PHOTOSYNTHETICA 46 (2): 185-192, 2008

Overexpression of tomato chloroplast omega-3 fatty acid desaturase gene alleviates the photoinhibition of photosystems 2 and 1 under chilling stress

X.-Y. LIU[*,**], B. LI[**], J.-H. YANG[***], N. SUI[**], X.-M. YANG[**], and Q.-W. MENG[**,+]

School of Medicine, Zhejiang University, Hangzhou, 310058, P.R. China[*]
College of Life Science, State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, 271018, P.R. China[**]
Department of Horticulture, Zhejiang University, Hangzhou, 310029, P.R. China[***]

Abstract

In transgenic (TG) tomato (*Lycopersicon esculentum* Mill.) overexpressed ω-3 fatty acid desaturase gene (*LeFAD7*) was identified, which was controlled by the cauliflower mosaic virus 35S promoter and induced increased contents of unsaturated fatty acids in thylakoid membrane. Under chilling stress at low irradiance (4 °C, 100 μmol m^{-2} s^{-1}) TG plants with higher linolenic acids (18 : 3) content maintained a higher O_2 evolution rate, oxidizable P700 content, and maximal photochemical efficiency (F_v/F_m) than wild type (WT) plants. Low temperature treatment for 6 h resulted in extensive changes of chloroplast ultrastructure: in WT plants most chloroplasts became circular, the number of amyloids increased, appressed granum stacks were dissolved, grana disappeared, and the number of grana decreased, while only a few grana were found in leaves of TG plants. Hence the overexpression of *LeFAD7* could increase the content of 18 : 3 in thylakoid membrane, and this increase alleviated the photoinhibition of photosystem (PS) 1 and PS2 under chilling at low irradiance.

Additional key words: chlorophyll fluorescence induction; linolenic acids (18 : 3); *Lycopersicon esculentum*; Northern blot; oxygen evolution; photoinhibition.

Introduction

When plants are exposed to low temperatures in the dark, photodamage is rarely observed immediately after chilling stress, even in the extremely thermophilic species (Martín *et al.* 1981, Flexas *et al*. 1999, Allen and Ort 2001). However, chilling stress under low irradiance could cause obvious photoinhibition of photosystem (PS) 1 and PS2 (Li *et al.* 2003b, 2004). D1 protein of PS2 is the target of photoinhibition when plants were exposed to high or medium irradiance (Allen *et al.* 2000). PS1 is more sensitive to chilling stress than PS2 under low irradiance (Sonoike 1996). The membrane of plants is the primary target for chilling stress (Kratsch and Wise 2000).

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 stresses (Graham and Patterson 1982, Nishida and Murata 1996, Iba 2002). It has been hypothesized that chilling stress may impair membrane permeability by the transition of membrane lipids from a liquid-crystalline phase to a gel phase (Lyons and Raison 1970). A greater abundance of unsaturated fatty acids was found in chilling resistant plants than in chilling sensitive plants (Somerville 1995, Nishida and Murata 1996, Murata and Los 1997). Under chilling stress, the activities of desaturase increase and the proportion of unsaturated fatty acids rises (Palta *et al.* 1993). Tolerance to chilling

Received 26 July 2007, *accepted* 6 December 2007.
[+]Corresponding author; fax: +86 538 8226399; e-mail: qwmeng@sdau.edu.cn
Abbreviations: DAs – dienoic fatty acids; DGDG – digalactosyldiacylglycerol; F_v/F_m – maximal photochemical efficiency of photosystem 2; JA – jasmonic acid; *LeFAD7* – *Lycopersicon esculentum* ω-3 fatty acid desaturase gene; MGDG – monogalactosyldiacylglycerol; P700 – photosystem 1 reaction centre; PC – phosphatidylcholine; PFD – photon flux density; PG – phosphatidylglycerol; PI – phosphatidylinositol; SQDG – sulfoquinovosyldiacylglycerol; TAs – trienoic fatty acids; TG – transgenic; 16 : 1(3t) – trans-hexadecenoic acids; 16 : 3 – hexadecatrienoic acids; 18 : 1 – oleic acid; 18 : 2 – linoleic acid; 18 : 3 – linolenic acids.
Acknowledgements: This research was supported by the State Key Basic Research and Development Plan of China (2006CB100100), the Natural Science Foundation of China (30471053), and the Doctoral Foundation of Shandong Province (2004BS06005).

stress is closely connected with the fatty acid desaturation of plant membrane lipids (Browse and Somerville 1991, Moon *et al.* 1995, Sakurai *et al.* 2003).

Chloroplast membrane in higher plants contains generally 70 % of fatty acids (Browse and Somerville 1991) and exhibits distinct characteristic of the higher content of polyunsaturated fatty acids, especially trienoic fatty acids (TAs), namely linolenic (18 : 3) and hexadecatrienoic (16 : 3) acids. There are two pathways for polyunsaturated fatty acid biosynthesis in higher plants: the prokaryotic and eukaryotic pathways, of which 18 : 3 is synthesized by linoleic acid (18 : 2) *via* oleic acid (18 : 1) in the prokaryotic pathway, and 16 : 3 is generally synthesized in the eukaryotic pathway. However, these two pathways are not mutually exclusive (Ohlrogge and Browse 1995). Roles of TAs, especially 18 : 3, have been widely described in higher plants. For example, the increase of TAs in the membrane lipids was considered to be an adaptation to low temperature during acclimation to chilling (Somerville and Browse 1991). Meanwhile, 18 : 3 can convert to jasmonic acid (JA) *via* the octadecanoid pathway, which consequently protects plants from the attacks of insects and pathogens by expressing some related response genes (Vick and Zimmerman 1984, Vijayan *et al.* 1998, Martín *et al.* 1999). In addition, there are several reports about the role of TAs in response to either salt, ABA, fungal infection, or irradiance stresses (Zou *et al.* 1995, Kirsch *et al.* 1997, Berberich *et al.* 1998). In some desert and evergreen plants an increase in the growth temperature leads to a reduction of TAs, α-linolenic acid (18 : 3), and 16 : 3 (Pearcy 1978, Raison *et al*. 1982).

ω-3 fatty acid desaturase is a key enzyme in the synthesis of TAs and catalyses the desaturation of lipid-linked dienoic fatty acids (DAs). In *Arabidopsis thaliana*, three genes, *fad3*, *fad7*, and *fad8* encoding the *ω*-3 fatty acid desaturase mediate the synthesis of TAs from 18 : 2 and 16 : 2. *Fad3* is located in the microsome membrane, while *fad7* and *fad8* are located in the plastid membrane (Browse *et al.* 1986, Lemieux *et al.* 1990, McConn *et al.* 1994, Somerville and Browse 1996). Increased content of 18 : 2 and slightly chlorotic leaves were found in tomato chloroplast *ω*-3 fatty acid desaturase mutants (Li *et al.* 2003a). It is possible to improve the cold tolerance of plants by genetic modifications. When a *ω*-3 fatty acid desaturase gene was silenced in potato leaf, transgenic (TG) plants exhibited lower linolenic acid content and activation in response to wounding (Martín *et al.* 1999). TG tobacco with silenced chloroplast ω-3 fatty acid desaturase gene contained less of TAs than WT plants and was able to acclimate to high temperature (Murakami *et al.* 2000). Overexpression of the *Arabidopsis fad7* in tobacco induced a decrease in the DAs content and a corresponding increase in the TAs (Murakami *et al.* 2000).

Tomato is a chilling sensitive vegetable. We investigated the capacity of its chilling tolerance through the increase of TAs by overexpression of *LeFAD7*.

Materials and methods

Plants: The seeds of WT and TG tomato plants (T_1 generation, overexpression of *LeFAD7*) germinated on moistened filter paper at 25 °C 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 25–30/15–20 °C (day/night) under a 14-h photoperiod (300–400 μmol m^{-2} s^{-1} PFD) in a greenhouse. Fully expanded sixth leaves were harvested from one-month-old tomato plants. Attached leaf petioles were soaked in water, directed to light, and treated with low temperature stress under low irradiance for 6 h (4 °C, 100 μmol m^{-2} s^{-1}). Then the treated leaves were immediately frozen in liquid nitrogen and stored at –80 °C.

Plasmid construction and *Agrobacterium*-mediated transformation of tomato plants: The full-length *LeFAD7* (AY157317, Liu *et al.* 2006) cDNA was subcloned into the pBI121 vector downstream of the 35S-CaMV promoter to form sense constructs (35S-pBILeFAD7). The constructs were firstly introduced into *A. tumefaciens* LBA4404 by the freezing transformation method (Gu *et al.* 1995) and verified by PCR and sequencing. Leaf disk transformation using wild-type (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 for inducing roots. Both media contained cefotaxime sodium (250 g m^{-3}) and kanamycin (50 g m^{-3}). TG plants were screened using kanamycin with the marker on the vector from shoots generated by the incubation of transformed tomato leaf disks. Then the TG plants were detected in PCR with PBI260 (located at 260 bp before being inserted at the pBILeFAD7 site) (5') TCCTACAAATGCCATCATTG (3') and P3 (5'-GGCT GGATTTAGTTACCTATTTGC-3') after the first screening with kanamycin (50 g m^{-3}). In order to further assess the expression of *LeFAD7* in screened TG plants, the one-month-old seedlings were subjected to genome PCR and Northern blotting analysis. Compared with seedlings of WT and control line (PBI: TG lines into which the construct without an insert was introduced), high expression of *LeFAD7* was detected in TG plants grown at 25 °C.

Fatty acid analysis: 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 described by Su *et al.* (1980).

O_2 evolution: The plants were placed in an irradiation incubation chamber (*GXZ-260C*) maintained for 6 h at 24 °C and under 100 μmol m^{-2} s^{-1} PFD. O_2 evolution rates were determined using a modified Clarke-type O_2 electrode unit (*Hansatech*, Kings Lynn, UK) as described by Walker (1990). After treatments, leaf discs (2.25 cm^2) were vacuumized with 0.1 M $NaHCO_3$, and then were dissected into pieces of about 1 mm^2. A reaction mixture of 0.1 M $NaHCO_3$ was used to maintain a high concentration of CO_2 under PFD of 800 μmol m^{-2} s^{-1}.

Chlorophyll *a* fluorescence: The maximum photochemical efficiency of PS2 expressed as F_v/F_m was measured with a portable fluorometer (*FMS2*, *Hansatech*, England, UK) according to the protocol described by Van Kooten and Snel (1990). NPQ was estimated as $NPQ = F_v/F_m' - 1$, where F_m is the maximum fluorescence measured after dark adaptation (Schreiber *et al.* 1994), F_m' is the maximum yield of fluorescence in irradiation-adapted leaves, and F_0' is the original fluorescence in irradiation-adapted leaves. The actual photochemical efficiency of PS2 under irradiation was estimated as $\Phi_{PS2} = (F_m' - F_s)/F_m'$, where F_s is the steady-state fluorescence yield.

820-nm absorbance: Oxidation and reduction of P700 (PS1 reaction centre) was measured at 820 nm with a PEA (Plant Efficiency Analyzer) senior (*Hansatech*, Kings Lynn, UK) as described by Schansker *et al.* (2003).

Electron microscopy: Both the TG lines (T_1-7 and T_1-11) and WT plants were used for microscopic analysis. Leaf samples were collected from five plants of each genetic source after chilling 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, briefly post-fixed in 1 % osmium tetroxide, dehydrated in an ascending ethanol series from 10 to 70 % ethanol preceding the endosmosis, embedment, and polymerization of material into *Epon812* resin. Thin sections were cut from the embedded samples using a *LKB-V* ultramicrotome. Sections were stained with uranium acetate and lead citrate and examined under a transmission electron microscope (*JEOL-1200EX*).

Results

Screening of TG tomato: The putative TG plants were detected by PCR after being screened with kanamycin (50 g m^{-3}) firstly. Then, four TG lines, one positive control (the PBI vector without *LeFAD7*), and WT were collected to evaluate the expression of *LeFAD7* (Fig. 1). Varied expression levels of *LeFAD7* were observed in these four TG plants at 25 °C by Northern blot analysis, of which T_0-7 exhibited most abundant expression of *LeFAD7* than other TG plants. Meanwhile, expression of *LeFAD7* was not influenced in positive control and WT plants (Fig. 1). In addition, about 3 : 1 ratio segregation was observed in the T_1 generation, of which 287 individuals were TG plants and 113 segregation plants. According to the difference of *LeFAD7* transcripts in the four TG plants, T_1-7 and T_1-11 lines were chosen as experimental materials.

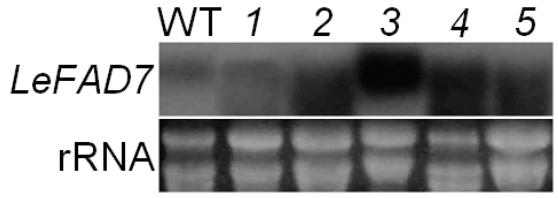

Fig. 1. Northern blot analysis of *LeFAD7* in WT and TG tomato leaves. Total RNA was extracted from WT and transgenic plants. The probe of the RNA gel blot was labelled with [α-^{32}P]-dCTP. About 20 μg of total RNA was analyzed by RNA gel blot. WT – wild type; *1*: pBIHFAD (+) plasmid; *2–5* – transgenic lines T_1-1, T_1-7, T_1-8, and T_1-11, respectively.

Changes of fatty acid composition in TG and WT plants: To clarify the relationship between different expression levels of *LeFAD7* and fatty acid composition, the fatty acid composition of whole lipids was detected in leaves from T_1-7, T_1-11, and WT plants. High proportions of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and low proportions of phosphatidylinositol (PI) were observed in WT and TG plants. Proportions of sulfoquinovosyldiacylglycerol (SQDG), phosphatidylglycerol (PG), and phosphatidylcholine (PC) were all about 10 % in WT and TG plants (Table 1). In TG plants, the percentage of total lipids was markedly higher for SQDG, PG, and PC, and the percentage of DGDG slightly increased compared to WT plants. Meanwhile, the percentages of MGDG and PI were almost indistinguishable in the three lines.

The total DAs/TAs of T_1-7, T_1-11, and WT plants was 15.0, 29.9, and 55.2 %, respectively. Compared with WT plants, the DAs/TAs of T_1-7 was most decreased (to 27.2 %) and in T_1-11 (middle expression of *LeFAD7*) it was decreased to 54.2 %. The polyunsaturated fatty acid index (IUFA, IUFA = 18 : 1×1 + 18 : 2×2 + 18 : 3×3) was 191.8, 185.8, and 184.8 % in T_1-7, T_1-11, and WT plant leaves, respectively.

The fatty acid components in lipids were obviously

Table 1. Fatty acid composition of lipids in WT and TG tomato leaves. – present at trace levels (<0.1 % of total fatty acids). $p<0.05$, significant level (*t*-test). Means±SD ($n = 4$) expressed as mol%. Standard deviations between triplicates was <3 % of the indicated values.

Lipid	Genotype	% of total polar lipids	Fatty acids 16 : 0	16 : 1(3t)	18 : 0	18 : 1	18 : 2	18 : 3
	WT	45.03±0.14a	2.30±0.79a	0	0.33±0.04b	22.10±0.80a	12.51±0.40a	62.76±1.5a
MGDG	T_1-7	45.27±1.70a	4.73±0.04b	0	0.57±0.06a	11.93±0.55b	1.57±0.20b	81.20±1.16b
	T_1-11	45.07±0.55a	3.46±0.50c	0	0.35±0.05b	17.48±0.40c	5.11±0.30c	73.60±0.60c
	WT	23.48±1.07a	14.29±0.90c	0	4.19±0.92a	2.42±0.16a	12.39±1.00a	66.71±1.65b
DGDG	T_1-7	24.22±1.55a	21.44±0.80a	0	2.21±0.36b	1.18±0.07b	3.72±0.40b	71.45±1.50a
	T_1-11	23.61±0.98a	16.55±0.61b	0	3.32±0.25ab	2.04±0.08c	8.14±0.09c	69.95±0.80a
	WT	7.96±0.12b	35.48±1.43a	0	1.85±0.30a	4.05±0.32c	20.64±0.81a	37.98±1.35a
SQDG	T_1-7	8.52±0.26a	36.25±0.69a	0	2.12±0.08a	4.72±0.10b	15.77±0.60b	41.14±0.70a
	T_1-11	8.20±0.8ab	32.30±1.10b	0	1.98±0.20a	9.00±0.20a	16.32±0.5b	40.40±1.0a
	WT	8.40±0.48b	21.74±0.98a	26.28±1.37b	4.43±0.70a	5.68±0.66a	16.46±1.07a	25.41±1.15c
PG	T_1-7	9.47±0.30a	20.6±1.34a	29.48±0.70a	–	3.12±0.11b	8.83±0.32c	37.97±1.75a
	T_1-11	9.14±0.42ab	20.89±0.70a	27.23±0.60b	1.75±0.10b	3.57±0.12b	14.89±0.70b	31.67±0.80b
	WT	12.94±0.50a	25.23±0.90a	0	2.56±0.11a	13.14±0.80a	30.10±0.54a	28.97±0.20c
PC	T_1-7	10.43±0.33b	27.75±1.95a	0	–	6.40±0.70c	6.79±0.45c	59.06±0.50a
	T_1-11	11.98±0.68ab	26.68±1.09a	0	1.64±0.41b	9.89±0.06b	18.41±0.70b	43.38±1.40b
	WT	2.19±0.40a	17.43±0.90c	0	0.60±0.20	16.18±0.62a	43.04±1.44a	22.75±0.80c
PI	T_1-7	2.09±0.31a	27.65±0.61a	0	–	12.95±0.51b	13.75±0.92c	45.65±0.78a
	T_1-11	2.00±0.30a	24.53±0.81b	0	–	15.60±1.43a	24.98±0.71b	34.89±0.20b

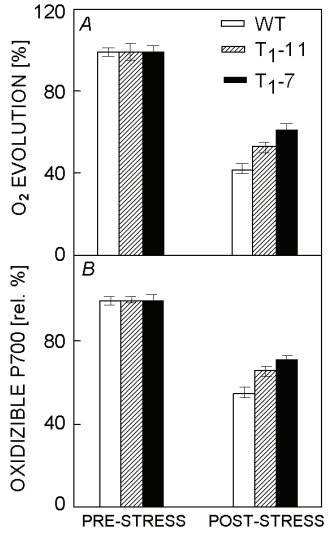

Fig. 2. Oxygen evolution (*A*) and changes of the oxidizable P700 (*B*) of TG and WT plant leaves treated at 4 °C for 6 h under 100 μmol m^{-2} s^{-1} PFD. In *B*, leaves were dark adapted for 15 min before measurement. *Each bar* represents the means±SD of 5 measurements on separate leaves. Means±SD, $n = 5$.

changed. Compared with WT plants, the most remarkable change in fatty acid composition of TG plants was higher content of 18 : 3 and corresponding low content of 18 : 2. In addition, the 18 : 1 content was reduced slightly. And the content of trans-hexadecenoic acids [16 : 1(3t)], the peculiar component of PG, was increased by 3.2 and 1.0 % in T_1-7 and T_1-11 lines, respectively.

Oxygen evolution under chilling stress: To test whether higher 18 : 3 content in thylakoid membrane could protect the photosynthetic apparatus from chilling stress under low irradiance, O_2 evolution rates were measured in intact leaves from TG plants pre-treated at 4 °C. During chilling stress, the decrease in oxygen evolution was 51.3, 47.8, and 38.1 % in WT, T_1-11, and T_1-7, respectively (Fig. 2*A*). O_2 evolution rates of plants decreased markedly at 4 °C for 6 h in both WT and TG plants, yet the decrease of the O_2 evolution rates in TG plants was slower than that in WT plants. Besides, O_2 evolution rates declined much slower in T_1-7 line with higher expression level of *LeFAD7* (Fig. 2*A*), which indicated that overexpression of *LeFAD7* could protect the photosynthetic apparatus from chilling stress.

Photoinhibition of photosystems under chilling stress: The oxidizable P700 was measured to investigate the photoinhibition of PS1 by the absorbance at 820 nm. After 6-h chilling stress, the oxidizable P700 decreased by 44.8, 34.2, and 29.3 % in WT, T_1-11, and T_1-7, respectively, thus overexpression of *LeFAD7* could alleviate the photoinhibition of PS1 (Fig. 2*B*).

Photoinhibition of PS2 in the leaves of TG and WT plants was estimated by measuring the maximum photochemical efficiency (F_v/F_m) and other relative parameters when exposed to chilling stress under low irradiance. Both F_v/F_m and F_0 values decreased in WT and TG plants. However, F_0 was maintained stable after 2 h of chilling stress (Fig. 3*A,B*). F_v/F_m in WT plants was reduced faster than in the TG plants whereas the change of F_0 was similar. At the end of chilling stress, the F_v/F_m of WT, T_1-11, and T_1-7 decreased to 0.57, 0.67 and 0.72, respectively. Namely F_v/F_m decreased by 30.6, 19.4, and 12.8 % in WT, T_1-11, and T_1-7, respectively. Among WT and TG lines, the Φ_{PS2} of T_1-7 line with highest expression of *LeFAD7* decreased least, while the Φ_{PS2} of WT was reduced most (Fig. 3*C*). NPQ of T_1-7 was

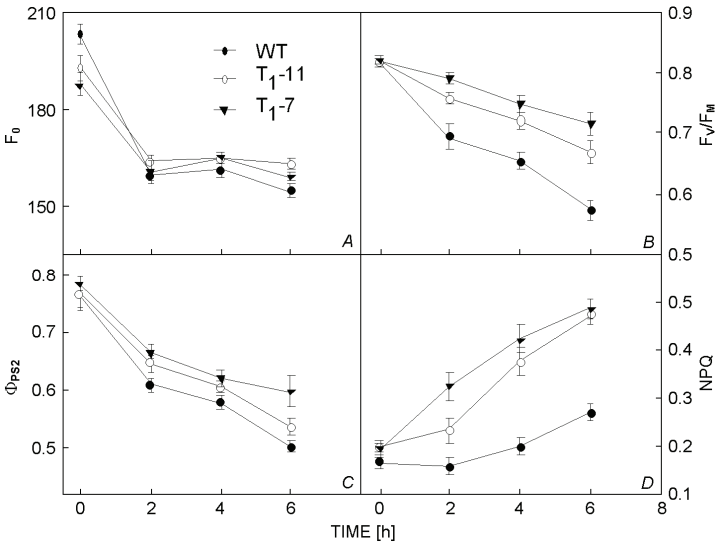

Fig. 3. Changes of F_0 (*A*), F_v/F_m (*B*), NPQ (*C*), and Φ_{PS2} (*D*) of leaves from TG and WT plants under chilling temperature (4 °C) at low irradiance (100 μmol m^{-2} s^{-1}). Before measurement, plants were treated for 0, 2, 4, 6, and 8 h at 4 °C. Before chilling stress, plants were adapted in darkness for more than 2 h to measure F_v/F_m. During chilling stress, plants were adapted in darkness for 15 min prior to F_v/F_m measurement. Means±SD, n = 5.

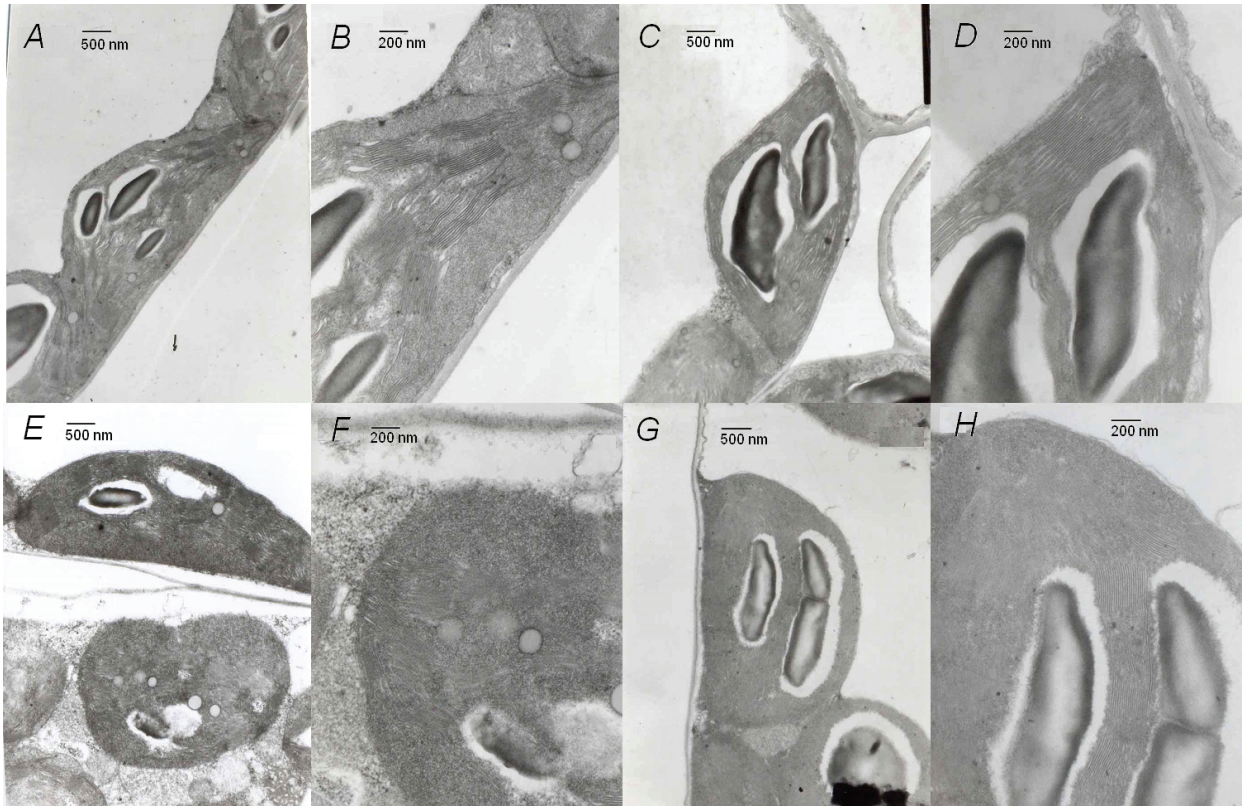

Fig. 4. Changes of chloroplast ultrastructure in T_1-7 and wild type (WT) leaves. Transmission electron micrographs of chloroplast ultrastructure in WT (*A, B, E, F*) and T_1-7 tomato leaves (*C, D, G, H*). *A–D*: plants were under normal conditions (25 °C, 100 μmol m^{-2} s^{-1}). *E–H*: plants were treated for 6 h at 4 °C under low irradiance (100 μmol m^{-2} s^{-1}).

highest, while NPQ of WT was lowest (Fig. 3*D*). Photoinhibition of PS2 under chilling stress under low irradiance was more obvious in WT than TG plants. And the increase of 18 : 3 content as a result of overexpression of *LeFAD7* alleviated the photoinhibition of PS2 during chilling stress.

Changes of chloroplast ultrastructure under chilling stress: Chloroplast ultrastructure and organization were similar in WT and T_1-7 plants before chilling stress (Fig. 4*A–D*). However, chilling stress induced extensive changes of chloroplast ultrastructure in WT plants. Most chloroplasts became circular and the number of amyloids increased. At the same time, appressed granum stacks were dissolved, grana disappeared, and the number of grana decreased in WT plants (Fig. 4*E,F*). Compared

with WT plants, chloroplast ultrastructure changed relatively less in T_1-7 plants when plants were exposed to chilling. After chilling 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 TG plants (Fig. 4*G,H*).

Discussion

Frequently, plants have to endure unfavourable environment factors such as chilling or freezing temperatures. Therefore, plants have developed unique mechanisms of acclimation to such stresses, which are associated with complex biochemical and physiological alterations in plants, including changes in gene expression (Thomashow 1994), ultrastructure (Ristic and Ashworth 1993), membrane lipid composition (Miquel *et al.* 1993, Somerville 1995, Nishida and Murata 1996, Murata and Los 1997), enzyme activities, contents of sugars and polyamines (Levitt 1980, Strand *et al.* 1997), and ion channel activities (Knight *et al.* 1996). A high TA content may reflect an adaptation to low temperature (Somerville and Browse 1991). This evidence of the relationship between chilling-tolerance and high content of TAs is consistent with several reports (Somerville and Browse 1991, Nishida and Murata 1996, Murata and Los 1997). We detected many biochemical and physiological alterations and evaluated the relationship between capacity of chilling-tolerance and TAs in tomato with overexpression of *LeFAD7*. The TG plants had higher content of TAs than WT, which was accompanied by the decrease of DAs content (Table 1), and exhibited higher chilling tolerance than WT plants (Fig. 2*A*). In addition, the level of *LeFAD7* transcripts was connected closely with the component of fatty acid in TG plants, especially the content of 18 : 3 in leaves. Moreover, the change of unsaturated fatty acid content in thylakoid membrane affects the photosynthetic electron transport (Rawyler and Siegenthaler 1981). The content of TAs was enhanced with *LeFAD7* overexpression and the photosynthesis rate was relatively higher than in WT plants under chilling stress (Fig. 2*A*).

SQDG and PG, the negative lipids, are important for the structure and function of thylakoid membrane (Yu and Benning 2003). PG is a major factor determining the temperature of phase transition of membrane lipids (Huner *et al*. 1989). The main role of SQDG is to substitute PG thus ensuring the balance of negative lipids being short of the phosphorus (Güler *et al.* 1996, Yu *et al.* 2002). And the contents of SQDG and PG in cucumber decrease when plants are exposed to chilling stress under low irradiance (Dai *et al.* 2004). Meanwhile, it would affect the structure and function of PS1 and PS2 that are located in the thylakoid membrane (Sato *et al.* 2003). In our study, the relative contents of SQDG and PG increased and the change of SQDG content was much larger than that of PG in the leaves of TG plants at 25 °C. The temperature of phase transition of membrane lipids might be elevated due to the relatively higher PG content in TG plants. And the relatively higher SQDG and PG contents ensured the balance of negative lipids during chilling stress. The 16 : 1(3t) is a special component of PG, associated with the synthesis of poly-LHC2 (light-harvesting chlorophyll *a/b* protein of PS2). During chilling stress, the contents of PG and 16 : 1(3t) are reduced and LHC2 is unpolymerized, then the light-harvesting efficiency decreases (Huner *et al*. 1989).

PS1 is more sensitive to chilling stress than PS2 and thus PS2 suffers less photoinhibition (Havaux and Davaud 1994, Li *et al*. 2003b). In addition, photoinhibition of PS1 is responsible for reduced photosynthesis rate in chilling-sensitive plants under chilling and low irradiance (Sonoike 1996). Although PS1 was more sensitive to chilling stress than PS2 under low irradiance (Figs. 3 and 4*B*), photoinhibition of PS1 might not be the main factor reducing photosynthetic rate under chilling stress. The limited electron acceptors might cause the decrease of the oxidizable P700, and 100 μmol m^{-2} s^{-1} PFD was excessive to tomato plants when Calvin cycle was inhibited under low temperature.

Greer *et al.* (1986) point out that photodamage and repair of PS2 are coinstantaneous under chilling stress and the extent of photoinhibition depends on the rates of damage and recovery. The recovery of PS2 requires the synthesis and insertion of D1 protein. F_v/F_m was lower in TG plants than in WT plants (Fig. 3*B*). The relatively lower photoinhibition of PS2 was due to the increase of 18 : 3 caused by the overexpression of *LeFAD7*. Φ_{PS2} of TG plants was higher than that of WT (Fig. 3*C*). NPQ could reflect a very important mechanism for the protection of PS2 against excess irradiance (Xu *et al.* 1999, Li *et al.* 2003b, 2004). This mechanism alleviates the excitation pressure on PS2 centres by diverting photon energy into heat (Gray *et al.* 1996). The higher NPQ in TG plants suggested that the increased energy dissipation protecting PS2 reaction centres (Fig. 3*D*).

Chloroplast thylakoid membrane maintains the stability of photosynthetic machinery (Routaboul *et al*. 2000). Increased TAs caused by overexpression of *LeFAD7* could protect chloroplast ultrastructure and organization during chilling stress (Fig. 4). The dissolving of appressed granum stacks during chilling stress may change some biochemical processes. The improvement of stability of the chloroplast ultrastructure enables plants to maintain higher membrane-binding enzyme activities and alleviate photoinhibition of PS1 and PS2 during chilling stress under the low irradiance. In conclusion, overexpression of *LeFAD7* increased the content of TAs, and then higher TAs content protected the chloroplast ultrastructure and alleviated photoinhibition of PS1 and PS2 during chilling under low irradiance.

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