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Enhancing the thermotolerance of tomato seedlings by heat shock treatment

Z.Q. YANG[*,**], C. XU[*,+], M.T. WANG[***,#,+], H.L. ZHAO[*], Y.J. ZHENG[*], H.J. HUANG[*], F. VUGUZIGA[*], and M.A. UMUTONI[*]

Collaborative Innovation Center on Forecast and Evaluation of Meteorological Disasters, Nanjing University of Information Science and Technology, Nanjing, Jiangsu Province, China[*]
Binjiang College, Nanjing University of Information Science and Technology, Nanjing, Jiangsu Province, China[**]
Sichuan Meteorological Observatory, Chengdu, Sichuan Province, China[***]
Water-Saving Agriculture in Southern Hill Area Key Laboratory of Sichuan Province, Chengdu, Sichuan Province, China[#]

Abstract

The heat tolerance of tomato seedlings was significantly enhanced after heat shock treatment at 40°C for 4 h. Compared with the control, the heat-shocked tomato seedlings, on one hand, had a higher net photosynthetic rate (P_N), stomatal conductance, intercellular CO_2 concentration, water-use efficiency, maximal quantum yield of PSII photochemistry (F_v/F_m), electron transport rate, actual photochemical efficiency of PSII, and activity of antioxidant enzymes, on the other hand, had lower nonphotochemical quenching, relative conductivity, malondialdehyde content (MDA), and accumulation of reactive oxygen species (ROS). In addition, heat shock induced production of heat shock proteins (HSPs) in tomato seedling leaves. HSP70 was significantly negatively correlated with P_N, F_v/F_m, catalase, superoxide dismutase, and peroxidase and was significantly positively correlated with MDA and ROS. Overall, short-term heat shock treatments, inducing the production of HSPs, helped improve the thermal tolerance of tomato seedlings.

Additional key words: chlorophyll fluorescence; high temperature stress; lipid peroxidation; *Lycopersicon esculentum*; photoinhibition.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important horticultural crops and food preference in China, with the cultivation area reaching about a third of the total area of protected crop cultivation (He *et al.* 2016). It has been reported that tomato plants are sensitive to temperature, especially at vegetative stages, and its optimum growth temperature is between 15 and 32°C (Hatfield and Prueger 2015). The term 'heat stress' is generally used for temperatures above the optimum, which produce irreversible injury to plant growth and development (Hütsch *et al.* 2019). During the summer horticultural cultivation, temperatures tend to reach above 35°C or even above 40°C in a greenhouse. Therefore, high temperature is considered as the major abiotic stress that limits growth and yield of tomato production in the greenhouse cultivation (Kissoudis *et al.* 2015).

Xu *et al.* (2016) showed that tomato plants exposed to temperatures above their normal growth temperature typically synthesize a group of proteins known as 'heat shock proteins' (HSPs). The synthesis of HSPs has been associated with the development of heat tolerance in a wide variety of organisms (Li *et al.* 2011). Keeler *et al.* (2000) found that the expression level of chloroplast HSPs cloned from lima bean (*Phaseolus lunatus* Linn.) is related to the heat tolerance of plants. Many other studies showed that the rate of thermotolerance development is similar to the rate of HSPs accumulation (Lindquist and Craig 1988, Mahat *et al.* 2016). In addition, when the plants return to normal temperatures, the decline in heat resistance is synchronized with the degradation of HSPs (Tedeschi *et al.*

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[+]Corresponding author; e-mail: xuchao@nuist.edu.cn, wangmt0514@163.com
Abbreviations: CAT – catalase; C_i – intercellular CO_2 concentration; E – transpiration rate; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; FM – fresh mass; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; HSPs – heat shock proteins; L_s – stomatal limitation; MDA – malondialdehyde content; NPQ – nonphotochemical quenching; P_N – net photosynthetic rate; POD – peroxidase; ROS – reactive oxygen species; SOD – superoxide dismutase; WUE – water-use efficiency; Φ_{PSII} – actual photochemical efficiency of PSII.
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2016). HSP70, HSP90, and HSP110, the major heat shock proteins, which are highly conserved among plant species, are correlated to the extent of heat stress, and also play an important role in the response to heat tolerance (Usman *et al.* 2014). Lurie and Klein (1991) found out that synthesis of HSPs confers heat tolerance in the tissue in which they were formed, so that subsequent exposure to a higher temperature does not cause damage. However, there are few reports on the synthesis of these HSPs that could increase the heat tolerance of tomato seedlings.

Photosynthesis plays an essential role in plant survival, growth, and production (Liang *et al.* 2018). Key components of the photosynthetic apparatus such as PSII are generally considered to be the most unstable and easily damaged photosynthetic component during a high temperature stress (Camejo *et al.* 2006). Chlorophyll (Chl) fluorescence is an effective probe for photosynthesis and fluorescence parameters, such as maximum quantum yield efficiency of PSII (F_v/F_m), effective quantum yield of PSII photochemistry (Φ_{PSII}), electron transport rate (ETR), and nonphotochemical quenching (NPQ). Chl fluorescence measurement can be used to assess changes of PSII photochemistry, linear electron flux, and CO_2 assimilation *in vivo* (Maxwell and Johnson 2000, Baker 2008). Lu *et al.* (2017) demonstrated that the photosynthetic capacity of tomato plants declines gradually and then sharply with leaf senescence under heat stress. During this process, the cell structure, metabolism, and gene expression of leaf cells undergo reversible or irreversible changes. At the same time, the content of reactive oxygen species (ROS) and lipid peroxidation in leaves increases, which in turn causes photoinhibition (Takahashi and Murata 2008). However, in biological systems, antioxidant enzymes, such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD), produced in different organelles, play an important role in the fight against oxidative stress and help maintain normal cellular components and metabolic functioning (Begara-Morales *et al.* 2016). Therefore, measuring the above indicators can effectively clarify whether the plant's growth physiological activities are affected by high temperature stress.

Until now, the photosystem activity and antioxidant response of tomato plants have been studied in response to high temperature stress (Camejo *et al.* 2005), but studies on enhancement of the thermotolerance of tomato seedlings using heat shock treatment are still rare. Therefore, it was of our interest to see if tomato seedlings exposed to heat shock could protect themselves from high temperature injury. In this paper, the relative expression levels of HSPs in tomato seedlings, induced by heat shock, and the photosynthetic characteristics were investigated after heat stress treatment and recovery period. We believe that this study can reveal new insights in the understanding of mechanisms responsible for heat tolerance in tomato plants.

Materials and methods

Plant material and growth conditions: The experiments were performed from September to October 2018 in the *Venlo*-type glasshouse of the Agricultural Meteorological Experiment Station located in Nanjing University of Information Science and Technology (NUIST), China (32°14'N, 118°42'E). The *Venlo*-type glasshouse, with a north-south length of 30 m, is composed of 12 spans, each 6-m wide in the east-west direction. The height of gutter and ridge was 4 and 4.73 m, respectively. A popular tomato cultivar, 'JinGuan 5' (*Lycopersicon esculentum* Mill. cv. 'JinGuan 5'), was used as plant material. Tomato seedlings planted in plastic pots (pot depth was 18 cm; pot diameter at the top and bottom was 24 and 16 cm, respectively) were provided by *Linyi Yuhong Seedling Planting Co.*, *Ltd.*, China. The pots were filled with loam rich in organic matter with a pH of 6–7, and the soil moisture was always maintained at 60–80%, which were the most suitable conditions for tomato seedlings growth. When ten true leaves appeared on tomato seedlings, healthy and uniform seedlings were selected for the experimental treatment.

Experimental design and treatments: Healthy tomato seedlings with 15-cm height and ten true leaves were transferred into artificial climate chambers (*A1000*, *Conviron*, Canada). One group exposed to 40°C for 4 h as the heat shock group, and another group with a temperature of 25°C for 4 h as the control group. The illumination and relative humidity of the artificial climate chamber were set to 1,000 μmol(photon) m^{-2} s^{-1} and 65%, respectively. The relative expression levels of heat shock proteins (HSP70, HSP90, and HSP110) in both groups were measured.

Both groups were then placed in a climate chamber with the temperature of 38/28°C (day/night, day from 6:00 to 18:00 h) for 48 h and then recovered at the temperature of 25/15°C for 48 h. The photoperiod, illumination intensity, and relative humidity of the climate chamber were set to 12/12 h, 1,000 μmol(photon) m^{-2} s^{-1}, and 65%, respectively. All indicators (photosynthetic parameters, Chl fluorescence parameters, electrolyte leakage, lipid peroxidation, ROS contents, and antioxidant enzymes activity) were measured 4 h after heat shock, 48 h after high temperature stress, and 48 h after recovery. All analyses were conducted in five replicates.

Relative expression levels of heat shock proteins: The HSP70, HSP90, and HSP110 gene sequences of tomato leaves were obtained from NCBI. *PrimerQuest* was used to design quantitative primers, and internal reference primers were designed based on tomato *β*-actin gene with determination of relative expression of three heat shock protein genes by qRT-PCR. Detailed determination of the relative expression level of three heat shock proteins were based on the method of Hu *et al.* (2010) and Piterková *et al.* (2013).

Gas-exchange parameters of tomato leaves were measured between 9:00–11:00 h by using the portable photosynthesis system *LI-6400* (*LI-COR Biosciences Inc*., USA), and the 5th to 8th mature leaves from top to bottom were selected. The PAR was set to 1,000 μmol(photon) m^{-2} s^{-1}, and the determination of each index was performed

automatically by the *LI-6400* built-in program. P_N, transpiration rate (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured. Stomatal limitation (L_s) and water-use efficiency (WUE) were calculated as follows: $L_s = 1 - C_i/C_a$, where C_a represents atmospheric CO_2 concentration; $WUE = P_N/E$.

Chl fluorescence parameters: The 5th to 8th fully expanded healthy leaves from the top were used to measure Chl fluorescence parameters by using a portable fluorometer (*FMS 2*, *Hansatech*, King's Lynn, UK). Minimal fluorescence (F_0), maximum fluorescence (F_m), and variable fluorescence (F_v) were automatically recorded by exposing the leaves to actinic light of 3,500 μmol(photon) m^{-2} s^{-1} after 20-min dark adaptation (leaves were shaded with clips). Then, the leaves were continuously illuminated with the actual growth light. Steady-state fluorescence in the light-adapted state (F_s) and maximal fluorescence level in the light-adapted leaves (F_m') were collected. Based on the measurements described aboved, F_v/F_m, Φ_{PSII}, ETR, and NPQ were calculated by using following formulas: $F_v/F_m = (F_m - F_0)/F_m$, $\Phi_{PSII} = (F_m' - F_s)/F_m'$, $ETR = 0.84 \times \Phi_{PSII} \times PAR \times 0.5$, $NPQ = (F_m - F_m')/F_m'$, according to Zhang *et al.* (2018) and Maxwell and Johnson (2000).

Electrolyte leakage: Ten leaf samples with 10 mm in diameter were placed in 25-ml glass test tubes and rinsed three times with distilled water. We added 20 ml of distilled water to test tubes and placed them in the dark for 18 h at room temperature. The electrical conductivity (EC_1) was measured by using an electrical conductivity meter (*Jenway-4520*, *Bibby Scientific US*, Burlington, USA). Then, test tubes were heated in the boiling water bath for 15 min and then cooled to room temperature, and the electrical conductivity (EC_2) was determined. Finally, electrolyte leakage was calculated according to the formula by Karlidag *et al.* (2010): $(EC_1/EC_2) \times 100\%$.

Lipid peroxidation: Estimation of lipid peroxidation was based on MDA content. The MDA content was determined using the thiobarbituric acid (TBA) method (Camejo *et al*. 2006). The MDA content was calculated and expressed as nmol g^{-1}(FM).

Active oxygen species analysis: Hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) were extracted and determined based on the method of Chen *et al.* (2013) and Wang *et al.* (2018) with minor improvements. Here, samples of 0.5 g of fresh leaf tissue were ground to homogenate with 5 ml of 5% (w/v) trichloroacetic acid and then centrifuged at 3,000 rpm at 4°C for 10 min. The supernatant, with 17 M NH_4OH added, was neutralized to pH 7.5, and used for measurements.

For determination of H_2O_2 content, the extract was divided into two aliquots with 100 μl each. One was added to 20 unit of CAT and the other was not added. Both two aliquots were added to 0.5 ml with 100 mM Tris-HCl (pH 8.0) and kept at room temperature for 10 min, and then 0.5 ml of colorimetric reagent was added made by mixing an equal volume of 0.3 mM 4-(2-pyridylazo) resorcinol monosodium salt and 0.3 mM potassium titanium oxalate. The absorbance of the two reaction mixtures at a wavelength of 508 nm was determined using an ultraviolet spectrophotometer (*UV-2450*, *Shimadzu*, Japan) and then the difference of absorbance between the two aliquots was used to calculate the concentration of H_2O_2 using an extinction coefficient of 39.4 mM^{-1} cm^{-1}. The concentration of H_2O_2 was expressed as μmol g^{-1}(FM).

For determination of $O_2^{\bullet-}$ production rate, the extract was divided into two aliquots with 100 μl each. One of which was added to 50 unit of SOD and the other was not added. Both of the two aliquots were added to 0.9 ml of 100 mM Tris-HCl (pH 7.5), and kept at room temperature for 10 min, following the addition of 100 μl of 5 mM 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt. The absorbance of the two assay mixtures was analyzed at a wavelength of 470 nm using an ultraviolet spectrophotometer (*UV-2450*, *Shimadzu*, Japan), and then the difference of absorbance between the two aliquots was used to calculate the concentration of $O_2^{\bullet-}$ using an extinction coefficient of 21.6 mM^{-1} cm^{-1}. The concentration of $O_2^{\bullet-}$ was expressed as mmol min^{-1} g^{-1}.

Antioxidant enzyme assays: The 5th to 8th fully expanded leaves from the top were selected and placed in liquid nitrogen for freezing, and then stored at –40°C. Leaf samples (0.5 g) were ground in a mortar with 5 mL of phosphate buffer pH 7.8 (0.2 mol L^{-1} KH_2PO_4 and 0.2 mol L^{-1} K_2HPO_4) and a small amount of quartz sand placed in an ice bath. The homogenate was poured into a centrifuge tube and centrifuged at 4°C; 4,000 rpm for 20 min. The supernatant was used for assay immediately.

Peroxidase (POD, EC 1.11.1.7) activity was determined by the increase in absorbance at 470 nm due to guaiacol oxidation (Meloni *et al*. 2003). One unit of POD activity was defined as the amount of the enzyme causing a change in absorbance at 470 nm of 0.01 per min. The specific POD activity was expressed as U g^{-1}(FM) min^{-1}.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was measured by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Zhu *et al*. 2004). One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions. The specific SOD activity was expressed as U g^{-1}(FM).

Catalase (CAT, EC 1.11.1.6) activity was assayed by monitoring the decline in absorbance at 240 nm due to decomposition of H_2O_2 (Luna *et al*. 2005). One CAT unit was defined as the amount of enzyme necessary to decompose 1 mM(H_2O_2) min^{-1} under the above-mentioned assay conditions. The specific CAT activity was expressed as U g^{-1}(FM) min^{-1}.

Statistical analysis: Data were analyzed with the *SPSS version 17.0* for *Windows* (*SPSS*, Chicago, IL, USA). One-way analysis of variance (*ANOVA*) followed by *Duncan*'s multiple range test between the treatments was used to detect the significance ($P<0.05$). Figures were drawn with the *GraphPad Prism version 7.05* for *Windows*

(*GraphPad Software*, San Diego, CA, USA). The reported data were presented as the mean value ± standard deviation (SD) of five replications.

Results

Relative expression levels of heat shock proteins: As it can be clearly seen from Fig. 1, the relative expression levels of HSP70, HSP90, and HSP110 in tomato leaves increased significantly after heat shock at 40°C for 4 h. After 38/28°C regime for 4 h, the contents of HSP70, HSP90, and HSP110 increased significantly in the control group. The content of HSP70 in heat shock group was significantly higher than that in the control group, but the contents of HSP90 and HSP110 were not significantly different from the control. After 25/15°C regime for 48-h recovery, the contents of HSP70, HSP90, and HSP110 in the control group were not different from those before the high temperature treatment. The content of HSP70 in the heat shock group remained at a high level, and

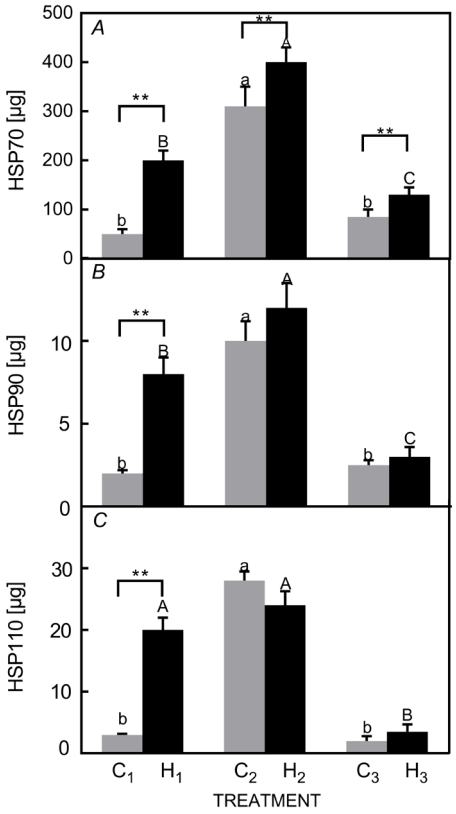

Fig. 1. Relative expression levels of heat shock protein (HSP) 70 (HSP70) (*A*), HSP90 (*B*), and HSP110 (*C*) in tomato seedlings after heat shock, heat stress, and recovery. C_1 – tomato seedlings being kept at 25°C for 4 h (control); H_1 – tomato seedlings being kept at 40°C for 4 h (heat shock); C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. *Different uppercase* and *lowercase letters* represent significant differences at 0.01 and 0.05 level by *Duncan*'s test, respectively. Values are means ± SD, $n = 15$. ** – significant difference at 0.01 level by *Duncan*'s test.

was significantly different from the control group, while the contents of HSP90 and HSP110 were lower and no significant difference was found from the control group.

Gas-exchange parameters: As it shown in Table 1, the heat shock at 40°C for 4 h significantly reduced P_N, g_s, C_i, WUE, and L_s, but increased E compared with control. Similarly, P_N, g_s, C_i, WUE, and L_s in the heat shock group were higher than those of the control group, but E was lower than that in the control group after heat stress and recovery.

Chl fluorescence parameters: As shown in Fig. 2, compared with control, the heat shock at 40°C for 4 h slightly reduced the values of F_v/F_m, Φ_{PSII}, and ETR, but significantly increased NPQ. The values of F_v/F_m, Φ_{PSII}, and ETR in the heat shock group were higher than those of the control group, but NPQ was significantly lower than that in the control group after heat stress and recovery.

Electrical conductivity and MDA content: Heat-shocked tomato seedlings reduced the electrical conductivity and MDA content in the leaves under high temperature stress and recovery (Fig. 3). Compared with the control, the heat shock at 40°C for 4 h slightly increased the electrical conductivity and MDA content. After 38/28°C for 48 h, the electrical conductivity and MDA content in the control group were higher than those of the heat shock group, but the difference was not significant. However, after 48-h recovery at 25/15°C, the electrical conductivity and MDA content of the control group were significantly higher than those of the heat shock group.

H_2O_2 content and $O_2^{\bullet-}$ production rate: Compared with the control, heat-shocked tomato seedlings decreased the H_2O_2 content and $O_2^{\bullet-}$ production rate in tomato leaves under high temperature stress and recovery (Fig. 4). After 40°C for 4 h of heat shock, there was no difference in H_2O_2 content and $O_2^{\bullet-}$ production rate between the heat-shock group and the control group. After high temperature stress, the content of H_2O_2 and $O_2^{\bullet-}$ production rate in the control group and the heat-shock group increased significantly, but the control group was slightly higher than the heat-shock group. After 48 h recovery at 25/15°C, both H_2O_2 content and $O_2^{\bullet-}$ production rate in the control and heat-shock groups were reduced, but the control group was significantly higher than the heat-shock group.

Antioxidant enzymes activities: As it can be seen from Fig. 5, tomato seedlings exposed to heat shock at 40°C for 4 h slightly increased the activities of CAT, SOD, and POD compared with the control. After 38/28°C for 48 h, the activities of CAT, SOD, and POD in the heat-shock group were higher than those in the control group, but the difference was not significant. After 48 h recovery at 25/15°C, the activities of CAT, SOD, and POD in the control and heat-shock groups increased, but the heat-shock groups were higher than that of the control group.

Correlation analysis: *Pearson*'s correlation analysis was

Table 1. Changes of gas-exchange parameters in tomato leaves after heat shock, heat stress, and recovery. *Different capital letters* in the same column represent significant differences at the level of 0.01 by *Duncan*'s test; *different lowercase letters* in the same column represent significant differences at the level of 0.05 by *Duncan*'s test. Values are means ± SD, $n = 15$. C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. P_N – net photosynthetic rate; g_s – stomatal conductance; C_i – intercellular CO_2 concentration; E – transpiration rate; WUE – water-use efficiency; L_s – stomatal limitation value.

	P_N [μmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [μmol(CO_2) mol^{-1}]	E [mmol(H_2O) m^{-2} s^{-1}]	WUE [μmol(CO_2) mmol(H_2O)$^{-1}$]	L_s
C_1	16.00 ± 0.65^{a}	1.02 ± 0.05^{a}	370.73 ± 2.03^{a}	3.11 ± 0.27^{c}	5.27 ± 0.39^{a}	0.120 ± 0.015^{a}
H_1	14.86 ± 0.23^{A}	0.80 ± 0.02^{C}	363.18 ± 6.54^{C}	3.27 ± 0.17^{B}	4.50 ± 0.23^{B}	0.117 ± 0.012^{A}
C_2	8.32 ± 0.34^{b}	0.56 ± 0.02^{c}	340.16 ± 6.52^{b}	3.89 ± 0.24^{a}	3.28 ± 0.17^{c}	0.086 ± 0.014^{b}
H_2	12.54 ± 0.56^{B}	0.88 ± 0.04^{B}	350.19 ± 4.12^{B}	3.54 ± 0.21^{A}	4.47 ± 0.21^{B}	0.087 ± 0.013^{B}
C_3	10.23 ± 0.12^{c}	0.74 ± 0.04^{b}	360.22 ± 2.11^{c}	3.44 ± 0.17^{b}	4.75 ± 0.03^{b}	0.093 ± 0.011^{A}
H_3	15.69 ± 0.15^{A}	0.90 ± 0.03^{A}	369.76 ± 3.46^{A}	3.12 ± 0.13^{BC}	5.12 ± 0.14^{A}	0.102 ± 0.014^{ac}

performed between photosynthetic parameters, MDA content, reactive oxygen species, antioxidant enzymes, and HSPs after high temperature stress. Table 2 shows that there was a significant negative correlation between HSP70 and P_N, F_v/F_m, SOD, POD, and CAT, and a significant positive correlation with MDA, H_2O_2, and $O_2^{\bullet-}$.

Discussion

Under high temperature stress, changes in the internal and external environment of plants inevitably affect the metabolism of proteins. High temperature-induced transcription and translation of HSPs has become a thermal protection mechanism for plants (Bita and Gerats 2013). In many plant species, heat tolerance of cells and tissues after high temperature stress depends almost on the induction of HSP70, though HSP100 has also been proven to be crucial (Gurley 2000). However, some studies showed that although some HSPs are essential for heat resistance, others play a less important role, for instance HSP101, HSA32, and HSFA3, because the knockout variants of these have a little effect on heat resistance (Sakata and Higashitani 2008, Yoshida *et al.* 2011). The results of this study indicated that HSPs are present in tomato seedlings under normal growth conditions, and heat shock can induce an increase in their synthesis. The HSPs content of heat-induced tomato seedlings after high temperature stress was significantly higher than that of the control group; also after 48 h of recovery, the HSPs content of the heat-shock treatment group was still quite higher. Because the synthesis of HSPs and heat resistance are consistent, it is believed that heat-shock treatment increases the synthesis of HSPs and improves the heat tolerance of tomato seedlings. It is hypothesized that some of the HSPs play an important protective role in heat stress, protecting the structure of the cell membrane and the antioxidant system from damage, making tomato seedlings resistant to heat stress (Nover *et al.* 2001). Our experiments confirmed that heat shock-induced synthesis of HSP70 was closely related to the improvement of heat tolerance of tomato seedlings (Table 2), which is consistent with the study of Hahn *et al.* (2011).

P_N is an important indicator of photosynthesis and light-use efficiency, which directly reflects the state of photosynthetic apparatus (Lang *et al.* 2013). Transpiration is described as a method of cooling leaves subjected to thermal shock. In our study, the heat-treated tomato seedlings had higher P_N, C_i, WUE, g_s, L_s, and had lower E (Table 1), indicating that heat shock can improve the photosynthetic efficiency of tomato seedlings and resist the damage caused by high temperature stress. The Φ_{PSII} is an indicator of the photochemistry effective quantum yield of PSII reaction center. The decrease of Φ_{PSII} is considered to be the decrease of electron transfer rate and CO_2 assimilation ability (Maxwell and Johnson 2000). The ETR reflects the electron transport rate in photochemical reactions. In this study, compared with the control, the heat-treated tomato seedlings had higher Φ_{PSII} and ETR after high temperature stress and recovery, indicating that the heat-shock treatment can improve the light energy

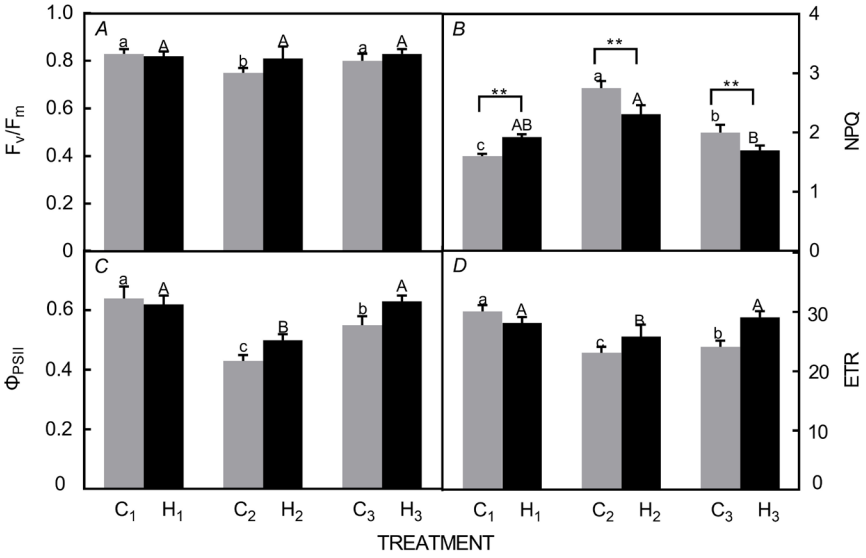

Fig. 2. Changes of maximal quantum yield of PSII photochemistry (F_v/F_m) (*A*), nonphotochemical quenching (NPQ) (*B*), actual photochemical efficiency of PSII (Φ_{PSII}) (*C*), and electron transport rate (ETR) (*D*) in tomato seedlings after heat shock, heat stress, and recovery. C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. *Different uppercase* and *lowercase letters* represent significant differences at 0.01 and 0.05 level by *Duncan*'s test, respectively. Values are means ± SD, n = 15. ** – significant difference at 0.01 level by *Duncan*'s test.

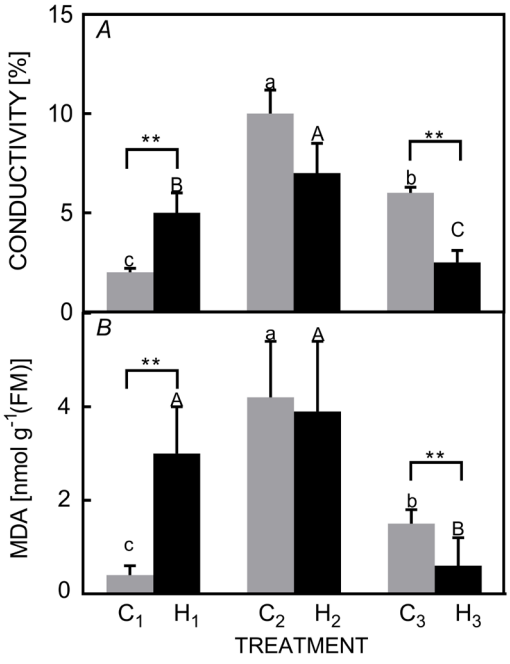

Fig. 3. Changes in relative conductivity (*A*) and malondialdehyde (MDA) content (*B*) of tomato seedlings after heat shock, heat stress, and recovery. C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. *Different uppercase* and *lowercase letters* represent significant differences at 0.01 and 0.05 level by *Duncan*'s test, respectively. Values are means ± SD, n = 15. ** – significant difference at 0.01 level by *Duncan*'s test.

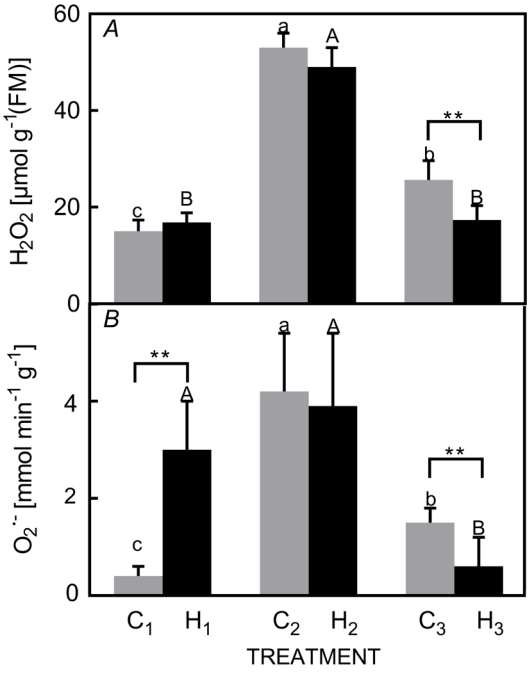

Fig. 4. Changes of H_2O_2 (*A*) and $O_2^{\bullet-}$ (*B*) in tomato leaves after heat shock, heat stress, and recovery. C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. *Different uppercase* and *lowercase letters* represent significant differences at 0.01 and 0.05 level by *Duncan*'s test, respectively. Values are means ± SD, n = 15. ** – significant difference at 0.01 level by *Duncan*'s test.

conversion efficiency, electron transport rate, and CO_2 assimilation ability of the PSII reaction centers. The NPQ reflects the ability of plants to dissipate excess light energy into heat, avoiding light damage, and also reflects the ability of plants to achieve photoprotection (Kanazawa and Kramer 2002). We found that compared with the control, the NPQ of tomato seedlings treated by heat shock was smaller after high temperature stress and recovery, indicating that the heat-shock treatment increased the proportion of light energy absorbed by the antenna pigment used for the photochemical reaction, reducing the proportion for heat dissipation. F_v/F_m reflects the quantum

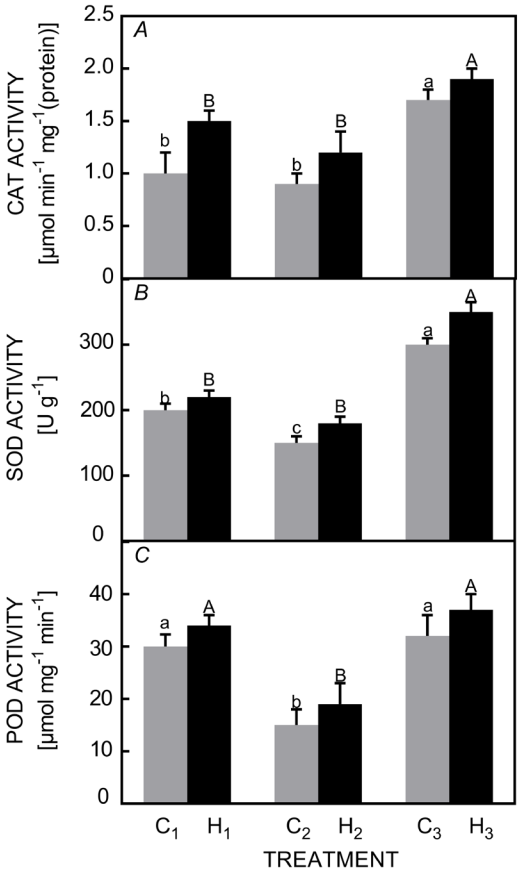

Fig. 5. Changes of catalase (CAT) (*A*), superoxide dismutase (SOD) (*B*), and peroxidase (POD) (*C*) activity in tomato leaves after heat shock, heat stress, and recovery. C_2 – C_1 followed by 38/28°C for 48 h; H_2 – H_1 followed by 38/28°C for 48 h; C_3 – recovery of tomato seedlings at 25/15°C for 48 h after C_2; H_3 – recovery of tomato seedlings at 25/15°C for 48 h after H_2. *Different uppercase* and *lowercase letters* represent significant differences at 0.01 and 0.05 level by *Duncan*'s test, respectively. Values are means ± SD, n = 15.

yield when all PSII reaction centers are in an open state (Zhou *et al.* 2015). Under normal conditions, the value of F_v/F_m is between 0.80 and 0.84. When the plant is stressed, the F_v/F_m is significantly lowered, which is considered to be a decrease in the efficiency of light energy conversion and a photoinhibition of the PSII reaction center (Maxwell and Johnson 2000, Prieto *et al.* 2009). In this paper, the heat-treated seedlings had higher F_v/F_m compared with the control, indicating that the heat-treated seedlings had the higher heat tolerance.

Cell membrane is a sensitive site for high temperature damage in plants, and relative conductivity is an important indicator for describing the degree of injury to cell membranes (Zarrin *et al.* 2011). The MDA is one of the most important products of membrane lipid peroxidation with the production of free radicals that exceeds the removal ability of protective enzyme system (Tsikas 2017). Both leaf conductivity and MDA contents are important parameters reflecting stress tolerance of plants. In this paper, the results showed that tomato seedlings that had not been subjected to heat shock treatment had higher contents of active oxygen species, higher electrical conductivity, and MDA content after high temperature stress and recovery. We can conclude that high temperature seriously damaged the cells of tomato seedlings, and heat shock could alleviate heat stress. Antioxidant enzymes, such as POD, CAT, and SOD, are the most important enzymes for scavenging free radicals, maintaining the balance between free radical production and elimination in plant cells (Xie *et al.* 2018). There have been many reports on the comparative effects of SOD, CAT, and POD activities exposed to high temperature stress (Zhu *et al.* 2010, Yuan *et al.* 2011, Wang *et al.* 2014). For instance, high activities of SOD, CAT, and POD seem to be associated with the development of heat shock-induced heat tolerance in tomato seedlings (Camejo *et al.* 2006). Our results also found that the activities of antioxidant enzymes, such as CAT, SOD, and POD, of heat-shocked tomato seedlings were higher after heat-stress treatment

Table 2. Correlation analysis of various indicators after high temperature stress in tomato seedlings. * and ** indicate a significant correlation at the level of 0.05 and 0.01 by *Duncan*'s test, respectively. P_N – net photosynthetic rate; F_v/F_m – maximum photochemical efficiency of PSII; SOD – superoxide dismutase; POD – peroxidase; CAT – catalase; MDA – malondialdehyde content; HSP110 – heat shock protein 110; HSP 90 – heat shock protein 90; HSP70 – heat shock protein 70.

	P_N	F_v/F_m	SOD	POD	CAT	MDA	H_2O_2	$O_2^{\bullet-}$	HSP110	HSP90	HSP70
P_N	1	0.965	0.846	0.995*	0.945	–0.866	–0.964	–0.866	–0.822	–0.946	–0.990**
F_v/F_m		1	0.956	0.933	0.997*	–0.967	–0.859	–0.967	–0.943	–0.998*	–0.963**
SOD			1	0.787	0.977	–0.999*	–0.672	–0.999*	–0.999*	–0.973	–0.843**
POD				1	0.901	–0.812	–0.986	–0.812	–0.761	–0.908	–0.995**
CAT					1	–0.985	–0.814	–0.985	–0.967	–0.905**	–0.938**
MDA						1	0.701	0.968**	0.997*	0.982	0.864*
H_2O_2							1	0.701	0.641	0.825	0.965*
$O_2^{\bullet-}$								1	0.997*	0.982	0.864*
HSP110									1	0.963	0.825
HSP90										1	0.944
HSP70											1

and recovery, suggesting that heat shock-induced heat tolerance of tomato seedlings occurred.

Therefore, we concluded that short-term heat shock treatments induced the production of HSPs, which helped to improve the thermal tolerance of tomato seedlings.

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