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

Sohair K. Ibrahim[1*] and Lulwa A. El- Muqadam[2]

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 responses within the plant cell. These results are expected and described by many researchers especially in the case of growing organs, since all the reactions 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).

* Correspondence: sohair.k@hotmail.com
[1]Department of Botany, National Research Centre, Dokki, Cairo, Egypt
Full list of author information is available at the end of the article

Protection of plants from high temperature stress can be achieved by several mechanisms; one of these 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 radicale protrusion through the seed coat (Heydeker 1977). Planting hardened seeds in the field gives 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 and 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 dishes each contained 20 seeds.

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

Experiment II

The previous experiment results indicated that the best results were obtained and the seeds were then soaked for 5 min (moisture content 20.6%) and exposed to for 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 (7) cubes, and when the seeds were attained 30 days, they were transferred to 30 cm in diameter pots filled with equal amounts of soil (consisted of clay + paet moss + 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 outdoors in the screen of the Girls College of Science, Damman, Saudi Arabia, for two successive seasons. The maximum and minimum temperatures as well as relative humidity are shown in Table 1.

Samples were collected at random 30 days after transplanting (DAT). Each treatment was 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 analysed according to Boulter and Barber (1963). Protein extraction followed Anderson and Beardall (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), and ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were estimated according to the method of Winzler (1955) and Burton (1956), respectively.

Fatty acids were analysed 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 °C to 130 °C and kept finally for 25 min. Injector and detector temperatures were 220 °C and 260 °C, respectively. Gases' flow rates were 30, 30 and 300 cm/min for N2, H2 and air, respectively. The flow rate inside the column was adjusted as 1 ml/min.

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

Month	1st season				2nd 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 the analysis of variance with interaction. The values of the 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 analysed according to Duncan (1955) at the probability of 5%. Mean value followed by the same letter within each column is not significant.

By applying the Steel and Torrie (1966) test, the results showed the same trend. Therefore, the combined analysis of the two seasons was calculated. The combined analysis of the two seasons was calculated after Steel and Torrie (1966) 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 the 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 the period of exposure to different degrees of temperature on the radicale 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 radicale 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 the 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).

Radicale fresh weight did not show any significant increase due to 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, radicale length (cm) and hypocotyl length (cm)

Treatment		Germination %			Radicale 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
LSD of interaction		–			0.49			0.72		

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

Treatment		Radicale 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
LSD 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 an opposite trend as all treatments decreased the fresh weight of the seedling (Table 3).

Growth responses

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

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

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

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

Shoot fresh weight showed a 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 the case of fresh weight. Meanwhile, the increase with treatment at 50 °C for 0.5 h was not significant (Table 4).

Biochemical analyses

Results recorded in Table 5 show the effect of hardening on photosynthetic pigments and the studied carbohydrate fractions. Chlorophylls 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 at 60 °C or 70 °C for 1 h. Chlorophylls b content significantly increased with treatment at 60 °C for 2 h and 70 °C for all used exposure periods. Total chlorophylls (a + b) showed a significant increase by all hardening treatments except 50 °C or 60 °C for 0.5 h.

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}
LSD 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/gm 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}
LSD at 5%		0.014	0.011	0.020	0.009	1.32	1.76	5.07

Carotenoid content showed a 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 at 50 °C for 0.5 h, 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 treatment at 70°C for 2 h, and treatment at 60 °C for 1 h or 2 h resulted in the highest significant increase.

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

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

Amino acids significantly increased with all treatments at 60 °C or 70 °C. The 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 at 60 °C for 1 h followed by the treatment for 2 h.

Nucleic acid (RNA) recorded a 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 at 60 °C for 1 h or 2 h. DNA content showed a 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. The analysis shows an increase of myristic acid percentage, while it shows a decrease of palmitic oleic and palmitoleic

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, ug/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}
LSD at 5%		2.12	0.56	1.37	0.050	0.004

Table 7 Effect of seed hardening on the 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 (hr)	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 nyristoleic 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; C18:3 linolenic acid

percentage. Total saturated fatty acid percentage was increased obviously compared to untreated plants. Maximum increments were recorded by treatment at 60 °C for 2 h.

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 modulates the physiological and biochemical nature of seeds that lead to induction of the ability of seeds to stand higher temperature for a prolonged period (Sujatha et al. 2013).

It is clear that exposure of seeds to suitable high temperature and period of soaking improved radicale and plumule length as well as seedling fresh weight. However, the increase of temperature and period of exposure caused a harmful effect (Tables 2 and 3). These results coincide with the findings of other investigators (Farooq et al. 2004, 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 stand more easily under unsuitable conditions (Matsushima and Sakagami 2013). Increase of radicale 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 a 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 radicale through the seed coat (Heydeker 1977) and provides a faster and synchronized germination (Nawaz et al. 2009).

Improvement of vegetative growth represented by enhancement of branching and increase of number and area of leaves as well as fresh and dry weight of tomato leaves and shoots indicates a generally positive effect. Pre-sowing heat hardening of tomato seeds with 50 °C and 60° 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 a 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 and El-Moursi et al. 2012) who proved that heat hardening of seeds promoted the growth of different plants. In addition, Souza and Devaraj (2013) reported an accumulation of biomass in heat-acclimated *Dilchos libalab* under heat stress condition.

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were increased by the 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 chlorophyll accumulation is the first process occurring in the plastids due to high temperature. Anjum et al. (2011) reported that the decrease of chlorophylls under drought stress may be a result of pigment photooxidation and chlorophyll degradation. Other researchers attributed a decrease of chlorophylls to the 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 chlorophyll synthesis under high temperature stress. High chlorophyll content due to hardening in the present study improved protection of tomato plants from heat stress as susceptible genotypes showed a higher reduction of total chlorophylls than the tolerant ones (Gosavi et al. 2014 and Zhou et al. 2017). Moreover, other researchers stated that the accumulation of chlorophylls has been used to characterize the

variability of thermotolerance for many crop species (Selvaraj et al. 2011).

The 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 directly quenching the excited triplet state of chlorophyll molecule and dissipate as a heat (Pallet and Young 1993). Kuczyriska et al. (2012) reported that xanthophylls play a key role in minimizing the overoxidation 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 and 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 and 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 a pronounced decrease of polysaccharide 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 especially sucrose concomitant to high activity of sucrose–phosphate synthetase compared to sensitive ones (Kerr et al. 1987 and Basu et al. 1991). Previously, Dinar and Rudich (1985) showed an increase of sucrose accompanied by a decrease of starch in a 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 an 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 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 Sreenivasulu 2014 and 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 an accumulation of proline under high temperature stress allows the plants to cope with heat stress (Chakraborty and Tongden 2005 and Rasheed et al. 2011).

It is 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 of 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 maintain turgor (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; Han et al. 2013; Devi and Sujatha 2014 and 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 and treatment at 60 °C for 2 h showed the highest level (Table 6). The same observation was recorded by other researchers (Gulen and Eris 2004 and He et al. 2005). It is worth to mention that tomato plants in the present study were 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 at 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 a decrease of DNA and RNA in wheat plants due to high temperature (Sadak and Orabi 2015). Heat stress injury involves water deficit and cell turgor. Other studies showed a decrease of nucleic acid associated with a rise of RNase activity under deficient water supply (Mukherjee and Mukherjee 2015).

High temperature stress induces changes of lipid membranes; 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 Huang 2004).

The present study showed that heat hardening of seeds increased the percentage of saturated fatty acids treatment at 50 °C for 2 h as well as treatment at 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 and 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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Authors' contributions
SKI preformed the laboratory analysis and wrote the paper, and LAE performed the data and coordinated the data collection. Both authors read and approved the final manuscript.

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The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]Department of Botany, National Research Centre, Dokki, Cairo, Egypt.
[2]Department of Botany, Girls College of Science, Damam University, Damam, Saudi Arabia.

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