Antisense expression of tomato chloroplast omega-3 fatty acid desaturase gene (*LeFAD7*) enhances the tomato high-temperature tolerance through reductions of trienoic fatty acids and alterations of physiological parameters

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Abstract

We studied how the reductions of trienoic fatty acids (TAs) and increases of dienoic fatty acids (DAs) enhanced high-temperature tolerance in antisense expression of tomato chloroplast omega-3 fatty acid desaturase gene (*LeFAD7*) transgenic tomato (*Lycopersicon esculentum* Mill.) plants. In transgenic plants, the content of linolenic acid (18:3) was markedly decreased, while linoleic acid (18:2) was increased correspondingly and the similar changes were observed under high-temperature stress as well. Under high-temperature stress, transgenic plants can maintain a relatively higher level of net photosynthetic rate (P_N) and chlorophyll (Chl) content than that of wild type (WT) plants. A decreased Chl/Carotenoids (xanthophylls and carotenes, Car) ratio and Chl *a*/*b* ratio were observed in transgenic plants. Transgenic plants exhibited visible decrease in the relative electrolyte conductivity, higher activities of antioxidative enzymes and lower reactive oxygen species correspondingly than WT. In addition, high-temperature stress for 24 h caused more extensive changes of chloroplast ultrastructure in WT than in transgenic plants. We therefore suggested that the enhancement of high-temperature tolerance in antisense expression of *LeFAD7* transgenic plants might be raised from the reduction of TAs and increase of DAs subsequently leading to series of physiological alterations.

Additional keywords: high-temperature tolerance; chloroplast omega-3 fatty acid desaturase; *Lycopersicon esculentum* Mill.; trienoic fatty acids.

Introduction

In the past several decades, extensive attention has been paid to the mechanism of temperature response in higher plants because of the agricultural demands for improvement of the tolerances to low- or high-temperature stress (Iba 2002, Raison *et al*. 1979, Wahid *et al*. 2007). Plants in tropical and sub-tropical areas may, conceivably, have to cope with high-temperature stress, which may be more serious than before with the global warming. Tomato (*Lycopersicon esculentum* Mill.) is a chilling-sensitive crop, however, it is still subjected to high temperature during summer cultivation.

Membrane lipid desaturation is closely linked to many biological and physiological phenomena including maintenance of chloroplast function, pollen development, cold tolerance and production of the plant hormone jasmonic acid (Gibson *et al*. 1994, Kodama *et al*. 1995, McConn and Browse 1996, Routaboul *et al*. 2000). Many researches had been performed on the role of membrane lipid desaturation in the tolerance of plants to cold, because cold is a detrimental environmental factor that

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Abbreviations: APX – ascorbate peroxidase; *AtFAD7* – *Arabidopsis thaliana* omega-3 fatty acid desaturase; DAs – dienoic fatty acids; DGDG – digalactosyldiacylglycerol; F_v/F_m – maximal photochemical efficiency of photosystem II; *LeFAD7* – *Lycopersicon esculentum* omega-3 fatty acid desaturase gene; MGDG – monogalactosyldiacylglycerol; OEC – oxygen evolving complex; PFD – photon flux density; PG – phosphatidylglycerol; SOD – superoxide dismutase; SQDG – sulfoquinovosyldiacylglycerol; TAs – trienoic fatty acids; 16 : 1(3t) – trans-hexadecenoic acids; 16 : 3 – hexadecatrienoic acids; 18 : 1 – oleic acid; 18 : 2 – linoleic acid; 18 : 3 – linolenic acids.
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causes severe damage to many agricultural crops (Raison *et al*. 1979). However, only a few reports have addressed the possible role of high temperature with membrane lipid desaturation. The pioneer research of membrane lipid desaturation pointed out that an increase in growth temperature leads to a reduction of TAs, such as α-linolenic acid and hexadecatrienoic acid (16:3), in some desert and evergreen plants (Pearcy 1978).

In higher plants, there are two pathways for polyunsaturated fatty acid biosynthesis, prokaryotic and eukaryotic pathways, respectively. 18:3 can be synthesized by 18:2 *via* oleic acid (18:1) in the prokaryotic and eukaryotic pathways, whereas 16:3 can be synthesized only in the prokaryotic mode. Omega-3 fatty acid desaturase is a key enzyme for the formation of TAs that catalyses the desaturation of lipid-linked DAs. It was reviewed that decreased contents of TAs play an important role in high-temperature tolerance by investigating transgenic tobacco plants with antisense-expressed omega-3 fatty acid desaturase (Murakami *et al*. 2000). In soybean (*Glycine max*), STR7 mutant, an atrazine-resistant mutant, exhibited unusual tolerance to high temperature, of which the chloroplast membrane of STR7 accumulated an unusually high content of 16:0 and reduced level of 16:1 and 18:3 compared with WT plants (Alfonso *et al*. 2001). Recently, it was reported that the content of 18:3 was reduced about 7–32% and 18:2 was increased in all of the transgenic rice lines with antisense-expressed the chloroplast-localized *Arabidopsis thaliana* omega-3 fatty acid desaturase (*AtFAD7*). Transgenic rice plants in which the content of DAs was increased as a result of co-suppression of fatty acid desaturase had higher tolerance to high temperature (35ºC) than WT plants, as judged by the growth rate and Chl content (Sohn and Back 2007). Higher content of TAs was related to the tolerance to cold stress. On the contrary, reduced content of TAs was linked to tolerance to high-temperature stress, although it was not completely confirmed up to date.

In our previous studies, we observed that expression of *LeFAD7* was inhibited by high temperature in tomato. Furthermore, transgenic plants with antisense-expressed *LeFAD7* exhibited lower 18:3 content and were supposed to endure high temperature because of higher maximal photochemical efficiency (F_v/F_m) and O_2 evolution rate than WT (Liu *et al*. 2006). Here, we showed that antisense-mediated depletion of *LeFAD7* enhanced the high-temperature tolerance through reductions of trienoic fatty acids and alterations of physiological parameters.

Materials and methods

Plant materials and treatments: Transgenic tomato plants with anti-expression of *LeFAD7* gene (AY157317) under the control of 35S-CaMV promoter were developed and described previously (Liu *et al*. 2006). The seeds of WT and transgenic tomato plants (T_1 generation) were germinated on moistened filter paper at 25ºC for 3 d. Sprouted burgeons were then planted in 13.5 cm-diameter plastic pots (one plant per pot) filled with sterilized soil and grown at 25–30/15–20ºC (day/night) under a 14 h photoperiod (300–400 μmol m^{-2} s^{-1} PFD) in greenhouse. The sixth fully expanded leaves were harvested from one-month-old tomato plants. Intact plants were treated with high-temperature stress under low light for 24 h (40ºC, 100 μmol m^{-2} s^{-1}). Then the treated leaves were immediately frozen in liquid nitrogen and stored at –80ºC.

Fatty acid analysis: Tomato leaves were harvested from one-month-old tomato plants and frozen immediately in liquid nitrogen. Lipids were extracted as described by Siegenthaler and Eichenberger (1984), and separated by two-dimensional TLC (Xu and Siegenthaler 1997). For quantitative analysis, individual lipids were separated by thin layer chromatography, scraped from the plates, and used to prepare fatty acid methyl esters (improved by Su *et al.* 1980). Fatty acid composition of individual lipids was determined by gas chromatography as previously described (Chen 1994, Su *et al*. 1980).

Membrane permeability determinations: Membrane permeability was determined by measuring the relative electrolyte conductivity following the method as described previously (Santos *et al*. 2001), with some modifications. Briefly, leaf samples were washed in deionized water to remove surface-adhered ions. Fresh samples (10 leaf discs) were added to glass flask containing 20 ml of deionized water. The flasks were shaken at 120 cycle min^{-1}. Samples were incubated 6 hours at 25ºC and the conductivity of solution (ECo) was measured with a microdigital conductivity meter (*DDS-11D, FEILO*, Shanghai, China). After the measurement, samples were killed by autoclaving at 110ºC for 10 min. When the temperature of the flasks declined to 25 ºC, the flasks were filled up with deionized water to 20 ml, and the total conductivity (ECt) was then measured. Relative electrolyte conductivity was calculated as (Eco/ECt) × 100, and the index of injury was calculated as [(Eco-ECt)/(1-ECt)] × 100.

Analysis of activities of antioxidative enzymes and reactive oxygen species: The leaves were homogenized in a blender in 200 cm^3 ice-cold medium containing 330 mM sorbitol, 30 mM 2-N-morpholino-ethanesulfonic acid (pH 6.5), 2 mM ascorbic acid, and 0.1 % bovine serum albumin. The homogenate was filtered through six layers of cheese cloth and centrifuged at 2,000 × *g* for 3 min. The pellet was suspended with 4 ml PBS (Phosphate Buffered Saline) for measurement of chloroplastic APX and SOD activities. APX activity was

determined according to Jimenez *et al*. (1997). SOD assay was performed as described by Giannopolitis and Ries (1977). The soluble protein content was measured following the method of Bradford (1976).

The assay for $O_2^{\cdot-}$ was performed as described by Wang and Luo (1990). Fresh leaves (5 g) without midrib were thoroughly ground in an ice bath in a grinding medium (4 ml) containing 0.05 M phosphate buffer (pH 7.8). The homogenate was centrifuged at 5,000 × *g* for 10 min at 4ºC. The supernatant with phosphate buffer (pH 7.8) and 10 mM hydroxylammonium chloride was incubated at 25ºC for 20 min, then 17 mM p-amino-benzene sulfonic acid and 7 mM α-naphthylamine were added, and the mixture was incubated at 25ºC for 20 min. Finally, similar volume of ethylether was added into the mixture that was centrifuged at 1,500 × *g* for 5 min. The water phase was used to determine the absorbance at 530 nm. The $O_2^{\cdot-}$ generation was calculated per g fresh mass (FM) of leaves. H_2O_2 content was determined according to the method of Sairam and Srivastava (2002). The concentration of H_2O_2 was estimated by measuring the absorbance of the titanium-hydroperoxide complex and using a standard curve plotted with known concentration of H_2O_2.

Assay of P_N and determination of pigment content: Plants were treated at 40ºC for 24 h and P_N was measured with a portable photosynthetic system (*CIRAS-2, PP Systems*, Herts, UK) at 25ºC and 40ºC under a concentration of ambient CO_2 (360 μmol mol^{-1}) and a PFD of 600 μmol m^{-2} s^{-1}. Before P_N measurement, tomato plants were adapted at 25ºC and a PFD of 100 μmol m^{-2} s^{-1} for about 30 min to make the stomata open and then adapted for about 15 min at a PFD of 600 μmol m^{-2} s^{-1}.

The procedure was carried out at 4ºC and dark. A leaf sample (0.25 g) was mashed in a mortar and pestle with 5 ml 80% acetone (v/v), the extract was filtered through two layers of nylon and added to 25 ml with 80% acetone. Then it was centrifuged in sealed tubes at 15,000 × *g* for 5 min. The supernatant was collected and read at 663 and 645 nm for Chl *a* and Chl *b*, respectively, and at 470 nm for Car content. The concentrations for Chl *a*, Chl *b*, and the sum of leaf Car were given in μg ml^{-1} extract solution according to the equations of Bruinsma (1961).

Electron microscopy: T_1-8 and WT plants were used for microscopic analysis. Leaf samples were collected from five plants of each genetic source after high-temperature stress. Whole leaves were pinned onto Silgard-coated plastic and overlaid with a fixing solution (3.5% glutaraldehyde). Thereafter, samples were washed with 0.1 M PBS buffer, then briefly postfixed in 1% osmium tetroxide, dehydrated in an ascending ethanol series from 10 to 70% ethanol, preceded the endosmosis, embedment and polymerization of material into Epon812 resin. Thin sections were cut from the embedded samples using a *LKB-V* ultramicrotome (*LKB-V*, *Bromma*, Stockholm, Sweden). Sections were stained with uranium acetate and lead citrate, and examined under a transmission electron microscope (*JEOL-1200EX, JEOL UK Ltd*., Basingstoke, UK).

Results

Fatty acid composition in leaves under high temperature: To elucidate the effects of antisense expression of *LeFAD7* on fatty acid compositions, we detected the fatty acid composition in leaves of transgenic and WT plants. In transgenic plants, 18:3 contents were significantly decreased and 18:2 contents were increased correspondingly compared with WT plants (Fig. 1). Under high-temperature stress, 16:0 and 18:2 contents were upregulated in both transgenic and WT plants, and 16:1(3t) and 18:3 contents were decreased correspondingly, of which the increases of 18:2 contents were more significant in transgenic plants than those in WT plants and the decrease of 18:3 contents as well (Fig. 1). At the same time, the index of unsaturated fatty acid

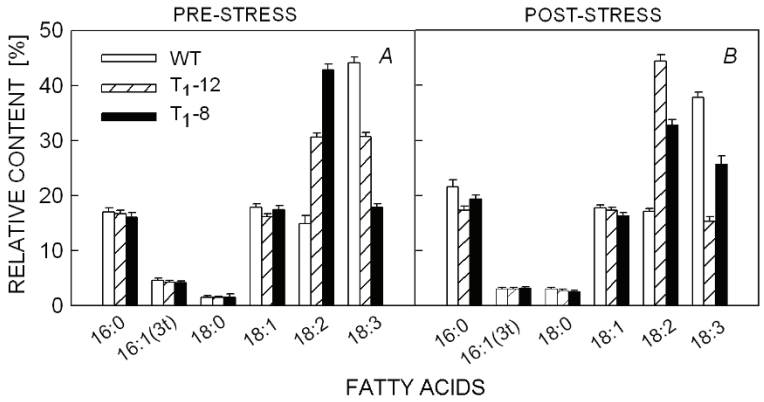

Fig. 1. Fatty acid composition of leaf tissue of WT and transgenic (T_1-12, T_1-8) tomato leaves at different conditions. *A*: The plants were grown at 25ºC; *B*: The plants were treated with 40ºC for 24 h. Means ± SD; $n = 3$.

Table 1. The effect of high temperature (40°C, 24 h) on fatty acid composition of thylakoid membrane lipids of wild type (WT) and transgenic tomato (T_1) leaves. - Present at trace levels (<0.1% of total fatty acid). Means±SD (n = 3) of three measurements on each of three plants are presented as mole percentage. P<0.05. Significant level (*Duncan*-test). Standard deviations between triplicates were <3% of the indicated values.

Lipid	Genotype	Fatty acid composition					
		16:0	16:1(3t)	18:0	18:1	18:2	18:3
MGDG	WT	7.14 ± 0.5^a	0	-	29.05 ± 0.7^a	13.22 ± 0.5^c	50.59 ± 1.7^a
	T_1-12	6.31 ± 0.6^a	0	-	23.28 ± 0.5^b	28.55 ± 0.8^b	41.86 ± 0.3^b
	T_1-8	4.90 ± 0.4^b	0	0.76 ± 0.2	17.99 ± 0.6^c	55.43 ± 1.5^a	20.92 ± 0.9^c
DGDG	WT	19.69 ± 0.5^a	0	1.54 ± 0.4^a	8.79 ± 0.8^a	9.34 ± 0.5^b	60.64 ± 1.1^a
	T_1-12	18.43 ± 0.4^b	0	1.66 ± 0.2^a	4.4 ± 0.5^b	41.37 ± 1.0^a	34.14 ± 0.7^b
	T_1-8	18.08 ± 0.5^b	0	1.83 ± 0.1^a	6.59 ± 0.5^c	42.66 ± 1.1^a	30.84 ± 0.9^c
SQDG	WT	41.54 ± 0.5^a	0	5.36 ± 0.5^a	11.03 ± 0.2^c	15.25 ± 0.3^c	26.82 ± 0.6^a
	T_1-12	38.42 ± 0.6^b	0	5.43 ± 0.1^a	14.23 ± 0.6^b	23.3 ± 0.8^b	18.62 ± 0.5^b
	T_1-8	33.51 ± 0.4^c	0	$6.01 \pm 0.5a$	17.17 ± 0.6^a	36.94 ± 1.1^a	6.38 ± 0.2^c
PG	WT	17.68 ± 0.6^a	12.38 ± 0.5^a	5.22 ± 0.3^a	21.97 ± 0.5^c	30.73 ± 0.8^c	13.02 ± 0.4^a
	T_1-12	14.25 ± 0.2^b	12.86 ± 0.4^a	3.18 ± 0.1^b	23.42 ± 0.4^b	38.00 ± 1.1^b	8.29 ± 0.2^b
	T_1-8	12.60 ± 0.3^c	12.27 ± 0.5^a	2.43 ± 0.2^c	27.57 ± 0.5^a	42.24 ± 1.0^a	2.89 ± 0.3^c

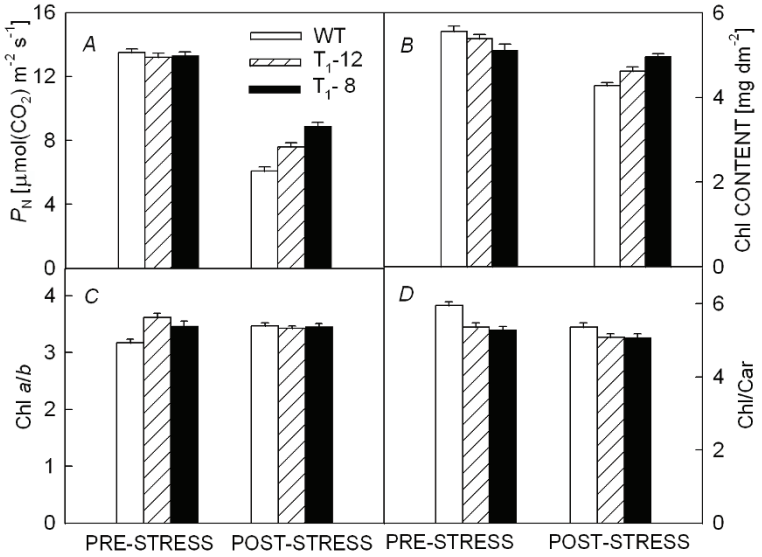

Fig. 2. Effects of high temperature (40ºC, 24 h) on P_N, chlorophyll (Chl) content, Chl *a*/*b* ratio and Chl/carotenoid (Car) ratio in WT and transgenic tomato leaves. Means ± SD; n = 5.

(IUFA = 18:1 × 1 + 18:2 × 2 + 18:3 × 3) was decreased after the treatment at 40ºC for 24 h. It was 156.8%, 169.7%, and 180.0% in leaves of T_1-8 and T_1-12 lines, and WT plants, respectively, before treatment. Under high-temperature stress, we observed that the IUFA was 151.7%, 159.1%, and 165.3% in T_1-8 and T_1-12 lines, and WT plants, respectively.

We determined the fatty acid composition of main polar lipid in leaves of transgenic and WT plants, of which MGDG and DGDG with primary component 18:3 had higher proportions than other lipids. Under high-temperature stress, we observed that 18:3 content of transgenic and WT plants was decreased with a weak increase of 18:2 in MGDG, DGDG, SQDG and PG compared with pre-stress. Also we found 18:3 content of T_1-8, in which expression level of *LeFAD7* was lowest, was about half of WT in MGDG and DGDG, about 25% of WT in SQDG and PG after treatment (Table 1).

Effects of high temperature on P_N and pigment content: When the expression of *LeFAD7* was inhibited in tomato, the decreased 18:3 content did not significantly influence P_N and Chl content under normal growth condition (Fig. 2). After the treatment of 40ºC for 24 h, P_N and Chl content in WT were markedly decreased, while decreased slowly in transgenic plants compared with WT plants, especially in T_1-8. The Chl content in T_1-8 plants was similar with pre-stress under high-temperature stress. A decreased Chl/Car ratio in both transgenic and WT plants and increased Chl *a*/*b* ratio only in WT were detected after treatment (Fig. 2).

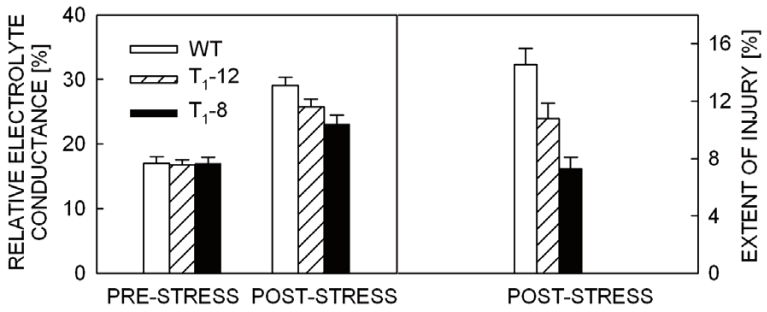

Fig. 3. Effects of high temperature (40ºC, 24 h) on relative electrolyte conductivity and index of injury in WT and transgenic tomato leaves. Means ± SD; $n = 5$.

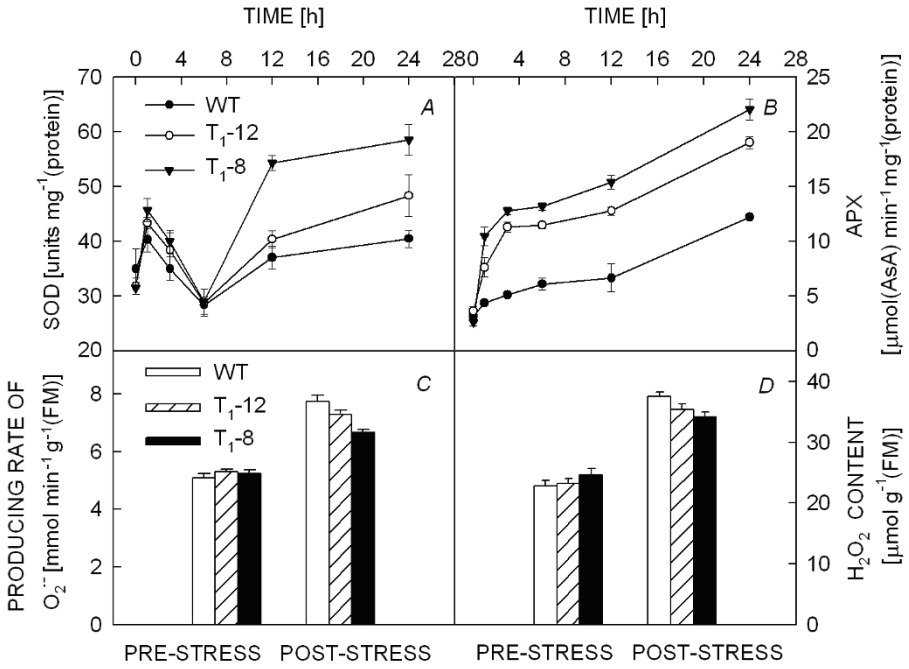

Fig. 4. Superoxide dismutase (SOD), ascorbate peroxidase (APX) activity and reactive oxygen species in WT and transgenic tomato leaves during high temperature stress (40ºC) under the light of 100 μmol(photon) m^{-2} s^{-1}. Reactive oxygen species in WT and transgenic tomato leaves were obtained after the treatment of 40ºC for 24 h. Means ± SD; $n = 5$.

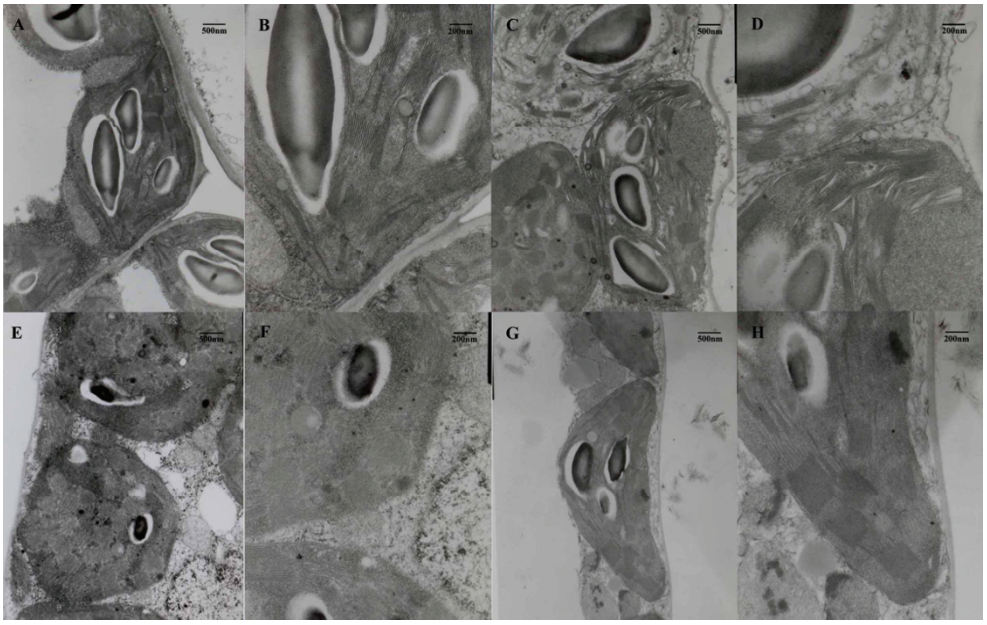

Fig. 5. Chloroplast ultrastructure in WT and transgenic tomato leaves. Transmission electron micrographs of chloroplast ultrastructure in WT (*A,B,E,F*) and T_1-8 tomato leaves (*C,D,G,H*). *A,B,C,D* were under normal conditions (25ºC, 100 μmol m^{-2} s^{-1}) and *E,F,G,H* were treated for 24 h at 40ºC under the low irradiance (100 μmol m^{-2} s^{-1}). *A, C, E, G*: ×10,000; *B, D, F, H*: ×25,000.

Effects of high temperature on membrane permeability and index of injury in leaves: Under normal growth conditions, transgenic plants exhibited similar relative electrolyte conductivity. When plants were under high-temperature stress (40ºC for 24 h), the relative electrolyte conductivity of WT plants showed a much greater increase compared with transgenic plants. Correspondingly, the extent of injury was much lower in transgenic plants than that of WT plants (Fig. 3).

Effects of high temperature on activities of antioxidative enzymes and reactive oxygen species content in leaves: After high-temperature treatment, the activity of SOD was firstly increased within 1 h, then decreased, and afterwards increased again after 6 h both in transgenic and WT plants (Fig. 4*A*). The activity of APX constantly increased during the high-temperature stress both in transgenic and WT plants (Fig. 4*B*). In transgenic plants, the activities of SOD and APX were retained a relative higher level than that of WT plants during high-temperature stress (Fig. 4*A,B*). After the treatment of 40ºC and 24 h, we detected lower level of reactive oxygen species, $O_2^{\cdot-}$ and H_2O_2 in transgenic plants compared with the WT ones (Fig. 4*C,D*).

Effects of high temperature on chloroplast ultrastructure in leaves: Chloroplast ultrastructure and organization appeared similar in WT and T_1-8 plants before high-temperature stress (Fig. 5*A,B,C,D*). However, high-temperature stress resulted in extensive changes of chloroplast ultrastructure in WT plants. Most of chloroplasts became circular and the number of amyloid increased. At the same time, appressed granum stacks were dissolved, grana disappeared and the number of grana decreased in WT plants (Fig. 5*E,F*). Compared with WT plants, chloroplast ultrastructure changed relatively less in T_1-8 plants when plants were exposed to high-temperature stress. After high-temperature stress, a few chloroplasts became circular and most of the grana retained normal appressed granum stacks. Only a few of them were obscure in leaves of transgenic plants (Fig. 5*G,H*).

Discussion

Omega-3 fatty acid desaturase has been considered to be the rate-limiting enzyme for the production of TAs (Iba *et al*. 1993). Therefore, the control of the expression of omega-3 fatty acid desaturase genes by genetic techniques is one of the most effective methods for the modification of the content of DAs and TAs. Previously, we developed antisense-expressed *LeFAD7* transgenic tomato that was supposed to exhibit tolerance to high-temperature stress (Liu *et al*. 2006). Here, we studied how antisense-expressed *LeFAD7* could alleviate tomato high-temperature stress.

Several different mechanisms related to protection against high temperature have been reported (Wahid *et al* 2007, Guo *et al*. 2006, Havaux and Tardy 1997, Heckathorn *et al*. 1998). Saturation/unsaturation of thylakoid membrane lipids was considered to be extraordinarily associated with temperature stress, of which saturation of thylakoid membrane lipids by catalytic hydrogenation increases the thermal stability of membranes (Thomas *et al*. 1986). It had been proposed that increased saturation of fatty acids from membrane lipids may raise the phase-separate temperature at which lipids such as MGDG become into nonbilayer structures, which disrupt membrane organization (Murakami *et al*. 2000, Thomas *et al*. 1986). If so, the tolerance to high temperature might be improved by reducing the content of unsaturated fatty acids such as TAs and increasing the content of saturated fatty acids (Hugly *et al*. 1989, Kunst *et al*. 1989). Here, we demonstrated that antisense-expressed *LeFAD7* in tomato reduced the level of TAs and increased the level of DAs under high-temperature stress and exhibited high-temperature tolerance. It should be noted that in some species high-temperature tolerance does not correlate with the degree of lipid saturation, suggesting that factors rather than membrane stability might limit the growth at high temperature.

Sustained function of cellular membranes under stress is pivotal for physiological processes such as photosynthesis and respiration (Blum 1988). PSII is highly thermolabile and its activity is greatly reduced or even stopped under high-temperature stress (Camejo *et al*. 2005 missing), which may be due to the changes on thylakoid membranes where PSII is located (Mcdonald and Paulsen 1997). Several reports suggested that increased saturated fatty acids in membranes maintained relative higher photosynthesis and growth rate (Murakami *et al*. 2000, Sohn and Back 2007). However, still transgenic tobacco plants with increased 16:0 levels in PG lipid class showed no difference in PSII activity at low or high temperature (Moon *et al*. 1995). PG and the light-harvesting Chl *a*/*b* protein complex of PSII (LHCII) are linked by a covalent bond. In our studies, transgenic tomato plants could retain higher P_N (Fig. 2), F_v/F_m and O_2 evolution (Liu *et al*. 2006). Compared with WT plants, the content of PG of total polar lipids was increased to 2.25-fold in the T_1-8 line and 1.71-fold in the T_1-12 line (data not shown). Transgenic tomato plants with a relatively higher content of PG may maintain a relatively higher photosynthesis and high-temperature tolerance.

The integrity and functions of biological membranes are sensitive to high temperature because high-temperature stress alters the tertiary and quaternary structures of membrane proteins. The electrolytes leakage, as an indication of decreased cell membrane thermostability, has long been used as an indirect measure of high-

temperature stress tolerance in diverse plant species, such as potato and tomato (Chen *et al*. 1982), soybean (Martineau *et al*. 1979) and barley (Wahid and Shabbir 2005). In our investigations, we showed decreased relative electrolyte conductance in transgenic plants compared with WT, though it was increased in both transgenic and WT plants under high-temperature stress (Fig. 3). The lower permeability of membranes in transgenic plants may stabilize tertiary and quaternary structures of membrane proteins, and thus, antioxidative enzymes can maintain relatively higher activities than that of WT under high-temperature stress (Fig. 4). In addition, several physiological parameters, such as Chl *a*/*b* ratio, Chl/Car ratio and the contents of active oxygen species were suggested to be associated with tolerance to high temperature (Camejo *et al*. 2005, Camejo *et al*. 2006, Guo *et al*. 2006, Wahid and Ghazanfar 2006). Here, we observed Chl/Car ratio was decreased while Chl *a*/*b* ratio was increased in transgenic plants before treatment, however, no increase for transgenic plants was detected under high-temperature stress (Fig. 2).

In conclusion, in this study we demonstrated that antisense-mediated depletion of *LeFAD7* enhanced the high-temperature tolerance of tomato plants through reduction of TAs. As the global warming, the omega-3 fatty acid desaturase enzyme, which is expressed in almost all the plant species, may be widely useful in genetic modification of high-temperature tolerance in plants.

References

Alfonso, M., Yruela, I., Almárcegui, S., Torrado, E., Pérez, M.A., Picorel R.: Unusual tolerance to high temperatures in a new herbicide-resistant D1 mutant from *Glycine max* (L) Merr. cell cultures deficient in fatty acid desaturation. – Planta **212**: 573-582, 2001.

Blum, A.: Plant Breeding for Stress Environments. – CRC Press Inc, Boca Raton 1988.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. – Anal. Biochem. **72**: 248-254, 1976.

Bruinsma, J.: A comment on the spectrophotometric determination of chlorophyll. – Biochemica and Biophysica Acta. **52**: 576-578, 1961.

Camejo, D., Jiménez, A., Alarcón, J.J., Torres, W., Gómez, J.M., Sevilla, F.: Changes in photosynthetic parameters and antioxidant activities following heat-shock treatment in tomato plants. – Funct. Plant Biol. **33**: 177-187, 2006.

Camejo, D., Rodríguez, P., Morales, M.A., Dell'amico, J.M., Torrecillas, A., Alarcón, J.J.: High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. – J. Plant Physiol. **162**: 281-289, 2005.

Chen, T.H.H., Shen, Z.Y., Lee, P.H.: Adaptability of crop plants to high temperature stress. – Crop Sci. **22**: 719-725, 1982.

Chen, Z.Q., Xu, C.H., Chen, M.J., Xu, L., Wang, K.F., Lin, S.Q.: Effect of chilling acclimation on thylakoid membrane protein of wheat. – Acta Bot. Sin. **36**: 423-429,1994.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. I. Occurrence in higher plants. – Plant Physiol. **59**: 309-314, 1977.

Gibson, S., Arondel, V., Iba, K., Sommerville, C.: Cloning of a temperature-regulated gene encoding a chloroplast omega-3 desaturase from *Arabidopsis thaliana*. – Plant Physiol. **106**: 1615-1621, 1994.

Guo, Y.P., Zhou, H.F., Zhang, L.C.: Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. – Sci. Hort. **108**: 260-267, 2006.

Havaux, M., Tardy, F.: Thermostability and photostability of photosystem II in leaves of the Chlorina-f2 barley mutant deficient in light-harvesting chlorophyll *a*/*b* protein complexes. – Plant Physiol. **113**: 913-923, 1997.

Heckathorn, S.A., Downs, C.A., Sharkey, T.D., Coleman, J.S.: The small, methionine-rich chloroplast heat-shock protein protects photosystem II electron transport during heat stress. – Plant Physiol. **116**: 439-444, 1998.

Hugly, S., Kunst, L., Browse, J., Somerville, C.: Enhanced thermal tolerance of photosynthesis and altered chloroplast ultrastructure in a mutant of *Arabidopsis* deficient in lipid desaturation. – Plant Physiol. **90**: 1134-1142, 1989.

Iba, K., Gibson, S., Nishiuchi, T., Fuse, T., Nishimura, M., Arondel, V., Hugly, S., Somerville, C.: A gene encoding a chloroplast omega-3-fatty-acid desaturase complements alterations in fatty-acid desaturation and chloroplast copy number of the fad7 mutant of *Arabidopsis thaliana*. – J. Biol. Chem. **268**: 24099-24105, 1993.

Iba, K.: Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. – Annu. Rev. Plant Biol. **53**: 225-245, 2002.

Jimenez, A., Hernandez, J.A., del Rio, L.A., Sevilla, F.: Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. – Plant Physiol. **114**: 275-284, 1997.

Kodama, H., Horiguchi, G., Nishiuchi, T., Nishimura, M., Iba, K.: Fatty-acid desaturase during chilling acclimation is one of the factors involved in conferring low-temperature tolerance to young tobacco-leaves. – Plant Physiol. **107**: 1177-1185, 1995.

Kunst, L., Browse, J., Somerville, C.: Enhanced thermal tolerance in a mutant of *Arabidopsis* deficient in palmitic acid unsaturation. – Plant Physiol. **91**: 401-408, 1989.

Liu, X.Y., Yang, J.H., Li, B., Yang, X.M., Meng, Q.W.: Antisense-mediated depletion of tomato chloroplast omega-3 fatty acid desaturase enhances thermal tolerance. – J. Integr. Plant Biol. **48**: 1096-1107, 2006.

Martineau, J.R., Specht, J.E., Williams, J.H., Sullivan, C.Y.: Temperature tolerance in soybean. 1. Evaluation of technique for assessing cellular membrane thermostability. – Crop Sci. **19**: 75-78, 1979.

McConn, M., Browse, J.: The critical requirement for linolenic acid is pollen development, not photosynthesis, in an arabidopsis mutant. – Plant Cell **8**: 403-416, 1996.

McDonald, G.K., Paulsen, G.M.: High temperature effects on photosynthesis and water relations of grain legumes. – Plant Soil **196**: 47-58, 1997.

Moon, B.Y., Higashi, S.I., Gombos, Z., Murata, N.: Unsaturation of membrane-lipids of chloroplasts stabilizes the photosynthetic machinery against low-temperature photo-

inhibition in transgenic tobacco plants. – Proc. Natl. Acad. Sci. USA **92**: 6219-6223, 1995.

Murakami, Y., Tsuyama, M., Kobayashi, Y., Hiroaki, K., Iba, K.: Trienoic fatty acids and plant tolerance of high temperature. – Science **287**: 476-479, 2000.

Pearcy, R.: Effect of growth temperature on the fatty-acid composition of the leaf lipids in *Atriplex lentiformis* (Torr) Wats. – Plant Physiol. **61**: 484-486, 1978.

Raison, J.K., Chapman, A., Wright, L.C., Jacobs, S.W.L.: The role of the membrane. – In: Lyons J.M., Graham D.J., Raison H.K. (ed.): Low Temperature Stress in Crop Plants. Pp. 177-186, Academic Press, New York 1979.

Routaboul, J.M., Fischer, S.F., Browse, J.: Trienoic fatty acids are required to maintain chloroplast function at low temperatures. – Plant Physiol. **124**: 1697-1705, 2000.

Sairam, R.K., Srivastava, G.C.: Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. – Plant Sci. **162**: 897-904, 2002.

Santos, C.L.V., Campos, A., Azevedo, H., Caldeira, G.: In situ and in vitro senescence induced by KCl stress: nutritional imbalance, lipid peroxidation and antioxidant metabolism. – J. Exp. Bot. **52**: 351-360, 2001.

Siegenthaler, P.A., Eichenberger, W.: Structure, function and metabolism of plant lipids. – In: Plant Lipids-Metabolism-Congresses. Pp. 485-488. Elsevier Science Publ., Amsterdam 1984.

Sohn, S.O., Back, K.: Transgenic rice tolerant to high temperature with elevated contents of dienoic fatty acids. – Biol. Plant. **51**: 340-342, 2007.

Su, W.A., Wang, W.Y., Li, J.S.: Analysis of plant lipid and fatty acid. – Plant Physiol. Commun. **3**: 54-60, 1980.

Thomas, P.G., Dominy, P.J., Vigh, L., Mansourian, A.R., Quinn, P.J., Williams, W.P.: Increased thermal stability of pigment-protein complexes of pea thylakoids following catalytic hydrogenation of membrane lipids. – Biochim. Biophy. Acta **849**: 131-140, 1986.

Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R.: High-temperature tolerance in plants: an overview. – Environ. Exp. Bot. **61**: 199-223, 2007.

Wahid, A., Ghazanfar, A.: Possible involvement of some secondary metabolites in salt tolerance of sugarcane. – J. Plant Physiol. **163**: 723-730, 2006.

Wahid, A., Shabbir, A.: Induction of high-temperature stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. – Plant Growth Regul. **46**: 133-141, 2005.

Wang, A.G., Luo, G.H.: Quantitative relation between the reaction of hydroxylamine and superoxide anion radicals in plants. – Plant Physiol. Commun. **6**: 55-57, 1990.

Xu, Y.N., Siegenthaler, P.A.: Low temperature treatments induce an increase in the relative content of both linolenic and delta(3)-trans-hexadecenoic acid in thylakoid membrane phosphatidylglycerol of squash cotyledons. – Plant Cell Physiol. **38**: 611-618, 1997.