Antisense-mediated suppression of tomato zeaxanthin epoxidase alleviates photoinhibition of PSII and PSI during chilling stress under low irradiance

N. WANG*, B. LI*, H.-L. FENG, Q.-Y. ZHANG, X.-H. YANG, and Q.-W. MENG+

College of Life Science, State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, 271018, P.R. China

Abstract

A tomato (*Lycopersicon esculentum* Mill.) zeaxanthin epoxidase gene (*LeZE*) was isolated and antisense transgenic tomato plants were produced. Northern, southern, and western blot analyses demonstrated that antisense *LeZE* was transferred into the tomato genome and the expression of *LeZE* was inhibited. The ratio of (A+Z)/(V+A+Z) in antisense transgenic plants was maintained at a higher level than in the wild type (WT) plants under high light and chilling stress with low irradiance. The value of non-photochemical quenching (NPQ) in WT and transgenic plants was not affected during the stresses. The oxidizable P700 and the maximal photochemical efficiency of PSII (F_v/F_m) in transgenic plants decreased more slowly at chilling temperature under low irradiance. These results suggested that suppression of *LeZE* caused zeaxanthin accumulation, which was helpful in alleviating photoinhibition of PSI and PSII in tomato plants under chilling stress.

Additional key words: antisense transgenic plants; chilling stress under low irradiance; photoinhibition; tomato; zeaxanthin epoxidase gene.

Introduction

Light is necessary for photosynthesis in higher plants, but if the absorbed light exceeds the plant's photosynthetic capacity, excessive irradiation, which is not promptly quenched, can lead to photoinhibition and photodamage (Foyer *et al*. 1994, Telfer *et al*. 1994, Pastenes *et al*. 2005). Photoinhibition occurs in the field in plants exposed to conditions of high light (Long *et al*. 1994). Low temperature sensitizes photosynthesis to photoinhibition, so that even low light may induce photoinhibition (Boese and Huner 1992, Feierabend *et al*. 1992, Long *et al*. 1994, Xu *et al*. 1999, Bertamini *et al*. 2006). This is partly because lower temperature generally reduces the rates of biological reactions, particularly carbon dioxide reduction and photorespiration, and therefore limits the sinks for the absorbed excitation energy (Allen and Ort 2001). The crucial events of PSII photoinhibition are the turnover of protein D1 in the reaction center (Aro *et al*. 1993, Zhang *et al*. 2000). Some reports suggested that PSI had a greater chilling sensitivity than PSII upon exposure to chilling under low irradiance, especially in chilling-sensitive plants, such as cucumber, tomato, *etc*. (Sonoike and Terashima 1994, Sonoike 1996, Li *et al*. 2004). During chilling stress, reactive oxygen species (ROS) are produced, which are associated with the photoinhibition of PSI (Sonoike *et al*. 1997, Li *et al*. 2003, 2004). Using thylakoids or intact chloroplasts of spinach, Jakob and Heber (1996) found that PSI photoinhibition occurred concomitant with the accumulation of ROS.

In their long-term evolution, plants have developed many protective mechanisms to balance the absorbed light energy with photosynthesis, thereby protecting the

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+Corresponding author; tel: +86 538 8249606, fax: +86 538 8226399; e-mail: qwmeng@sdau.edu.cn
Abbreviations: A – antheraxanthin; Chl – chlorophyll; F_m – maximum yield of fluorescence in darkness; F_m' – maximum yield of fluorescence during illumination; F_o – initial fluorescence; F_v – variable fluorescence; F_v/F_m – maximal photochemical efficiency of PSII; HPLC – high performance liquid chromatography; *LeZE* – *Lycopersicon esculentum* zeaxanthin epoxidase gene; NADPH – nicotinamide adenine dinucleotide phosphate; NPQ – non-photochemical quenching; PPFD – photosynthetic photon flux density; PSI – photosystem I; PSII – photosystem II; ROS – reactive oxygen species; V – violaxanthin; VDE – violaxanthin de-epoxidase; Z – zeaxanthin; ZE – zeaxanthin epoxidase.
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*Both these authors contributed equally to this work.

photosynthetic apparatus against photoinhibition (Horton *et al*. 1996, Niyogi *et al*. 1998, 2001). One of the protective mechanisms is the xanthophyll cycle-dependent thermal energy dissipation measured as non-photochemical quenching of chlorophyll fluorescence (NPQ), which protects the photosynthetic apparatus from inactivation and damage caused by excess excited energy (Demming-Adams and Adams 1996, Huner *et al*. 1998, Müller *et al*. 2001, Li *et al*. 2003, 2005). The xanthophyll cycle transforms the excitation energy into heat and thereby prevents the formation of damaging ROS. In this way it protects the photosynthetic apparatus against photodamage (Huner *et al.* 1998, Müller *et al.* 2001, Li *et al.* 2003, 2004). NPQ might be an efficient pathway for plants to alleviate PSII photoinhibition (Xu *et al*. 1999, Liu *et al*. 2001).

The xanthophyll cycle involves interconversions between the three pigments, violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). The conversion is catalyzed by two enzymes, violaxanthin de-epoxidase (VDE: EC1.10.99.3) and zeaxanthin epoxidase (ZE: EC1.14.13.90). In excess light conditions, VDE catalyzes the conversion of V to Z *via* A, whereas ZE catalyzes the reverse reaction (Yamamoto 1979, Hager 1980). A correlation between an increase in NPQ and zeaxanthin formation under excess light conditions suggests that zeaxanthin has a photoprotective function, dissipating excess energy by quenching. A stronger correlation was obtained when the sum of antheraxanthin and zeaxanthin was correlated with NPQ („Adams *et al*. 1995). It is widely thought that the main function of zeaxanthin is quenching the $^1Chl^*$ state directly through the process of NPQ (Müller *et al*. 2001). However, previous research has shown that zeaxanthin may protect the photosynthetic apparatus from light stress by directly quenching $^1O_2^*$ and other ROS (Havaux and Niyogi 1999). Moreover, zeaxanthin could also have the function of decreasing light-harvesting Chl antenna size and decreasing the fluidity of the membrane in order to lower the penetration of ROS inside the thylakoid (Havaux 1998, Müller *et al*. 2001, Havaux *et al*. 2004).

ZE is a member of the lipocalin family (Bugos *et al*. 1998), which localizes on the stromal side of the chloroplast thylakoid membrane and catalyzes the conversion of zeaxanthin to antheraxanthin and violaxanthin. ZE is also the key enzyme in abscisic acid (ABA) biosynthesis. Previous work concerning ZE has focused on its role in abscisic acid (ABA) biosynthesis during drought stress, in seed development and the relationship between impaired zeaxanthin epoxidase and photosynthesis using a mutant lacking ZE activity (Rock *et al*. 1991, Tardy and Havaux 1996, Hurry *et al*. 1997, Audran *et al*. 1998, Thompson *et al*. 2000). Questions that need to be resolved are whether the suppression of *LeZE* can change the components of the xanthophyll cycle, and whether zeaxanthin accumulation is helpful in alleviating photoinhibition of PSI and PSII in tomato plants under chilling stress.

To understand the physiological effects and functional mechanism of the xanthophyll cycle in tomato at chilling temperatures, the *LeZE* was isolated (Burbidge *et al*. 1997, Wang *et al*. 2008) and antisense *LeZE* was introduced to the same plants to inhibit the expression of *LeZE*. Using antisense transgenic tomato plants, we investigated the effect of *LeZE* depletion on the content of xanthophyll cycle pigments and the role of increased Z in protecting the photosynthetic apparatus from chilling stress.

Materials and methods

Plant materials and treatments: Seeds of tomato cultivar (*Lycopersicon esculentum* cv. Zhongshu 4) were germinated and treated as described previously (Wang *et al.* 2008). The 7-week-old WT and transgenic plants were exposed to high light and chilling stresses, respectively.

Northern blot analysis: *LeZE* was isolated as described as Wang *et al.* (2008). 20 μg of total RNA were separated on a 1.2% agarose formaldehyde gel and transferred to nylon membrane as described by Sambrook *et al*. (1989). RNA was fixed on the membrane by cross-linking with ultraviolet light. Pre-hybridization was performed at 65°C for 12 h. The 3′ partial cDNA 0.4 kb of *LeZE* was used as a gene-specific probe and labeled with [α-^{32}P]-dCTP by the randomly prime labeling method (*Prime-a-Gene-Labeling System, Promega*, Madison, USA). After 24 h of hybridization, filters were subsequently washed in 2 × standard saline citrate (SSC) (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) with 0.2% SDS and 0.2 × SSC with 0.2% SDS at 42 °C. Autoradiography was performed at –80°C.

Southern blot analysis: 10 g of genomic DNA from the leaves of WT and antisense transgenic line T_1-1 were digested with *Eco*RI, *Xho*I and *Bam*HI, separated by electrophoresis on a 0.7% agarose gel, and blotted onto a nylon membrane by the alkaline transfer method. Two probes were synthesized. One was the 5′ non-coding regions of *LeZE,* which was amplified by primers ZP51 (5'-GATATCAATCTTCCTTGC-3') and ZP52 (5'- CAG TCTTCTCTGTAGGAG-3'); the other was the full length of *LeZE*. The membrane was then hybridized with the two specific cDNA probes, washed with 0.1 × SSC and 0.1% sodium dodecyl sulfate (SDS) at 65°C for 1 h, and autoradiography was performed at –80°C.

SDS-PAGE and immunological analysis: A coding region of *LeZE* in the pMD18-T vector of about 2,010 bp

was subcloned into the pET-30a(+) vector between the *Nco*I and *Sal*I sites. A recombinant of prokaryotic expression vector pET-LeZE was constructed and transformed to *E. coli* BL21 and then expressed by inducing with IPTG. Depositions were dissolved in the presence of 2 × SDS loading buffer and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using 10% separate gels and 4% concentrated gels and containing 10% SDS. The strong induced fusion protein bands were collected into phosphate buffer solution (PBS) and were used to immunize white mice to obtain antiserum. The secondary antibody was peroxidase-conjugated goat anti-mouse IgG from *Santa Cruz Biotechnology, Inc*. (Santa Cruz, CA, USA). The antibody was used at a dilution of 1:500 and the secondary antibody was used at a dilution of 1:5000. For immunoblotting, polypeptides were electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane (*Millipore*, France). Proteins in the WT and transgenic lines were detected with antibodies raised against *LeZE*. The protein content was determined by dye-binding assay (Bradford 1976).

Plasmid construction and Agrobacterium-mediated transformation of tomato plants: The full-length *LeZE* cDNA was subcloned into the pBI121 downstream of the 35S-CaMV promoter to form antisense constructs (pBI-RVLeGPAT). The 35S-CaMV antisense *LeZE* constructs were first introduced into *Agrobacterium tumefaciens* LBA4404 by the freezing transformation method and verified by PCR and sequencing. Leaf disk transformation using WT tomato plants was performed as described by Horsch *et al.* (1985). Discs infected with *A. tumefaciens* were incubated on medium for inducing shoots. After a few weeks, the regenerated shoots were transferred to medium to induce roots. Both media contained cefotaxime sodium (250 μg mL^{-1}) and kanamycin (50 μg mL^{-1}). Antisense transgenic plants were screened using kanamycin selection generated by the incubation of transformed tomato leaf disks (Holsters *et al.* 1978). As a consequence, twenty individual kanamycin resistant lines were obtained from tissue culture. Each transgenic line seemed to represent an independent integration event since a specific DNA fragment in each line was observed by genomic DNA gel blot analysis (data not shown). In order to further assess the expression of *LeZE* in screened plants of antisense transgenic tomato, the 3-month-old seedlings were subjected to molecular and physiological analyses.

Pigment analysis: Leaves were immersed in liquid N_2 immediately after F_v/F_m determination and stored at –80°C until use. Photosynthetic pigments were extracted from leaves with 80% ice-cold acetone. The extracts were centrifuged at 20,000 × *g* for 5 min and supernatants were filtered through a 0.45-μm membrane filter before injection into reversed-phase high performance liquid chromatography (HPLC) using a *Shimadzu Series model SCL-10AVP* (Japan) equipped with an *Elite Hypersil ODS2 4.6*-250 mm cartridge column (China). Photosynthetic pigments were separated and qualified essentially following the method of Zhao *et al*. (1995). The relative epoxidation state of the xanthophyll cycle pigments was calculated as (V + A)/(V + A + Z).

O_2 evolution: The O_2-evolution rates were determined using a modified Clarke-type O_2 electrode unit (*Hansatech*, King's Lynn, UK) as described by Walker (1990). After treatments, leaf discs (2 cm^2) were vacuumized with 0.1 M $NaHCO_3$. Then the leaf disks were dissected into pieces of about 1 mm^2 for CO_2 infiltration in a liquid phase. The reaction mixture was 0.1 M $NaHCO_3$ to maintain a high concentration of CO_2. The measurement was done at 25°C under 800 μmol m^{-2} s^{-1} PPFD.

Measurement of chlorophyll (Chl) fluorescence: Chl fluorescence from the attached leaves was measured with a portable fluorometer (*FMS2, Hansatech*, King's Lynn, UK) according to the protocol described by van Kooten and Snel (1990). The initial fluorescence (F_o) of PSII with all reaction centres open was determined by a modulated light that was low enough not to induce any significant variable fluorescence (F_v). The maximal fluorescence (F_m) with all reaction centres closed was determined by a 0.8-s saturating light of 7,000 μmol m^{-2} s^{-1} on a dark-adapted (adapted 15 min in the darkness) leaf. The maximal photochemical efficiency (F_v/F_m) of PSII was expressed as: $F_v/F_m = (F_m–F_o)/F_m$. NPQ was calculated according to Schreiber *et al*. (1994). NPQ was estimated as NPQ = $(F_m–F_m')/F_m'$, where F_m was measured after dark adaptation for more than 2 h at a room temperature prior to treatments. For high-light treatment, F_m', the maximum yield of fluorescence in light-acclimated leaves, was measured when plants were exposed to 1,200 μmol m^{-2} s^{-1} PPFD for 0, 1, 3, 6, 9, and 12 h under the room temperature. For chilling stress, F_m' was measured when plants were exposed to low temperature (4°C) under 100 μmol m^{-2} s^{-1} PPFD for 0, 1, 3, 6, 9, and 12 h. The light was produced by a microwave sulfur lamp (*MSL-1000*, *MSL-1000, Youhe Light Source*, NingBo, China) which releases less heat and emits uniform light.

Absorbance at 820 nm: Oxidation and reduction of P_{700} was measured at 820 nm with a *Plant Efficiency Analyzer* (*PEA*) senior (*Hansatech*, UK) as described by Schansker *et al*. (2003).

Results

Molecular characterization of the transgenic plants: A cDNA was isolated from tomato and designated as *LeZE* (Burbidge *et al*. 1997, Wang *et al*. 2008). Transgenic plants carrying the antisense *LeZE* were detected by PCR after the first screening with kanamycin (50 μg mL^{-1}) (data not shown). Twenty-two individual kanamycin resistant lines were obtained from tissue culture. RNA gel blot analysis at 25°C indicated that the expression of *LeZE* was inhibited completely in the T_1-1 line, but only partially in the T_1-5 line (Fig. 1). T_1-1 and T_1-5 lines were chosen as the physiological experimental materials in the following analysis, and T_1-1 was used as the material for southern blot analysis. The three strong positive signals of southern blot in transgenic plants compared with the signal in the WT showed that antisense *LeZE* had been introduced into the tomato genome (Fig. 2).

Xanthophyll cycle changes in transgenic tomato plants: Higher Z content was detected in antisense transgenic plants before and after stress, but A contents were hardly detected. After chilling and high-light stresses, Z in WT plants significantly increased but was still obviously lower than that in antisense transgenic plants (data not shown). In WT plants, the de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/(V+A+Z) markedly increased after 12 h of chilling and high-light stresses. However, the ratio (A+Z)/(V+A+Z) in antisense transgenic plants was remarkably higher than that in WT (Figs. 3*A,* 4*A*). These results indicated that suppression of *LeZE* caused Z accumulation and thereby affected the de-epoxidation state of the xanthophyll cycle.

Energy dissipation in PSII of WT and transgenic plants: The dissipation of excess energy was monitored by measuring the development of NPQ. WT and antisense transgenic plants exhibited de-epoxidation kinetics as shown by A+Z formation. NPQ increased when WT and antisense transgenic plants were exposed to chilling stress under low irradiance and high-light stress for 12 h. In both WT and two antisense transgenic lines, the NPQ significantly increased during the first 1 h and then increased gradually. The maximal value of NPQ was noted at the end of chilling and high-light stresses but there was no evident difference between WT and antisense transgenic plants (Figs. 3*B*; 4*B*).

Suppression of *LeZE* alleviates PSI and PSII photo-inhibition under chilling stress: Under high-light stress for 12 h, the O_2-evolution rates of WT and transgenic plants decreased. There was no evident difference between WT and antisense transgenic plants. After high-light stress for 12 h, the O_2-evolution rates in the WT, T_1-1, and T_1-5 decreased to 36.2, 34.5, and 35.1%, respectively (Fig. 3*C*). F_v/F_m decreased in both WT and transgenic plants under high-light stress, and there was no evident difference between WT and antisense transgenic plants either (Fig. 3*D*). At the end of high-light stress, F_v/F_m in WT, T_1-10, and T_1-1 lines decreased 24.5%, 23.9%, and 24.1%, respectively. It was suggested that the suppression of *LeZE* did not significantly affect the protection by the xanthophyll cycle under high-light stress.

On the other hand, the O_2-evolution rates also decreased in both WT and antisense transgenic plants under chilling stress with low irradiance for 12 h, with WT plants showing the greater decrease (Fig. 4*C*). After 12-h chilling stress, the O_2-evolution rates in the WT,

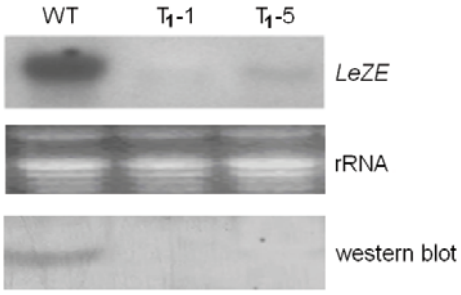

Fig. 1. Northern and western blot analyses of *LeZE* in transgenic tomato. Total RNA and protein were extracted from the WT and two antisense transgenic lines, respectively. The probe of northern blot was labeled with [α-^{32}P]-dCTP. About 20 μg of total RNA was analyzed by northern blot. The antibody against *LeZE* was produced by immunizing white mice and used at a dilution of 1:500. The dilution of the secondary antibody was 1:5,000. About 35 μg of protein was analyzed by the dye-binding assay.

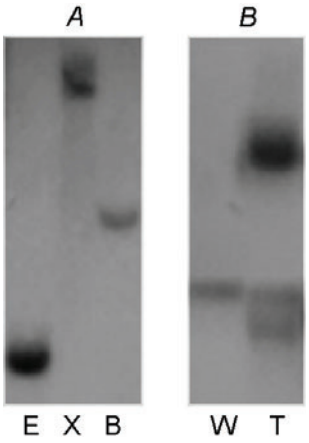

Fig. 2. Southern blot analysis of *LeZE*. DNA from a wild-type tomato plant (W) were cut with *Eco*R I (E), *Xho* I (X) and *Bam*H I (B), DNA from a transgenic line T_1-1 containing the LeZE antisense gene (T) were cut with *Eco*RI. *A*: probed with the 760 bp product of the 5′ noncoding regions of *LeZE*. *B*: probed with the full length of *LeZE*. Genomic DNA (25 μg) was digested using the indicated restriction enzymes. Separated on a 0.7% agarose gel and transferred to a nylon membrane.

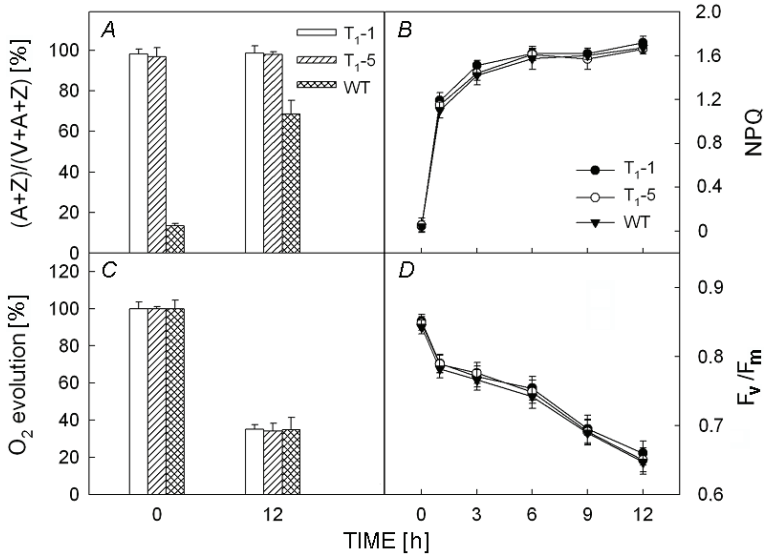

Fig. 3. Effect of high-light stress on de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/ (V+A+Z) (*A*), NPQ (*B*), oxygen evolution (*C*) and F_v/F_m (*D*) of WT and antisense transgenic plants. Oxygen evolution was measured 25°C under 800 μmol m^{-2} s^{-1} PPFD using tomato leaf discs treated under 1,200 μmol m^{-2} s^{-1} PPFD for 0 and 12 h. The original rates of control tomato plants before treatment were set at 100%. NPQ and F_v/F_m from tomato leaves treated under 1,200 μmol m^{-2} s^{-1} PPFD for 0, 1, 3, 6, 9, and 12 h. Before high-light stress, plants were adapted in the darkness for 2 h to measure F_v/F_m. During high-light stress, plants were adapted in, darkness for 15 min before F_v/F_m measurement. Each point represents the means±SD of five measurements on each of five plants.

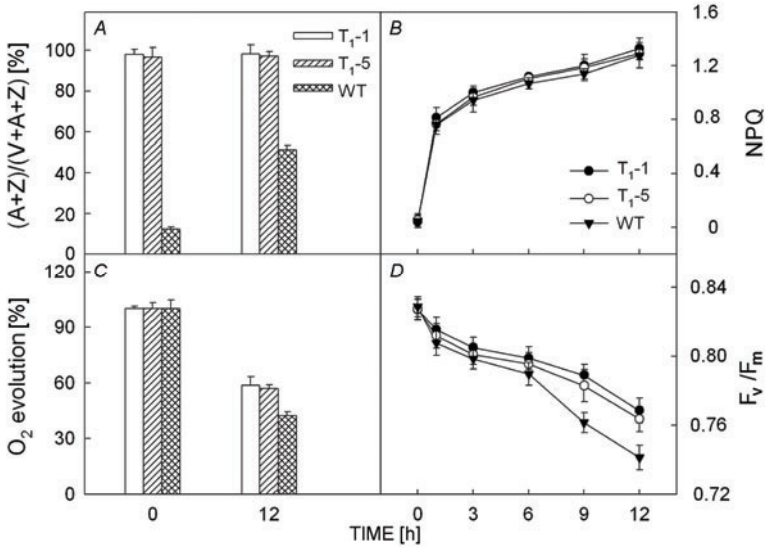

Fig. 4. Effect of chilling stress under low irradiance on de-epoxidation ratio of xanthophyll cycle pigments (A+Z)/ (V+A+Z) (*A*), NPQ (*B*), oxygen evolution (*C*) and F_v/F_m (*D*) of WT and antisense transgenic plants. Oxygen evolution was measured at 25°C under 800 μmol m^{-2} s^{-1} PPFD using tomato leaf discs treated under stress (4°C) in low irradiance (100 μmol m^{-2} s^{-1}) for 0 and 12 h. The original rates of control tomato plants before treatment were set at 100%. NPQ and F_v/F_m from tomato leaves treated with 100 μmol m^{-2} s^{-1} PFD at 4°C for 0, 1, 3, 6, 9, and 12 h. Before chilling stress, plants were adapted in the darkness for 2 h to measure F_v/F_m. During chilling stress, plants were adapted in the darkness for 15 min before F_v/F_m measurement. Each point represents the means±SD of five measurements on each of five plants.

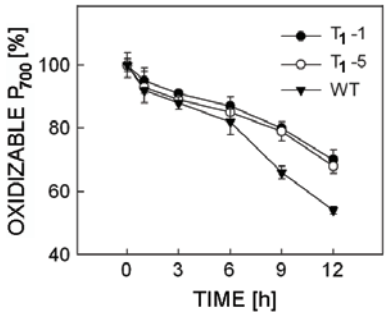

Fig. 5 The effect of chilling stress (4°C) under low irradiance (100 μmol m^{-2} s^{-1}) on oxidizable P_{700} in tomato leaves. Leaves were dark adaption for 15 min prior to measurement. For each line, the oxidizable P_{700} before treatment at 25°C was set at 100%. Means±SD of five measurements on each of five plants.

T_1-1, and T_1-5 decreased to 42.3%, 60.8%, and 58.7%, respectively. F_v/F_m also decreased significantly in WT plants during chilling stress relative to that in antisense transgenic plants (Fig. 4*D*). At the end of chilling stress, F_v/F_m in the WT, T_1-1, and T_1-5 decreased about 10.1%, 7.5%, and 7.9%, respectively. These results indicated that photoinhibition of PSII during chilling stress was alleviated in the antisense transgenic plants.

The oxidizable P_{700} decreased significantly both in WT and antisense transgenic plants under chilling stress with low irradiance (Fig. 5). This decrease was more obvious in WT than in transgenic plants. After 12-h chilling stress, the oxidizable P_{700} in WT, T_1-1, and T_1-5 decreased by about 46.3, 29.8, and 32.2%, respectively. These results indicated that suppression of *LeZE* was beneficial in protection from chilling-induced PSI photoinhibition.

Discussion

A cDNA encoding zeaxanthin epoxidase was previously isolated from a wilt-related tomato (Burbridge *et al*. 1997), but overexpression of the gene was studied only with a focus on ABA metabolism. In our previous study (Wang *et al*. 2008), overexpression of *LeZE* decreased the level of de-epoxidation and the NPQ under high-light- and chilling stresses (Wang *et al*. 2008). Here, we used antisense *LeZE* transformants as materials to investigate the relationship between zeaxanthin accumulation and photoinhibition of PSI and PSII under chilling stress. The expression of *LeZE* in transgenic tomato plants was inhibited completely or partially by an antisense-mediated approach (Fig. 1). The expression of *LeZE* may change the content of xanthophyll cycle pigments and therefore affect the level of de-epoxidation (A+Z)/(V+A+Z). The suppression of *LeZE* results in the accumulation of zeaxanthin, and the antisense transgenic plants maintained a high level of de-epoxidation (A+Z)/(V+A+Z) during high-light- and chilling stresses (Figs. 3*A*, 4*A*). It has been shown that zeaxanthin plays an important role in the formation of NPQ (Jin *et al*. 2003) with the ZE mutant (*zea1*). Although NPQ increased when WT and antisense transgenic plants were exposed to chilling stress under low irradiance and high light, there was no significant difference between WT and antisense transgenic plants (Figs. 3*B*, 4*B*).

The xanthophyll cycle-dependent NPQ is considered a very important mechanism for the protection of PSII against excess irradiance (Demmig-Adams and Adams 1996, Li *et al.* 2003, 2004, Xu *et al.* 1999). There was no evident difference in the O_2-evolution rates and F_v/F_m between WT and antisense transgenic lines during high-light stress (Fig. 3*C*,*D*). Nevertheless, the photoinhibition of PSII and PSI in WT was more severe than in transgenic plants at chilling temperature under low irradiance (Figs. 4*C*,*D*; 5). This suggests that there is a stronger photoprotection mechanism operating in transgenic lines T_1-1 and T_1-5 under chilling stress. Zeaxanthin is an antioxidant that functions to protect lipids under photooxidative stress and *Arabidopsis* mutants with zeaxanthin as the only xanthophyll are more resistant to photooxidative stress than WT exposed to high light and low temperatures (Havaux *et al*. 2004).

It has been established that xanthophyll cycle pigments exist not only in PSII but also in PSI (Thayer and Björkman 1992, Lee and Thornber 1995). Thermal dissipation in PSI antenna could be an efficient regulatory mechanism for Chl excitation in PSI (Croce *et al*. 1996) and could protect PSI from the adverse effects of excess excitation (Terashima *et al*. 1994, Tjus *et al*. 1998). PSI of the chilling-sensitive plants was more sensitive than PSII and easily damaged during chilling stress under low irradiance (Havaux and Davaud 1994), which might be caused by a decrease in the rate of carbon dioxide fixation and an accumulation of reducing power on the acceptor side of PSI (Terashima *et al*. 1994, Sonoike 1996). Under chilling stress, the oxidizable P_{700} of WT decreased faster than that in transgenic plants (Fig. 5). There are two possible explanations for this result. First, because zeaxanthin could affect membrane fluidity and decrease the rate of plastoquinone reoxidation at chilling temperatures (Gruszecki and Strzałka 1991, Havaux and Gruszecki 1993, Havaux 1998), more zeaxanthin in transgenic plants could prevent lipid peroxidation and increase the stability of PSI under chilling stress with low irradiance. Second, more zeaxanthin in transgenic plants could quench more ROS.

There are many mechanisms in higher plants that protect the photosynthetic apparatus against photoinhibition (Niyogi *et al*. 1998, 2001). However, these mechanisms might be impaired under chilling stress. It has been suggested that the membrane is the primary location damaged under chilling stress (Kratsch and Wise 2000). Chilling stress could impair membrane liquidity by the transition of membrane lipids from a liquid-crystalline phase to a gel phase, weakening the turnover of protein D1 (Allen and Ort 2001), and therefore the photodamage of PSII would be much more severe (Murata *et al*. 2007). The transition of membrane lipids from a liquid-crystalline phase to a gel phase can also affect the activity of thylakoid-bound APX and consequently affect the scavenging of ROS (Terashima *et al*. 1994, Havaux and Davaud 1994, Sonoike and Terashima 1994, Sonoike 1996). Furthermore, the activity of VDE was obviously inhibited under chilling stress, as VDE also localizes on the thylakoid membrane (Foyer and Lelandais 1996). So the function of the xanthophyll cycle in protecting the photosynthetic apparatus from chilling stress was impaired. In this study, we did not find any obvious difference in photoinhibition of PSII between WT and antisense transgenic plants under high-light stress.

In conclusion, suppression of *LeZE* increased the content of zeaxanthin in the xanthophyll cycle pigments of antisense transgenic tomato plants, and the de-epoxidation ratio was therefore increased. However, the change in the de-epoxidation ratio did not affect non-photochemical quenching. Accumulated Z in antisense transgenic plants played a vital role in alleviating photoinhibition of PSII and PSI under chilling stress with low irradiance.

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