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Suppression of *SlNAC1* reduces heat resistance in tomato plants

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Abstract

NAC (NAM, ATAF1,2, and CUC2) transcription factors play an important role in the responses of plants to various environmental stresses. To investigate the function of *SlNAC1*, which was found to be a member of the ATAF subfamily in tomato (*Solanum lycopersicum* L.) plants under heat stress conditions, transgenic tomato plants were generated using an antisense technology. After a treatment at 40 °C for 48 h, in comparison with wild-type (WT) plants, the transgenic plants were severely wilted and exhibited a lower net photosynthetic rate and a maximal photochemical efficiency of photosystem II. Moreover, the transgenic plants displayed a higher ion leakage and malondialdehyde content and a lower proline content. The content of reactive oxygen species (superoxide anion radicals and hydrogen peroxide) were higher, and activities of ascorbate peroxidase and superoxide dismutase lower in the transgenic plants than in the WT plants. The transgenic plants also exhibited a lower accumulation of the transcripts of some heat shock protein genes (*Hsp70, Hsp90, sHsp17.4,* and *sHsp17.6*). All of these results suggest that the suppression of *SlNAC1* could obviously reduce heat resistance in the tomato plants, and this indicates that *SlNAC1* played an important role in the thermal tolerance of the tomato plants.

Additional key words: ascorbate peroxidase, chlorophyll fluorescence, heat shock proteins, high temperature, net photosynthetic rate, reactive oxygen species, *Solanum lycopersicum*, superoxide dismutase, transgenic plants.

Introduction

Currently, global warming presents a very serious concern. Hence, the development of heat stress resistant crops by altering the critical genes is the most effective strategy. Suitable temperatures for the growth and development of tomato plants range from 13 to 33 °C, and most cultivars are unable to grow at a temperature of 38 °C or above even when exposed to such temperatures for only short durations (Abdul-Baki 1991). Thus, an understanding of the molecular mechanisms of thermotolerance is more urgent today than ever before (Bokszczanin *et al*. 2013).

Plants alter their metabolism in response to heat stress through various mechanisms including the production of compatible solutes for osmotic adjustment and protection of proteins and cellular structures, and a modification of the antioxidant system to re-establish the cellular redox balance and homeostasis (Valliyodan and Nguyen 2006, Munns and Tester 2008, Janska *et al*. 2010). Further, ion transporters, late embryogenesis abundant proteins, and factors involved in signalling cascades are significant for counteracting the effects of stress (Wang *et al*. 2004, Rodríguez *et al*. 2005). High temperature could induce accumulation of reactive oxygen species (ROS), which subsequently provokes oxidative stress causing peroxidation of membrane lipids and disruption of cell membrane stability by denaturation of proteins (Rodríguez *et al*. 2005, Camejo *et al*. 2006, Wang *et al*. 2009). This results in increased malondialdehyde (MDA) content and relative electrolytic conductance (REC). However, plants also evolved scavenging mechanisms to protect themselves from oxidative stress (Hu *et al*. 2010). The up-regulation of the expression of genes encoding

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Abbreviations: APX - ascorbate peroxidase; F_v/F_m - variable to maximum chlorophyll fluorescence ratio (the maximal photochemical efficiency of PS II); *HSGs* - heat shock genes; HSPs - heat shock proteins; H_2O_2 - hydrogen peroxide; MDA - malondialdehyde; $O_2^{\bullet-}$ - superoxide anion radical; P5CS - pyrroline-5-carboxylate synthase; PFD - photon flux density; P_N - net photosynthetic rate; PS - photosystem; REC - relative electric conductance; ROS - reactive oxygen species; sHSPs - small heat shock proteins; TBA - thiobarbituric acid; TCA - trichloroacetic acid; WT - wild type.

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antioxidative enzymes, and increase in their activities are considered to be one of the primary pathways of cell acclimation to heat stress (Almeselmani *et al*. 2009).

In general, heat resistance is responsible for the upregulation of several heat-inducible genes which are commonly referred as "heat shock genes" (*HSGs*). These genes encode heat shock proteins (HSPs; Rizhsky *et al*. 2004) which protect intracellular proteins from denaturation and act as chaperones (Chang *et al*. 2007). HSPs can be grouped into five different families: HSP100 (or ClpB), HSP90, HSP70 (or DnaK), HSP60 (or GroE), and HSP20 (or small HSP, sHSP) (Baniwal *et al*. 2004). Plants show the greatest diversity in sHSPs with a very low molecular mass of 12 - 40 kDa (Swindell *et al*. 2007, Morrow and Tanguay 2012). For example, the overexpression of the *Arabidopsis Hsp101* gene in transgenic rice successfully enhanced rice thermotolerance (Katiyar-Agarwal *et al*. 2003). Transforming plants with *Hsp24* from *Trichoderma harzianum* was found to confer a significantly higher resistance to heat stress when constitutively expressed in *Saccharomyces cerevisiae* (Liming *et al*. 2008).

The name of NAC is derived from *NAM* (for *no apical meristem*), *ATAF1*, *ATAF2*, and *CUC2* (for *cup-shaped cotyledon*) (Souer *et al*. 1996, Aida *et al*. 1997). Proteins belonging to this family share a highly conserved NAC domain in the N-terminal region which functions as DNA-binding domain, and a nonconserved C-terminal region that usually contains the transcriptional activation domain. *NAC* genes are involved in many developmental processes and biotic and abiotic stress responses (Delessert *et al*. 2005, Kim *et al*. 2009). *SiNAC* is induced by dehydration, salinity, and treatments with ethephon and methyl jasmonate in *Setaria italica* (Puranik *et al*. 2011). *OsNAC2/6* and *OsNAC10* enhance drought and salt tolerances in *Oryza sativa* (Nakashima *et al*. 2009, Jeong *et al*. 2010). We found that *SlNAC1* belongs to the ATAF subfamily, and that its overexpression can improve the chilling tolerance of tomato plants (Ma *et al*. 2013). Although the members of the ATAF subfamily are known to be stress-responsive genes (Lu *et al*. 2007, Wu *et al*. 2009), most studies have focused on drought, salt, and cold stresses, and few reports have described the role of the members of the ATAF under heat stress. In this study, we applied a heat stress to *SlNAC1* antisense transgenic and wild-type tomato plants to elucidate the role of *SlNAC1* in the heat stress response in tomato plants.

Materials and methods

Plants and treatments: Antisense transgenic plants were generated as previously described (Ma *et al*. 2013) with a modification that *SlNAC1* was inversely inserted into the expression vector pBI121. Screening and identification of antisense transgenic plants were performed as described by Kong *et al*. (2013). Moreover, the RT-qPCR analysis was performed to verify the expression level of *SlNAC1*. The primers are shown in Table 1 Suppl.

The wild-type (WT) tomato (*Solanum lycopersicum* L. cv. Zhongshu 6) plants and the transgenic plants of the T_2 generation were grown in a pot in a naturally lit greenhouse under a 12-h photoperiod and day/night temperatures of 25/20 °C. These plants were irrigated with a Hoagland nutrient solution every other day to ensure regular growth until the sixth leaf was fully expanded, when the plants were subjected to a heat stress. One half of the pots was transferred to an incubation chamber and exposed to 40 °C at a photon flux density (PFD) of 200 μmol m^{-2} s^{-1} for 48 h. The control plants were placed under the same conditions, with the exception that the temperature was 25 °C. After treatments, the plants were photographed, leaf fresh mass was determined, and the rate of water loss was measured relative to the initial fresh mass. Simultaneously, the leaves at the same position were detached, immediately frozen in liquid nitrogen, and stored at -80 °C until use.

Measurements of maximal photochemical efficiency (F_v/F_m) and net photosynthetic rate (P_N): A portable fluorometer (*FMS2*, Hansatech, Norfolk, UK) was used to measure chlorophyll fluorescence according to the protocol described by Van Kooten and Snel (1990). The experimental plants were placed in the dark for 15 min before measurement, and then, minimal fluorescence [F_0, the fluorescence when all photosystem (PS) II reaction centers are open] was determined under a low irradiance (approximately 10 μmol m^{-2} s^{1}). Maximal fluorescence (F_m, the fluorescence when all PS II reaction centers are closed) was measured under a saturating irradiance of 7 000 mol m^{-2} s^{-1} for 0.8 s and variable fluorescence (F_v) was determined as F_m - F_0. Maximal photochemical efficiency was F_v/F_m ratio.

A portable photosynthetic system (*CIRAS-2*, *PP Systems*, Herts, UK) with an open-flow gas exchange was used to measure P_N. This measurement was performed under an ambient CO_2 concentration (380 mm^3 cm^{-3}), PFD of 800 μmol m^{-2} s^{-1}, a temperature of 25 ± 1 °C, and a relative humidity of 80 %.

Measurements of electrolyte leakage and malondialdehyde content: To measure relative electric conductance (REC), eight leaf discs (0.8 cm^2 each) were placed into a 20-cm^3 glass tube containing 10 cm^3 of distilled water. The tube was evacuated for 30 min and surged for 3 h to measure the initial conductance (S1). The tubes were then boiled for 30 min and cooled to 25 °C to measure the final conductance (S2). The conductance of distilled water (S0) was also measured, and this

was used as blank control. The relative electric conductance (REC) was calculated as (S1 - S0)/(S2 - S0) × 100.

MDA content was measured to evaluate the degree of oxidative damage of membrane lipids. Approximately 0.5 g of leaves was ground to powder in a cold mortar containing 5 cm^3 of 10 % (m/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10 000 *g* and 4 °C for 10 min. A mixture of 2 cm^3 of the supernatant with 2 cm^3 of 0.6 % (m/v) a thiobarbituric acid (TBA) reagent (0.6 % TBA dissolved in 10 % TCA) was then heated in boiling water for 15 min, immediately cooled, and centrifuged at 3 000 *g* for 10 min. Samples that contained 2 cm^3 of distilled water instead of the extract served as controls. Finally, the supernatant was used to measure the absorbances at 450, 532, and 600 nm. The MDA content was calculated using a standard curve relating the MDA concentration to the absorbance.

Measurement of proline: Approximately 0.2 g of leaves was cut up and placed into a glass tube containing 5 cm^3 of 3 % (m/v) sulfosalicylic acid. After heating in boiling water for 10 min, the mixture was cooled to room temperature. and centrifuged at 8 000 *g* for 1 min. A mixture of 2 cm^3 of the supernatant with 2 cm^3 of ice acetic acid and 3 cm^3 of acidic ninhydrin was then heated in boiling water for 40 min. After cooling to room temperature, 5 cm^3 of toluene was added to the mixture and mixed, after which the mixture became stratified. The absorbance of the upper toluene layer was measured at 520 nm. The proline content was calculated using a standard curve.

Measurement of H_2O_2 and superoxide anion radical: To measure H_2O_2 content, approximately 0.5 g of leaves was ground to powder in liquid nitrogen and transferred into a centrifuge tube. After the addition of 3 cm^3 of a 50 mM cold phosphate buffer (pH 6.8), the samples were centrifuged at 6 000 *g* for 15 min, and a mixture of 3 cm^3 of the supernatant with 1 cm^3 of 0.1 % (m/v) titanium sulfate in 20 % (m/m) H_2SO_4 was transferred into a new tube and centrifuged at 6 000 *g* for 15 min. The absorbance was measured at 410 nm and the H_2O_2 content was calculated according to a standard curve.

To measure $O_2^{\bullet-}$ content, approximately 0.5 g of leaves ground in liquid nitrogen was transferred into a tube with 5 cm^3 of a 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 12 000 *g* for 20 min, and a mixture of 0.5 cm^3 of the supernatant with 0.5 cm^3 of the 50 mM phosphate buffer and 1 mM hydroxyl ammonium chloride was incubated at 25 °C for 20 min. After the addition of 1 cm^3 of 17 mM *p*-aminobenzene sulfonic acid and 1 cm^3 of 7 mM α-naphthylamine, the mixture was again incubated at 25 °C for 20 min. The absorbance was measured at 530 nm, and the $O_2^{\bullet-}$ content was calculated according to a standard curve.

Detection of antioxidant enzyme activities: To measure SOD activity, 3.3 cm^3 (V) of assay mixture contained 0.1 cm^3 (V_t) of the supernatant (the blank control was of the distilled water) which was used in $O_2^{\bullet-}$ detection, 1.5 cm^3 of a 50 mM phosphate buffer (pH 7.8), 0.3 cm^3 of 65 mM *L*-1-methionine, 0.3 cm^3 of 500 μM nitroblue tetrazolium, 0.3 cm^3 of 100 μM Na_2EDTA, 0.3 cm^3 of 200 μM riboflavin, and 0.5 cm^3 of distilled water in a transparent glass thimble tube. The mixture reacted under PFD of 100 μmol m^{-2} s^{-1} for 20 - 30 min and the absorbance was measured at 560 nm. One unit of SOD activity was calculated as:

$(A_{CK} - A_E) \times V / (0.5 \times A_{CK} \times V_t \times M)$,

where A_{CK} and A_E were the absorbances of treatment group and blank control group, respectively. APX activity was measured by monitoring the decrease in absorbance at 290 nm (Nakano and Asada 1981).

RT-qPCR analysis: Total RNA was extracted from leaves using *RNAprep* plant kits (*Tiangen Biotech*, Beijing, China) according to the manufacturer's instructions. RNA treated with DNase was reverse transcribed using *M-MLV* reverse transcriptase (*Tiangen Biotech*). A real-time quantitative polymerase chain reaction (RT-qPCR) was performed on a *CFX96*™ RT-PCR system (*Bio-Rad*, Hercules, USA) using a *SYBR* real master mix (*Tiangen Biotech*). PCR thermal cycle conditions were as follows: denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 10 s, and 68 °C for 10 s. *EF-1α* (GenBank accession No. X144491) was used as reference gene. The primers used in this study are shown in Table 1 Suppl.

Statistical analysis: Statistical analysis was conducted using data processing system (DPS) procedures (Zhejiang University, Zhejiang, China). Differences between the WT and transgenic tomato plants were evaluated using Duncan's multiple range tests at the probability of 0.05.

Results

Transgenic plants surviving in the MS medium with 50 μg cm^{-3} kanamycin were detected. The expected fragment was amplified in the transgenic lines but not in WT (Fig. 1). To evaluate the expression of *SlNAC1* in the transgenic lines, RT-qPCR was performed. Compared with the WT plants, the relative mRNA content of *SlNAC1* in these seven lines (A1 - A7) was suppressed to 0.48, 0.86, 0.92, 0.27, 0.77, 0.33, and 0.59, respectively (Table 1). Lines A1, A4, and A6 were selected for further experiments.

Both the transgenic and WT plants grew well, and no differences between them under normal conditions were observed (Fig. 2*A*). However, after the treatment at 40 °C for 48 h, leaves of both the transgenic and WT plants wilted, more severely leaves of the transgenic plants (Fig. 2*B*). The leaf fresh mass decreased under the heat stress more in the transgenic plants than in WT, and the transgenic plants exhibited a higher rate of water loss than WT (Table 2). These results suggest that the suppression of *SlNAC1* reduced high-temperature resistance in the tomato plants.

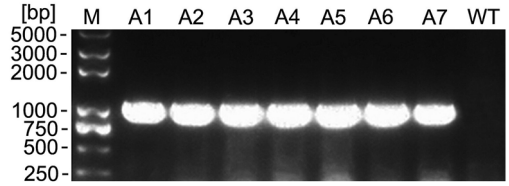

Fig. 1. Identification of antisense transgenic plants by PCR. A1 - A7 and WT indicate antisense transgenic tomato plants and wild-type tomato plants, respectively. M - marker.

Before the heat treatment, no significant difference between the WT and transgenic plants was observed with respect to the values of P_N and F_v/F_m. After the heat treatment for 48 h, P_N was reduced in both the WT and transgenic plants, with transgenic plants exhibiting a greater reduction (Table 2). Moreover, F_v/F_m exhibited a profile similar to that of P_N (Table 3). These results indicate that the WT plants had higher P_N and F_v/F_m values than the transgenic plants. This finding suggests that damage to PS II under the heat stress might be more severe in the transgenic plants than in the WT plants.

REC and the MDA content exhibited little difference between the WT and transgenic plants at 25 °C. After the exposure to 40 °C for 48 h, REC and the MDA content of the WT and transgenic plants increased (Table 3). Under normal conditions, the proline content in the WT plants was higher than in the transgenic plants. After the treatment at 40 °C for 48 h, the proline content increased more in WT than in the transgenic lines (Table 2). Pyrroline-5-carboxylate synthase (P5CS) is the key enzyme in the proline synthesis, and the accumulation of *P5CS* transcripts showed little difference between the WT and transgenic plants under normal conditions. The heat stress induced the *P5CS* expression in both the WT and transgenic plants; however, a higher expression was observed in the WT plants (Table 3). Based on these results, we can infer that the reduced heat stress tolerance of the transgenic plants was partly due to the lowered increase in the expression of *P5CS*.

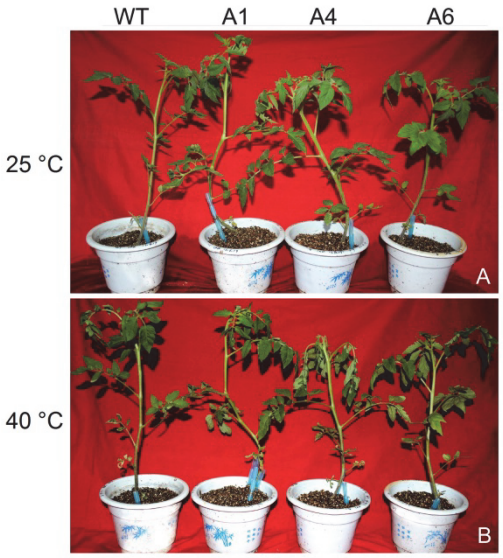

Fig. 2. Phenotypes of WT and transgenic plants A1, A4, and A6 under normal conditions (*A*) and a heat stress for 48 h (*B*).

The transgenic plants exhibited a slightly higher content of H_2O_2 and $O_2^{\cdot-}$ than the WT plants under normal conditions (Table 2). After the treatment at 40 °C for 48 h, the H_2O_2 and $O_2^{\cdot-}$ content increased more in the transgenic lines than in WT (Table 2). These results indicate that the suppression of *SlNAC1* could result in the accumulation of ROS, leading to a more severe injury to plants under the heat stress conditions.

The transgenic lines exhibited a slightly higher SOD activity but a lower APX activity than the WT plants under normal conditions. After the treatment at 40 °C for 48 h, the activity of both the antioxidant enzymes increased more in WT than in the transgenic lines. Consistent with these results, the transgenic plants exhibited lower amounts of *FeSOD* and *APX* mRNAs after the heat treatment (Table 3). We infer that the reduced resistance to the heat stress in the antisense transgenic plants was due to the downregulation of expression of genes encoding the primary antioxidant enzymes.

At room temperature, amounts of mRNA of *Hsp70*

Table 1. The RT-qPCR analysis of *SlNAC1* expression in WT and antisense transgenic plants. The expression level of *SlNAC1* in the WT plants was normalized to 1. A1 - A7 represent seven antisense transgenic lines. Means ± SD of four individual experiments. Different letters indicate statistically significant differences at $P \leq 0.05$.

	WT	A1	A2	A3	A4	A5	A6	A7
SlNAC1 expression	1.00±0.01a	0.48±0.04e	0.86±0.07b	0.92±0.03b	0.27±0.03f	0.77±0.07c	0.33±0.03f	0.59±0.04d

and *sHsp17.4* in the transgenic plants were lower than in the WT plants, whereas the expressions of *Hsp90* and *sHsp17.6* in the transgenic plants were higher than in the WT plants. After the treatment at 40 °C for 48 h, the expressions of these four genes demonstrated sharp increases in all plants, but the accumulation of transcripts was lower in the transgenic plants than in the WT plants (Table 3). These results suggest that *SlNAC1* positively regulated the expression of *Hsps* to counter the heat stress.

Table 2. Effects of a heat stress (40 °C for 48 h) on fresh mass and some physiological parameters in WT and transgenic plants A1, A4, and A6. During the heat stress, the plants were adapted in darkness for 15 min before the F_v/F_m measurement which was done at 40 °C. Before the P_N measurement, the plants were kept at 25 °C and PFD of 100 μmol m^{-2} s^{-1} for 30 min to induce stomata opening, and then were irradiated with PFD of 800 μmol m^{-2} s^{-1} for 15 min. The plants were exposed to 40 °C for 48 h before the measurements of REC, MDA, proline, ROS, and enzyme activities. Means ± SD of four individual experiments. Different letters indicate statistically significant differences at $P \leq 0.05$.

Parameter	Temperature [°C]	WT	A1	A4	A6
Fresh mass	25	0.84±0.01a	0.85±0.01a	0.85±0.01a	0.87±0.01a
	40	0.65±0.02a	0.56±0.01b	0.42±0.01d	0.53±0.01c
Leaf dehydration [%]	40	22.82±0.01d	33.68±0.01c	51.21±0.01a	39.23±0.01b
F_v/F_m	25	0.88±0.01a	0.87±0.02a	0.86±0.02a	0.87±0.01a
	40	0.80±0.02a	0.77±0.01ab	0.73±0.03b	0.71±0.01b
P_N	25	11.30±0.10ab	11.50±0.10a	10.33±0.29b	11.13±0.12ab
[μmol m^{-2} s^{-1}]	40	9.97±0.06a	8.40±0.06c	6.43±0.06d	9.17±0.20b
REC	25	25.44±1.95a	19.31±1.82b	27.72±1.12a	28.42±1.97a
[%]	40	39.66±2.23b	54.30±4.43a	56.98±2.62a	53.83±1.96a
MDA content	25	1.91±0.11a	2.02±0.14a	2.15±0.06a	2.08±0.27a
[μmol g^{-1}(f.m.)]	40	2.35±0.16b	2.83±0.20a	2.67±0.09a	2.67±0.19a
Proline content	25	9.72±0.69a	9.03±0.55a	6.83±0.56b	6.67±0.21b
[mg g^{-1}(f.m.)]	40	21.57±1.56a	13.73±0.81b	10.90±0.14c	11.76±0.84c
$O_2^{\bullet-}$ production	25	2.44±0.20b	2.56±0.07ab	2.83±0.22a	2.67±0.20ab
[μmol g^{-1}(f.m.) min^{-1}]	40	4.64±0.37c	7.51±0.56a	7.56±0.35a	6.63±0.04b
H_2O_2	25	4.71±0.07b	4.80±0.22b	4.94±0.18ab	5.08±0.03a
[μmol g^{-1}(f.m.)]	40	6.10±0.13c	6.87±0.42b	6.93±0.13b	8.52±0.38a
SOD activity	25	80.09±6.71a	87.94±6.37a	83.42±9.89a	80.39±4.02a
[U g^{-1}(f.m.)]	40	121.03±2.81a	103.60±7.16bc	93.87±2.26c	109.79±2.57b
APX activity	25	8.67±0.52a	7.03±0.28b	6.59±0.40b	7.00±0.20b
[U g^{-1}(f.m.)]	40	33.49±2.09a	12.64±0.35b	11.77±0.78b	12.18±0.46b

Table 3. Relative expressions of *P5CS, FeSOD, APX,* and *Hsp* genes. Means ± SD of four individual experiments. Different letters indicate statistically significant differences at $P \leq 0.05$.

Gene	Temperature [°C]	WT	A1	A4	A6
P5CS	25	1.00±0.06a	1.08±0.16a	0.96±0.12a	1.06±0.13a
	40	2.80±0.10a	2.11±0.17b	1.72±0.15c	1.79±0.30bc
FeSOD	25	1.00±0.04b	1.19±0.01a	0.75±0.01c	0.78±0.01c
	40	0.75±0.04a	0.46±0.02c	0.47±0.01c	0.54±0.03b
APX	25	1.00±0.01a	1.07±0.04a	0.96±0.08a	1.08±0.07a
	40	0.69±0.04a	0.45±0.02b	0.24±0.01c	0.24±0.02c
Hsp70	25	1.00±0.09a	0.83±0.04b	0.72±0.02c	0.63±0.02c
	40	15.71±0.69a	13.43±0.51b	12.27±0.41b	12.35±0.28b
Hsp90	25	1.00±0.10c	2.67±0.10a	1.41±0.08b	1.44±0.07b
	40	301.40±19.3a	114.59±6.54d	155.50±13.7c	202.58±7.98b
sHsp17.4	25	1.00±0.09a	0.30±0.04b	0.31±0.04b	0.22±0.02b
	40	35.26±2.77a	23.82±2.19b	26.03±1.98b	23.24±2.07b
sHsp17.6	25	1.00±0.10b	1.43±0.12a	1.46±0.13a	1.48±0.08a
	40	38.58±3.00a	32.47±2.56b	33.13±1.69ab	30.35±2.57b

Discussion

It is well known that heat stress is associated with the overproduction of ROS (Liu and Huang 2000). The heat stress induced excess accumulations of $O_2^{\cdot-}$ and H_2O_2, resulting in oxidative damage proved by increasing the ion leakage and MDA content and reducing P_N and F_v/F_m in both the transgenic and WT plants, but more in the transgenic plants (Table 3). Plants can ameliorate a possible heat stress damage induced by generation of ROS *via* enzymatic ROS scavenging. The lower expression of *SOD* and *APX* (Table 4) resulted in lower activities of SOD and APX (Table 3) in the transgenic plants indicating that the suppression of *SlNAC1* increased the photoinhibition of PS II and reduced the heat stress tolerance of the plants. The accumulation of proline occurs in response to various environmental stresses, and proline has been shown to have a protective role in some cases. In the present study, proline accumulated in all of the plants after the treatment by the heat stress, but it increased less in the transgenic plants than in the WT plants (Table 2). Moreover, the expression of *P5CS* (Table 3) followed the same pattern as the accumulation of proline (Table 2). These results suggest that *SlNAC1* positively regulated heat resistance in the tomato plants by maintaining higher antioxidant enzyme activities to scavenge excess ROS and by increasing the accumulation of proline.

The morphological and functional integrity of cells depends on the equilibrium of most, if not all, of the encoded proteins, also termed protein homeostasis which includes the control of synthesis, intracellular sorting, folding, functioning, and degradation of proteins (Hartl *et al*. 2011). Major *HSPs* encoding molecular chaperones are prominent examples in plants under heat stress conditions. Heat stress affects plants by changing membrane fluidity and disturbing protein homeostasis, leading to disturbed cellular homeostasis. *HSP* genes are ubiquitously and rapidly induced among all of the genes activated under heat stress (Li *et al*. 2013) and HSPs protect plants from damage by functioning as molecular chaperones in protein folding, assembly, translocation, and membrane stabilization (Lindquist and Craig 1988, Vierling 1991, Jakob *et al*. 1993, Boston *et al*. 1996, Waters *et al*. 1996, Wang *et al*. 2004). Under heat stress, the induced members of the *HSPs* and *sHSPs* families can work together to protect misfolded proteins from irreversible aggregation and can cooperate to enable the dissolution of protein aggregates or refolding denatured proteins during stress recovery. In our study, the major *HSPs* (*Hsp70*, *Hsp90*, *sHsp17.4*, and *sHsp17.6*) significantly increased in all the plants after the heat stress, and WT exhibited a higher expression than the transgenic plants (Table 4) suggesting that *SlNAC1* regulated heat tolerance of the tomato plants by upregulating the expression of *HSPs*. However, the mechanism by which *SlNAC1* regulates *HSPs* remains to be revealed.

In summary, our results confirm that *SlNAC1* participated in the responses of the plants to the heat stress. The transgenic plants with the suppressed expression of *SlNAC1* exhibited a reduced expression of *P5CS* and *HSPs* and a reduced antioxidative activity. This resulted in lower proline and HSPs content and in excess ROS, leading to membrane lipid peroxidation and damage to PS II. However, the specific mechanism of the function of *SlNAC1* in the response of plants to heat stress remains unclear.

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