmand VF) were soaked in water at 25 °C for 5 min, 3 h, and 6 h; dried quickly within two layers of filter paper; and each group was divided to two sets. The first set from each group was weighed then dried at 105 °C till constant weight according to Hart and Neustadt (1957) to measure moisture content that reached 20.6%, 44.33%, and 63.52% respectively. The second set from each group was subjected to 50, 60, and 70 °C for 0.5 h, 1 h, and 2 h. The seeds were germinated at 25 °C in Petri dish each containing 20 seeds.

Ten replicates were allotted for each treatment as well as control treatment (seeds soaked for 0.5 h or 1 h or 3 h at room temperature (25 °C)). Germination was recorded for 8 days, then germination data were recorded as follows: germination capacity, radicle and hypocotyl length, as well as radicle fresh weight and seedling fresh weight.

Experiment II

The previous experiment results indicated that the best results were obtained in seeds soaked for 5 min (moisture content 20.6%) and exposed to 50 °C, 60 °C, and 70 °C for 0.5 h, 1 h, and 2 h as well as seeds soaked for 5 min only as (control). These treatments were chosen to study their effect on vegetative growth and some biochemical parameters.

Growing technique and sampling

Tomato seeds were sown on March in JV (seven) cubes; when the seeds attained 30 days, they were transferred to 30 cm in diameter pots filled with equal amounts of soil (consisted of clay + petmoss + perlite at the ratio of 1: 3: 5 (*w*/*w*)). Fertilization was applied as the recommended dose (5 g superphosphate, 10 g mixture of ammonium sulphate and potassium sulphate at the ratio of 3:2) for each pot.

The plants were supplied with water according to their requirements which was governed by climatic conditions. The experiment was carried out doors in the screen of Girls College of Science, Damman, Saudi Arabia for two successive seasons. The maximum and minimum temperature as well as relative humidity are shown in Table 1.

Samples were collected at random 30 days after transplanting (DAT). Each treatment is divided into three replicates for recording vegetative characters as well as chemical analysis.

Biochemical analysis

Photosynthetic pigments [chlorophyll (a), chlorophyll (b), and carotenoids (car)] were determined in fresh leaves (Metzner et al. 1965).

The following parameters were estimated in dry shoots at 70 °C

Reducing sugars, sucrose, and polysaccharides were measured according to (Dubios et al. 1956). Total amino acids were analyzed according to Boulter and Barber (1963). Protein extraction followed Anderson and Beardal (1991) and estimated as described by Lowry et al. (1951).

Proline, nucleic acids, and fatty acids were determined in fresh shoots. Proline was measured according to Troll and Lidsley (1955). Nucleic acids were extracted according to Schmidt and Thaunhauser (1945), whereas ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were estimated according to the method of Winzler (1955) and Burton (1956), respectively.

Fatty acids were analyzed through four successive steps: (1) extraction by petroleum ether 60–40 °C, (2) saponification with NaOH (20%), (3) methylation by methyl alcohol, and (4) identification by GLC (Varian Model 6000 chromatography). The GLC condition was as follows: THE glass column filled with (15% DEGS). The column oven temperature was programmed at 6 °C/min from 80 to 130 °C and kept finally for 25 min. Injector and detector temperature were 220 °C and 260 °C, respectively. Gases flow rates were 30, 30, 300 cm/min for N_2, H_2, and air, respectively. The flow rate inside the column was adjusted to 1 ml/ min.

Table 1 Average of monthly maximum and minimum air temperature and relative humidity during the two season of study

Month	First season				Second season			
	Temperature		Relative humidity %		Temperature		Relative humidity %	
	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
March	30	10	95	17	30	11	95	20
April	38	14	85	10	36	14	95	17
May	43	16	98	3	44	18	90	8

Statistical analysis

The data of the first experiment was subjected to statistical analysis according to analysis of variance with interaction. The values of least significant difference (LSD) were calculated at 5% level of probability.

The data of the second experiment was arranged in complete randomized design. The obtained data were statistically analyzed according to Duncan (1955) at probability 5%. Mean value followed by the same letter within each column are not significant.

By applying the Steel and Torrie (1960) test, the results showed the same trend. Therefore, the combined analysis of the two seasons was calculated. Combined analysis of the two seasons was calculated after Steel and Torrie (1960) as the results obtained showed the same trend.

Results

Germination percentage (Table 2) showed the optimum values as the seeds soaked for 5 min, 3 h, and 6 h at room temperature compared to the other treatments at the different degrees (50, 60, and 70 °C). Moreover, these treatments decreased germination percentage by increasing degree of temperature from 50 → 60 → 70 °C as well as prolonging the period of soaking from 5 min → 3 h → 6 h. The lowest value of germination percentage was obtained by soaking the seeds for 3 h or 6 h before exposure for 70 °C for any period of exposure. Thus, the seeds did not tolerate the high percentage of moisture content concomitant to high temperature.

Results in Table 3 revealed the interaction of the soaking period of the seeds and period of exposure to different degrees of temperature on radicle length; their length significantly increased by exposing the seeds to 50 °C or 60 °C for all exposure periods after soaking the seeds for 5 min. However, soaking the seeds for 6 h before exposing to 70 °C at all used periods decreased significantly the length of radicle as compared to control. Hypocotyl length significantly increased by soaking tomato seeds for 5 min before exposing to the different used periods at 50 °C. On the other hand, increasing period of soaking to 3 h and 6 h accompanied with elevating the exposure temperature to 60 °C or 70 °C significantly decreased hypocotyl length. Maximum significant decrease was reached by soaking the seeds for 3 h or 6 h before exposing to different periods at 70 °C (Table 2).

Radicle fresh weight did not show any significant increase due for treatments. In addition, significant decrease was obtained by soaking the seeds for 3 h or 6 h before exposing to 60 °C or 70 °C for all exposure periods (Table 3).

Table 2 Effect of seed hardening on germination percentage, radicle length (cm), and hypocotyl length (cm)

Treatment		Germination %			Radicle length (cm)			Hypocotyl length (cm)		
Temp (°C)	Exposure period (h)	Period of soaking			Period of soaking			Period of soaking		
		5 min	3 h	6 h	5 min	3 h	6 h	5 min	3 h	6 h
Control		99.8	99.5	99.4	4.17	4.37	4.30	11.90	12.00	11.13
50	0.5	99.4	95.4	96.1	5.13	4.80	4.60	13.03	12.40	11.63
	1	98.0	92.8	92.7	5.33	5.03	4.50	12.65	11.40	11.17
	2	97.8	89.2	80.5	4.67	4.63	4.93	13.03	12.07	11.70
60	0.5	99.6	77.4	74.0	5.23	4.57	4.70	11.80	12.20	10.13
	1	97.4	75.2	61.0	5.47	4.23	4.23	12.23	11.80	10.17
	2	95.6	68.8	61.5	5.47	4.20	4.17	12.03	11.50	10.13
70	0.5	91.8	55.4	52.2	4.70	4.07	3.53	12.13	10.50	10.13
	1	84.9	51.5	50.4	4.63	3.90	3.10	11.70	8.50	8.63
	2	85.5	49.9	50.6	4.66	3.20	3.13	10.73	8.13	7.47
L.S.D of interaction		–			0.49			0.72		

Table 3 Effect of seed hardening on radicle fresh weight (g/plant) and seedling fresh weight (g/plant)

Treatment		Radicle fresh wt. g/plant			Seedling fresh wt. (g/plant)		
Temp (°C)	Exposure period (h)	5 min	3 h	6 h	5 min	3 h	6 h
Control		0.001	0.011	0.012	0.081	0.080	0.080
50	0.5	0.012	0.011	0.012	0.085	0.077	0.077
	1	0.012	0.011	0.011	0.087	0.075	0.077
	2	0.012	0.011	0.011	0.082	0.072	0.072
60	0.5	0.012	0.010	0.011	0.087	0.073	0.074
	1	0.012	0.009	0.010	0.088	0.074	0.072
	2	0.012	0.009	0.009	0.083	0.070	0.069
70	0.5	0.011	0.009	0.008	0.084	0.055	0.051
	1	0.011	0.008	0.008	0.082	0.056	0.051
	2	0.011	0.007	0.007	0.082	0.055	0.050
L.S.D of interaction		0.001			0.003		

Seedling fresh weight exhibited significant increase by soaking the seeds for 5 min before exposing to 50 °C or 60 °C for 0.5 h or 1 h. However, prolonging the period of soaking to 3 h and 6 h before exposing the seeds to different temperature degrees for all used periods of exposure (0.5 h, 1 h, and 2 h) showed opposite trend as all treatments decreased fresh weight of seedling (Table 3).

Growth responses

The studied parameters of growth were shown in Table 4. Stem length of tomato plants showed increase by exposing the seed for 60 °C or 70 °C. Significant increments were attained by treatment 60 °C for 2 h or 70 °C for 0.5 h.

Number of branches significantly increased by all treatments seeds for 50 °C for 0.5 h, and the highest number of branches was obtained by treatment 60 °C as well as treatment 70 °C for 0.5 h. Maximum significant increase was attained by exposing the seeds to 60 °C for 1 h or 2 h.

Number of leaves significantly increased by treatment 50 °C for 1 h or 2 h and 60 °C for all periods of exposure.

Area and fresh weight of leaves increased significantly by all treatments with the exception of seeds treatment 70 °C for 2 h. Maximum increase attained by treating the seeds for 2 h at 60 °C. Dry weight of leaves followed almost the same trend but increase with the treatment 50 °C for 0.5 was not significant (Table 4).

Shoot fresh weight showed significant increase with all treatments except treating the seeds at 70 °C for 2 h.

Shoot dry weight followed the same pattern of changes shown in case of fresh weight. Meanwhile, the increase with treatment 50 °C for 0.5 h was not significant (Table 4).

Biochemical analyses

Results recorded in Table 5show the effect of hardening on photosynthetic pigments and the studied carbohydrate fractions. Chl (a) significantly increased with all treatments except those exposed to 50 °C for 0.5 h. The maximum increase was given by exposing the seeds to 60 °C for 2 h followed by treatments 60 °C or 70 °C for 1 h. Chl (b) content significantly increased with treatment 60 °C for 2 h and 70 °C for all used exposure periods. Total chlorophylls (a + b) showed significant increase by all hardening treatments except 50 °C or 60 °C for 0.5 h.

Carotenoids content showed significant increase with all treatments except 50 °C for 0.5 h. The highest value was obtained by exposing the seeds to 60 °C for 1 h or 2 h.

Carbohydrate fractions showed that direct reducing sugars were significantly increased for all treatments except the treatment 50 °C for 0.5 h and 60℃ for 1 h or 2 h.

Sucrose content followed more or less the same trend of reducing sugars with all treatments except the

Table 4 Effect of seed hardening on vegetative growth

Treatments		Parameters							
Temp. (°C)	Exposure period (h)	Stem length (cm)	No. of branches/plant	No. of leaves/plant	Leaf area (cm^2/plant)	Leaves fresh wt. (g/plant)	Leaves dry wt. (g/plant)	Shoot fresh wt. (g/plant)	Shoot dry wt. g/plant
Control		36.5^{b}	0.67^{d}	6.33^{c}	199.27^{e}	6.01^{g}	0.691^{d}	11.76^{e}	1.41^{ef}
50	0.5	35.1^{b}	0.67^{d}	6.67^{c}	229.10^{bc}	6.51^{f}	0.714^{cd}	12.52^{d}	1.44^{de}
	1	36.0^{b}	1.00^{c}	7.67^{ab}	238.70^{b}	6.76^{ef}	0.743^{bc}	13.20^{c}	1.48^{cd}
	2	37.7^{b}	1.33^{b}	8.33^{b}	239.70^{b}	7.18^{cd}	0.779^{b}	13.53^{c}	1.51^{bc}
60	0.5	36.5^{b}	1.00^{c}	7.67^{c}	215.90^{cd}	7.02^{de}	0.764^{b}	13.69^{c}	1.49^{cd}
	1	36.9^{ab}	1.00^{c}	8.00^{c}	287.40^{a}	7.65^{ab}	0.764^{b}	14.79^{b}	1.51^{bc}
	2	39.3^{a}	1.67^{a}	8.33^{a}	279.00^{a}	7.95^{a}	0.829^{a}	15.77^{a}	1.63^{a}
70	0.5	39.0^{a}	1.33^{b}	7.67^{b}	252.0^{b}	7.46^{bc}	0.774^{b}	14.78^{b}	1.54^{b}
	1	37.4^{ab}	1.00^{c}	7.00^{c}	220.00^{cd}	7.73^{ab}	0.723^{c}	13.74^{c}	1.47^{cd}
	2	37.1^{ab}	1.00^{c}	6.67^{c}	208.30^{de}	6.10^{g}	0.683^{d}	12.05^{de}	1.36^{f}
L.S.D at 5%		2.40	0.25	0.88	15.31	0.883	0.031	0.53	0.05

Table 5 Effect of seed hardening on photosynthetic pigments (mg/g fresh weight of leaves) and carbohydrate content of shoots (mg/glucose/g dry wt.)

Treatments		Parameters						
Temp. (°C)	Exposure period (h)	Ch1a	Ch1b	Ch1 a + b	Carotenoids	Reducing sugars	Sucrose	Polysaccharides
Control		0.563^{e}	0.225^{d}	0.788^{fg}	0.217^{f}	18.26^{e}	32.11^{d}	137.90^{a}
50	0.5	0.565^{e}	0.210^{e}	0.785^{g}	0.222^{ef}	18.01^{e}	30.92^{d}	140.73^{a}
	1	0.580^{d}	0.232^{cd}	0.812^{e}	0.231^{de}	24.43^{b}	36.35^{b}	125.62^{c}
	2	0.603^{c}	0.230^{cd}	0.833^{d}	0.238^{d}	22.37^{cd}	34.55^{c}	112.06^{d}
60	0.5	0.581^{d}	0.226^{d}	0.807^{ef}	0.239^{d}	22.37^{cd}	38.07^{b}	114.14^{d}
	1	0.642^{b}	0.230^{cd}	0.872^{bc}	0.299^{a}	27.14^{a}	46.14^{a}	89.96^{f}
	2	0.683^{a}	0.250^{a}	0.933^{a}	0.308^{a}	28.22^{a}	44.92^{a}	103.15^{e}
70	0.5	0.611^{c}	0.248^{ab}	0.883^{b}	0.285^{b}	22.55^{c}	38.22^{b}	112.36^{d}
	1	0.642^{b}	0.241^{bc}	0.859^{c}	0.268^{c}	21.06^{d}	34.57^{c}	113.20^{d}
	2	0.617^{c}	0.238^{bc}	0.855^{c}	0.262^{c}	22.14^{cd}	31.91^{d}	131.09^{b}
L.S.D at 5%		0.014	0.011	0.020	0.009	1.32	1.76	5.07

treatment 70 °C for 2 h; treatment 60 °C for 1 h or 2 h resulted in the highest significant increase.

Polysaccharides content clearly showed opposite trend to reducing sugars and sucrose. Significant decrease recorded for all treatments except for treatment 50 °C for 0.5 h. Maximum decrease was recorded by treatment 60 °C for 1 h followed by 2 h (Table 5).

Table 6 shows the changes of proline, amino acids, protein, as well as nucleic acids RNA and DNA. Proline content exhibited significant increment, the exception for all treatments except for the treatment 50 °C for 0.5. Maximum increase was obtained by the treatment 60 °C for 1 h followed by 2 h.

Amino acids significantly increased with all treatments 60 °C or 70 °C. Highest value of increase was obtained by 60 °C for 2 h as well as 70 °C for 1 h.

Protein content exhibited significant increments by all treatments with the exception of two treatments 50 °C or 60 °C for 0.5 h. The highest value of significance was obtained by the treatment 60 °C for 1 h. Followed by treatment for 2 h.

Nucleic acid (RNA) recorded significant increase for most treatments except 50 °C for 0.5 h as well as 70 °C for 1 h or 2 h. Meanwhile, maximum significant increase was recorded by treatment 60 °C for 1 h or 2 h. DNA content showed significant increase by the following treatments: 50 °C for 1 h, 60 °C for 1 h or 2 h, and 70 °C for 1 h (Table 6).

Table 7 shows the effect of heat hardening of tomato seeds on the percentage of fatty acids. Analysis shows increase of myristic acid percentage, while it shows decrease of palmitic oleic and palmitoleic percentage. Total saturated fatty acids percentage was increased obviously compared to untreated plants. Maximum increments were recorded by treatment 60 °C for 2 h.

Table 6 Effect of seed hardening on proline, free amino acids, RNA, and DNA content of plant shoots

Treatments		Parameters				
Temp. (°C)	Exposure period (h)	Proline μg/g fresh wt.	Amino acids mg/g dry wt.	Proteins mg/g dry wt.	RNA mg/g fresh wt	DNA mg/g fresh wt.
Control		41.22^{g}	7.01^{ef}	29.21^{e}	1.01^{d}	0.211^{c}
50	0.5	42.22^{g}	6.61^{fg}	28.38^{e}	0.979^{d}	0.211^{c}
	1	44.77^{f}	7.22^{e}	30.00^{de}	1.07^{c}	0.219^{a}
	2	47.25^{e}	7.43^{de}	30.95^{cd}	1.10^{c}	0.214^{bc}
60	0.5	49.15^{c}	6.40^{g}	28.55^{g}	1.17^{b}	0.215^{abc}
	1	62.14^{b}	8.11^{bc}	32.04^{c}	1.23^{a}	0.218^{ab}
	2	64.48^{a}	9.52^{ab}	36.05^{a}	1.19^{ab}	0.218^{ab}
70	0.5	49.33^{e}	7.95^{cd}	33.93^{b}	1.09^{c}	0.217^{ab}
	1	55.15^{c}	9.92^{a}	31.45^{c}	1.01^{d}	0.211^{c}
	2	52.14^{d}	9.18^{b}	31.52^{c}	0.966^{d}	0.212^{c}
L.S.D at 5%		2.12	0.56	1.37	0.050	0.004

Table 7 Effect of seed hardening on percentage of fatty acids in plant shoots

Treatment	Percentage of saturated and unsaturated fatty acids[a]											Percentage of total saturated fatty acids
Temp. °C	Exposure period (h)	C12:0	C14:0	C14:1	C16:0	C16:1	C16:2	C18:0	C18: 1	C18:2	C18:3	
Control		0.69	1.32	–	36.61	31.61	1.45	7.26	16.88	–	1.86	45.88
50	2	0.29	58.70	–	12.85	4.99	0.93	0.97	13.82	–	3.36	72.81
60	1	2.81	50.64	5.07	7.00	11.45	0.64	10.26	1.51	–	2.38	70.71
60	2	0.07	67.43	3.14	8.21	4.47	1.23	3.89	3.98	1.82	1.10	79.60
70	0.5	0.30	57.94	–	11.31	11.5	0.32	0.34	10.60	3.10	1.58	69.88

[a]C12:0 lauric acid, C14: 0 myristic acid, C14: 1 myristoleic acid, C16:0 palmitic acid, C16:1 palmitoleic acid, C16:2 palmitolenic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, and C18:3 linolenic acid

Discussion

High temperature is considered one of the most important environmental factors that affect plant growth. It is the most influential factor which induces an increase of plant evaporation demand and indirectly contributes to water deficiency or salt stress (Karim et al. 1998). Seed hardening modulate the physiological and biochemical nature of seeds that lead to induction of the ability of seeds to withstand higher temperature for prolonged period (Sujatha et al. 2013).

It is clear that exposure of seeds to suitable high temperature and period of soaking improved radicle and plumule length as well as seedling fresh weight. However, increase of temperature and period of exposure caused harmful effect (Tables 2 and 3). This results coincide with the findings of other investigators (Farooq et al. 2004; Farooq et al. 2005; Rehman et al. 2014).

Other researchers stated that seed hardening can modify physiological and biochemical characters that enable seeds to tolerate environmental stress and good stand more easily under unsuitable conditions (Matsushima and Sakagani 2013). Increase of radicle and plumule length as well as fresh weight of seedlings indicated many alterations, such as changes within the cytoplasm as hydration of colloids and increase the viscosity and elasticity (Sujatha et al. 2013). Metabolic activity was also suggested by other investigators as soaking of seeds enhancing metabolites (Barsa et al. 2005) and inducing carbohydrate to become ready to be used for cell elongation (Farooq et al. 2006 on rice). All these changes lead to better start and uniform of the seedling that can endure environmental stress (Farahani et al. 2011). Thus, seed hardening stimulates pregermination metabolic process without protrusion of the radicle through the seed coat (Heydeker 1977) and provides faster and synchronized germination (Nawaz et al. 2009).

Improvement of vegetative growth represented by enhancement of branching, increase of number and area of leaves, as well as fresh and dry weight of tomato leaves and shoots indicates generally positive effect. Pre-sowing heat hardening of tomato seeds with 50 °C and 60 °C for 1 h or 2 h showed the highest increments (Table 4). These results may be attributed to healthy germination of seed, which in turn gave the plant better start and induced further growth of tomato seedlings. These results were supported by the findings of other researchers (Khalil and Moursy 1983; Gamal El-Din 1999; El-Moursi et al. 2012) who proved that heat hardening of seeds promoted growth of different plants. In addition, Souza and Devaraj (2013) reported accumulation of biomass in heat acclimated *Dilchos libalab* under heat stress condition.

Photosynthetic pigments (chl a, chb, and carotenoids) were increased by hardening of tomato seeds. Maximum level was attained by seeds treatment with 60 °C for 2 h (Table 5). Lower level of the photosynthetic pigments in control plants reflects the effect of high temperature stress as the ambient temperature above the threshold (43-44). The impairment of chlorophylls accumulation is the first process occurring in the plastids due to high temperature. Anjum et al. (2011) reported that decrease of chlorophylls under drought stress may be a result of pigment photooxidation and chlorophyll degradation. Other researchers attributed decrease of chlorophylls to reduction of their synthesis or acceleration of degradation or combination of both. In support to these finding, Dutta et al. (2009) and Reda and Mandura (2011) showed destruction of numerous enzymes involved in the mechanism of chlorophylls synthesis under high temperature stress. High chlorophylls content due to hardening in the present study improved protection of tomato plants from heat stress as susceptible genotypes showed higher reduction of total chlorophylls than tolerant ones (Gosavi et al. 2014 and Zhou et al. 2017). Moreover, other researchers stated that the accumulation of chlorophylls have been used to characterize the variability of thermotolerance for many crop species (Selvaraj et al. 2011).

Increase of carotenoids accompanied with high level of chlorophylls in treated plants pointed clearly to the effective role of carotenoids in protecting chlorophylls from the damage of singlet oxygen. Carotenoids scavenge them through direct quenching the excited triplet state of chlorophylls molecule and dissipate as a heat (Pallet and Young 1993). Kuczyriska et al. (2012) reported that xanthophylls play a key role in minimizing the over oxidation

in higher plants. Thus, tomato seed hardening increased photosynthetic pigments (ch1s and carotenoids of tomato plant), which in turn minimize the damage of light-absorbing efficiency of photosystems (PSI and PSII) (Murkowski 2001; Langium et al. 2006; Souza et al. 2004).

Plants use different strategies to maintain osmotic balance as the synthesis and accumulation of soluble sugars, amino acids, and proline (Shao et al. 2007; Hayat et al. 2012).

Reducing sugars as well as sucrose increased by hardening treatments in the present study especially by 60 °C for 1 h or 2 h treatments. On the other hand, the same treatments showed pronounced decrease of polysaccharides content (Table 5). Other researchers studied the correlation between soluble sugars and polysaccharides in tolerant and sensitive varieties of plants as the tolerant varieties contain high level of soluble sugars specially sucrose concomitant to high activity of sucrose–phosphate synthetase compared to sensitive ones (Kerr et al. 1987, Basu et al. 1991). Previously, Dinar and Rudich (1985) showed increase of sucrose accompanied by decrease of starch in tolerant variety of tomato (Robbin) compared to the sensitive one (Roma). Later, other investigators proved that sucrose has a crucial role in increasing the osmotic potential of stressed cells (Ruan et al. 2010). In addition, Greer and Weston (2010) reported accumulation of total soluble sugars in heat acclimated varieties of *vitis venefera.*

Proline is one of the most important amino acids which were accumulated under stress. The present investigation showed that proline content increased by most of the hardening treatments. Maximum increment was given by 60 °C for 1 h followed by exposure for 2 h. Several studies cleared a good relation between proline and increasing tolerance of plants under environmental stresses. These amino acids have different crucial roles and act as hydroxyl scavenger, stabilization of membranes and protein structure, as sink for carbon and nitrogen for stress recovery, and buffering cellular redox potential under stress (Hayat et al. 2012; Kavikishor and Speenivasulv 2014; Yaish 2015). Moreover, Li et al. (2013) reported inducing of tolerance of maize plants under high temperature stress concomitant to accumulation of proline through P_5C_5 (Δ′ ′ pyrrolidine -5-carbolate synthesis (2.7.2.11)) using hydrogen sulphide. Other researchers reported that accumulation of proline under high temperature stress allows the plants to cope with heat stress (Chakraborty and Tongden 2005; Rasheed et al. 2011).

Worth to mention that heat stress injury involves water deficit and cell turgor as high temperature cause increases in transpiration and in turn these changes lead to water deficit and increments of loss turgidity (Cansev 2012).

Protection against dehydration due to high temperature stress can occur via osmoprotectant (soluble sugars, amino acids, and proline as these metabolites act as stabilizer of cellular membranes and mentain turger) (Farooq et al. 2008). Many researchers proved that tolerant verities of different plants induced osmolytes as soluble sugars and proline under drought and high temperature (Arunkumar et al. 2012; Yingyan et al. 2013; Devi and Sujatha 2014; Solanki and Samangi 2014). Thus, accumulation of soluble sugars and proline are one of the potential biochemical indicators in selecting tolerant cultivars and allowing the plant to cope with heat stress.

Total protein showed significant increase due to heat hardening of tomato seeds treatment 60 °C for 2 h showed the highest level (Table 6). The same observation was recorded by other researchers (Gulen and Eris 2004; He et al. 2005). Worth to be mention that tomato plants in the present study exposed to high temperature during their growth as the ambient temperature reached (30–40°C) (Table 1).

Nucleic acid (DNA and RNA) contents of tomato shoots (Table 6) were increased due to hardening of seeds. Maximum increase was attained by treatment 60 °C for 1 h or 2 h. The promoting effect of heat hardening overcame the impairment of the prevailing high temperature on tomato plants. Other investigators reported decrease of DNA and RNA in wheat plants due to high temperature (Saduk and Orabi 2015). Heat stress injury involves water deficit and cell turgor. Other studies showed decrease of nucleic acid associated with rise of RNase activity under deficient water supply (Mukherjee and Mukherjee 2015).

High temperature stress induces changes of lipid membranes and it increases their fluidity via decreasing their lipid saturation (Horvath et al. 2012). Thus, it is important to increase the saturation of fatty acids for maintaining stability and enhancement of heat tolerance for membranes (Larkindale and Huany 2004).

The present study showed that heat hardening of seeds increased the percentage of saturated fatty acids treatment 50 °C for 2 h as well as treatment 60 °C for 1 h or 2 h increased the ratio of saturated fatty acids to 72.81%, 70.70%, and 79.60%, respectively compared to control.

Therefore, the present data can illuminate that saturation of fatty acids can share in enhancing heat tolerance of tomato plants (Bita and Gerats 2013; Ibrahim and El-Moqadam 2015).

Conclusion

Finally, it can be concluded that heat hardening of tomato seeds with 60 °C for 1 or 2 h could alleviate the harmful effect of high temperature prevailing during tomato plants growth, through the enhancement of their protective parameters such as carotenoids, proline, osmolytes, and saturated fatty acids. Thus, this protective mechanism helped the plants to induce their

tolerance against high temperature stress, which in turn was reflected on their growth.

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SKI preformed the laboratory analysis and wrote the paper and, LAE-M performed the data and coordinated the data collection. Both authors read and approved the final manuscript.

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