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Differential Protection of Photosynthetic Capacity in Trehalose- and LEA Protein-producing Transgenic Plants under Abiotic Stresses

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We previously demonstrated that both trehalose and LEA protein protect plants from damage by drought, salt, and heat. Here, we compared their effectiveness in preserving photosynthetic capacity under those abiotic stresses. Upon dehydration, the Pmax (maximal photosynthetic rate) of O_2 evolution decreased similarly in both nontransformants and *otsA* plants. Contrastingly, Pmax was maintained at a considerably higher level in *CaLEA6* plants. However, no significant differences in Chl fluorescence parameters were observed between transformants and nontransformants. Under salinity stress, *CaLEA6* plants were also better than *otsA* plants in terms of their values for Pmax, photochemical efficiency of PSII (*Fv*/*Fm*), and photochemical quenching (qP). After heat both *otsA* and *CaLEA6* plants maintained a higher Pmax as well as more favorable Chl fluorescence parameters, although the latter transformant performed slightly better overall. Therefore, despite the comparable effectiveness of trehalose and LEA protein in enhancing tolerance against those abiotic stresses, they confer differential protection in maintaining photosynthetic capacity. Compared with trehalose, the *Ca*LEA6 protein appears to be a more universal and effective agent under those stresses.

Keywords: *abiotic stresses, LEA protein, photosynthesis, transgenic plants, trehalose*

Plant growth and productivity are affected by various environmental factors, such as light, water, soil salinity, and temperature, all of which impose abiotic stresses when conditions are out of the optimal range (Yancey et al., 1982). The most common is water deficit, caused by dehydration, which adversely affects plant growth and development by altering metabolism and gene expression (Ingram and Bartels, 1996; Bray, 1997). During drought periods , therefore, plants undergo many physiological changes and induce a large number of genes for adaptation (Ingram and Bartels, 1996). Water deficit is also triggered by high salinity, chilling, or high temperatures. In response to these stresses, plants utilize a number of defense strategies to cope with unfavorable conditions. The early events include their sensing and subsequent signal transduction to initiate metabolic responses by activating various stress-responsive genes (Bray, 1997). A typical change in expression during a period of water deficit is the induction of genes involved in the synthesis of various osmolytes, e.g., proline, glycinebetaine, and trehalose, as well as of low-molecular-weight proteins, such as dehyrins and LEA protein (Ingram and Bartels, 1996; Ramanjulu and Bartels, 2002). Overproduction of these products in transgenic plants demonstrates their protective effects against not only drought stress but also high salt and chilling (Holmström et al., 1996; Xu et al., 1996; Romero et al., 1997; Jun et al., 2001, 2005; Garg et al. 2002; Jang et al., 2003; Park et al., 2003; Kim et al., 2005).

Among these, trehalose (α-D-glucopyranosyl-[1,1]-α-D-glucopyranoside) is a commonly found, non-reducing disaccharide that is a storage form of reduced carbon and also acts as a protectant against desiccation and heat stress in bacteria, yeast, and resurrection plants (Müller et al., 1999; Argüelles, 2000). Engineered tobacco plants expressing either yeast *TPS* or *E. coli ots*A showenhanced tolerance by maintaininghigher fresh weights and recovering after rehydration (Holmström et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998). Likewise, transgenic rice plants producing trehalose exhibit enhanced tolerance not only to drought but also to salt and chilling (Garg et al., 2002; Jang et al., 2003). Furthermore, our own studies have shown that the effectiveness of trehalose in conferring increased tolerance can be extended to high temperatures, dehydration, and salinity in transgenic tobacco and other plants (Jun et al., 2001; our unpublished data).

LEA proteins are a large group of plant proteins that are heavily synthesized and stored during seed maturation (Ingram and Bartels, 1996; Bray, 1997). They were first identified in the desiccation phase of seed development, and are reported to protect specific cellular structures or ameliorate the effect of drought stress by sequestering ions and maintaining minimum requirements for cellular water (Baker et al., 1988; Dure et al., 1989). LEA proteins are classified into seven subgroups based on their amino acid sequence homology and specific motifs, which presumably undertake different functions during times of water deficit (Dure, 1993; Ingram and Bartels, 1996). Wheat Em, a Group 1 LEA protein, induces enhanced tolerance against osmotic stress in yeast (Swire-Clark and Marcotte, 1999). Group 2 LEA proteins from tomato and Group 3 LEA pro-

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Abbreviations: Chl, chlorophyll; Fm, maximal fluorescence after dark-adaptation; Fo, initial fluorescence; Fv, variable fluorescence; Fv/Fm, maximum photochemical efficiency of PSII; LEA, late embryo abundant; NPQ, nonphotochemical quenching of chlorophyll fluorescence; PEG, polyethylene glycol; Pmax, maximal photosynthetic rate of O_2 evolution; PS, photosystem; qP, photochemical quenching of chlorophyll; T6P, trehalose-6-phosphate; TPP, trehalose-6-phosphate phosphatase; TPS, trehalose-6-phosphate synthase

teins from wheat seedlings also are correlated with dehydration tolerance (Ried and Walker-Simmons, 1993; Zhang et al., 2000). In addition, transgenic rice over-expressing *HVA1*, a Group 3 LEA gene from barley, shows increased tolerance to water and salt stresses (Xu et al., 1996). A Group 4 LEA gene, tomato *le-25*, confers increased tolerance by yeast against high salt and chilling (Imai et al., 1996). Most of these LEA proteins are cytosolic and hydrophilic, and contain random coil or a-helices (Swire-Clark and Marcotte, 1999; Zhang et al., 2000; Soulages et al., 2002).

However, some atypical LEA proteins are hydrophobic and belong to Group 6. These include soybean D95-4, cotton Lea14-A, tomato ER5, tomato Lemmi9, CaLEA6 of hot pepper, and pcP27-45 of the resurrection plant (Piatkowski et al., 1990; Galau et al., 1993; Maitra and Cushman, 1994; van der Eycken et al., 1996; Zegzouti et al., 1997; Kim et al., 2005). As such, they are likely to function differently from those that are hydrophilic, despite their involvement in the dehydration response (Zegzouti et al., 1997). We recently demonstrated that transgenic tobacco plants over-expressing *CaLEA6* exhibit enhanced tolerance to dehydration and high salinity (Kim et al., 2005). We also showed that transgenic plants of Chinese cabbage (*Brassica campestris*) over-expressing the *E. coli* TPS gene (*otsA*) or the *Capsicum annuum* LEA protein gene (*CaLEA6*) have greater tolerance to heat stress, drought, and high salinity (Park et al., 2003).

Although numerous studies have been conducted on the role of such substances in conferring tolerance, none has carefully examined the protective effects of both in terms of maintaining photosynthetic capacities. Changes in this capacity within trehalose-producing plants have previously been examined (Pilon-Smits et al., 1998; Pellny et al., 2004). Here, we focused on the protective effect of trehalose and LEA protein on the photosynthetic apparatus, comparing their influences on the maintenance of photosynthetic capacity under abiotic stresses. Our objective was to provide a tool for engineering stress-tolerant crop plants that will have higher productivity and better survival under unfavorable conditions.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wild-type and transgenic plants of tobacco (*Nicotiana tabacum* L. var. SR1) were grown for 4 to 5 weeks in a growth chamber maintained at 25±1°C, under a 16 h-photoperiod. Light was provided from four banks of True-lite II fluorescent lamps (Durotest, USA), at an intensity of 150 μmol m^{-2} s^{-1}.

Plasmid Construct and Plant Transformation

The *TPS* and *CaLEA6* overexpressors were constructed as follows: coding regions of *otsA* (Kassen et al., 1992) and *CaLEA6* (Kim et al., 2005) were obtained by PCR, using Vent polymerase (New England Biolabs, Beverly, MA, USA) and primers corresponding to either the 5′- or 3′- ends of *ots*A with *Xba*I or *Eco*RI sites, or both ends of *CaLEA6* with the *Eco*RI site. We then cloned them into a pWP90 vector containing the double 35S promoter and poly(A) terminator of *CaMV*. The entire fragment from each construct was again cloned into a pBIN90 vector, using the *Kpn*I and *Sal*I sites. Transgenic tobacco was generated by the *Agrobacterium*-mediated leaf disc transformation procedure, and transformants were selected on an MS medium containing kanamycin. The transgenic plants were first identified by PCR, then confirmed by northern hybridization. Expression levels for *otsA* or *CaLEA6* varied depending on the transgenic line. Because the degree of tolerance against various abiotic stresses depended on the transcript level of each gene, we chose three lines for our photosynthetic measurements: Lines 2 and 4 for low expression, Lines 6 and 7 for intermediate, and Lines 8 and 9 for high expression. These were selected from trehalose and LEA plants based on the results from our northern hybridization (Jun et al., 2005; Kim et al., 2005). Homozygous plants in the F_2 generation were used in all experiments, and did not exhibit any phenotypic alterations.

Water, Heat, and Salt Stress Treatments

Water stress was applied to induce dehydration by immersing the roots of whole plants in a Hoagland solution containing 10% (w/v) polyethyleneglycol (PEG)-6000, a chemically inert solution that lowers leaf water potential in a time-dependent manner (Michel, 1970; Lee et al., 1998, 2004). For our high-temperature treatment, whole plants were held for the indicated time in a dark chamber set at 45°C but under high humidity to prevent wilting. Salt stress was imposed by irrigating plants twice per week with water containing 250 mM NaCl. After each treatment, photosynthetic measurements were immediately performed using detached leaves or leaf discs.

Measurement of Photosynthesis

Photosynthetic activities were assessed by measuring Chl fluorescence, O_2 evolution, and CO_2 gas exchange. Pmax values and Chl fluorescence parameters were obtained by simultaneously measuring O_2 evolution and Chl fluorescence at 25°C from 3.5-cm leaf discs in a Hansatech LD2 chamber, with a Clark type electrode connected to a Hansatech O_2 electrode control box and a Walz Pulse Amplitude Modulator (PAM) Chl fluorometer (Effeltrich, Germany). Actinic light (600 μmol m^{-2} s^{-1}) was provided by a Schott illuminator. Non-photochemical quenching (NPQ; defined as Fm/F′m–1) and photochemical quenching (qP) were determined as described by Schreiber et al. (1994). All chosen leaves had approximately equal Chl contents when measured by a Minolta Chl meter (SPAD-502; Minolta, Japan), and were normalized to the control. CO_2 gas exchange was measured with an infra-red gas analyzer (IRGA, Walz Portable Photosynthesis System HCM-1000; Effeltrich, Germany).

RESULTS

Photosynthetic Responses to Dehydration

Transgenic plants engineered to produce trehalose or LEA

protein show enhanced drought tolerance (Holmström et al., 1996; Xu et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998; Jun et al., 2001, 2005; Garg et al., 2002; Jang et al., 2003; Park et al., 2003; Kim et al., 2005). The criteria for this tolerance are based on the maintenance of leaf turgidity, retention of leaf fresh weights, and Chl fluorescence parameters (Pilon-Smits et al., 1998; Garg et al., 2002; Jang et al., 2003; Park et al., 2003; Jun et al., 2005; Kim et al., 2005). However, no previous studies have carefully examined the protective effect of both substances in terms of maintaining photosynthetic capacities under dehydration. To address whether the production of trehalose or LEA protein improves photosynthetic performance under water stress, we monitored changes in photosynthetic activity by measuring Chl fluorescence parameters, Pmax of O_2 evolution, and CO_2 fixation after subjecting nontransformants and transgenic tobacco plants to water deficit through PEG-treatment. Although limiting irrigation is the usual way to elicit such a response, we chose to induce dehydration by immersing plants in a nutrition medium containing chemically inert PEG-6000 in order to provide more uniformity and physiological relevance (Michel, 1970). Our PEG-treatment has also been used to lower leaf water potential in hot pepper and tobacco plants and leaf water potential was decreased in a time-linear manner to PEG-treatment (Lee et al., 1998, 2004; Jun et al., 2001).

In trehalose-producing plants, Pmax in all transgenic plants (Lines 4, 6, and 8) decreased in a similar phase to that of the nontransformants. Regardless of expression level, Pmax values were diminished by about 40% and 60% after 1 and 2 h of treatment, respectively (Figure 1). After 4 h of treatment, O_2 evolution was completely inhibited in all plants (data not shown). Interestingly, the transgenic plants from Line 6 exhibited higher photosynthetic rates under all conditions despite a similar pace of reduction after treatment. Changes in leaf water potential in those plants showed a resemblant trend (our unpublished data). Although the pattern of CO_2 fixation, measured by IRGA under a saturating CO_2 level, was about the same, fixation under ambient CO_2 was completely stopped within 1 min of treatment in all plants due to complete stomatal closure (data not shown).

In contrast, some LEA protein-producing plants showed significantly different photosynthetic behavior after PEG-treatment. For example, while wild-type plants and those from Line 2 had less than 40% of the initial activity, plants from Line 9 retained nearly 60% of their initial activity after 2 h of treatment (Figure 1). Plants of Line 7 showed an intermediate response, maintaining about 50% activity. Furthermore, plants of Line 9 sustained about 20% of their activity after 4 h of PEG-treatment whereas the nontransformants showed no photosynthetic activity (data not shown). Therefore, it appears that LEA protein, in contrast to trehalose, is also effective in protecting the photosynthetic apparatus under dehydration in addition to conferring enhanced tolerance. These results are in sharp contrast to those from our tolerance test, where both trehalose and LEA transgenic plants showed a similar degree of protection against dehydration (Jun et al., 2001, 2005; Park et al., 2003; Kim et al., 2005). It is likely that LEA protein is either a more efficient osmoprotectant than trehalose or it exerts its protective role differently.

Chl fluorescence parameters, especially initial Chl fluorescence (F_o) and maximal photochemical efficiency of PSII (F_v/F_m), are often used to assess the impairment of PSII function (Renger and Schreiber, 1986). Here, both F_o and F_v/F_m in all plants were not greatly influenced after 2 h of PEG-treatment (Figure 2). This is not unexpected because PSII is not the primary site affected by dehydration (Ögren and Öquist, 1985; Angelopoulos et al., 1996). However, quenching parameters showed some differences; i.e., in trehalose plants, photochemical quenching decreased while nonphotochemical quenching increased, comparable to that of the wild type (Figure 3). Contrastingly, qP and NPQ were maintained in LEA plants of Line 9. These stabilized quenching parameters substantiated our conclusion that photosynthetic

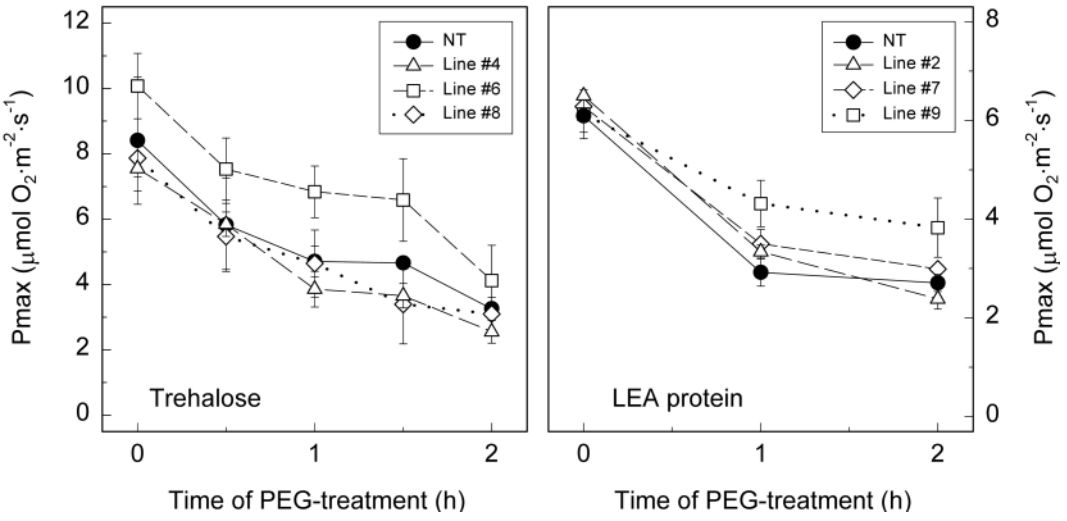

Figure 1. Changes in Pmax of O_2 evolution after PEG-treatment in nontransformants (NT), trehalose- (Lines 4, 6, and 8), and LEA protein-producing transgenic tobacco plants (Lines 2, 7, and 9). Roots of whole plants were immersed in 10% (w/v) PEG solution to induce dehydration for the indicated time prior to measuring photosynthesis. Data are means ± SE ($n = 4$).

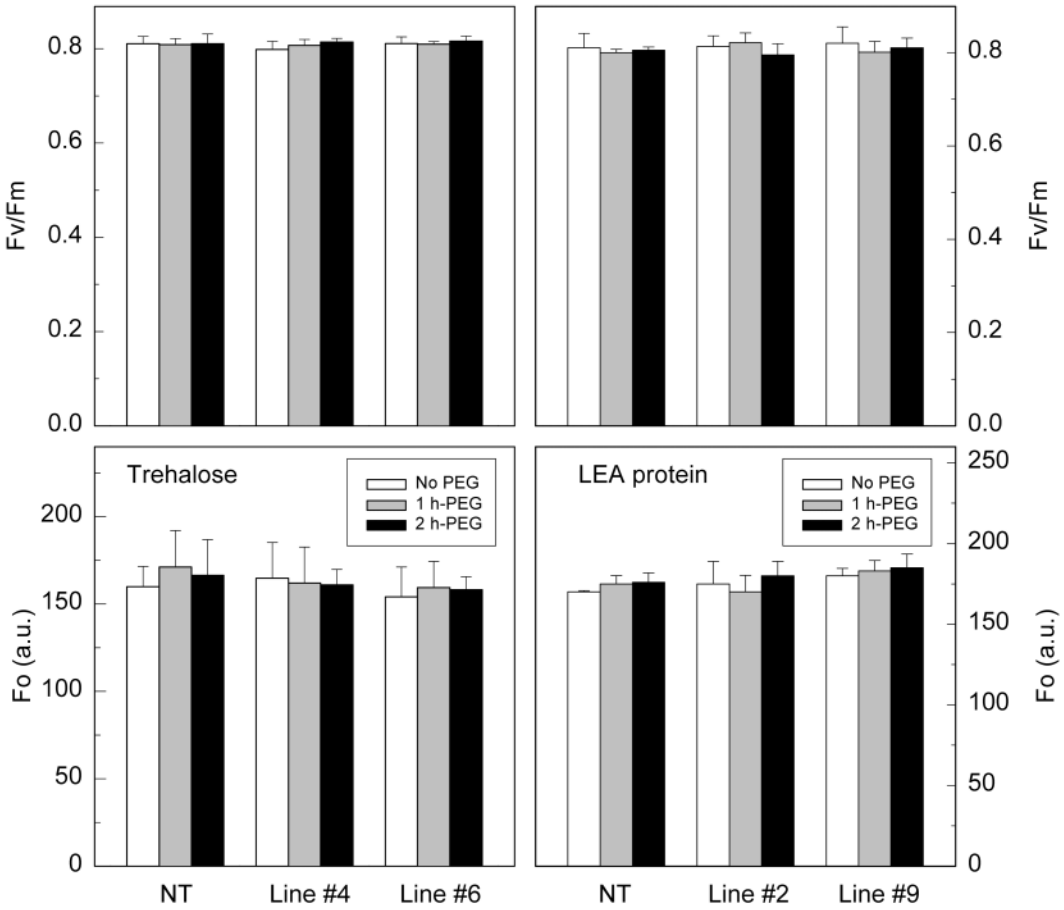

Figure 2. Changes in initial Chl fluorescence (F_o) and maximal PSII quantum yield (F_v/F_m) after PEG-treatment in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Roots of whole plants were immersed in 10% PEG solution for the indicated time prior to measuring Chl fluorescence. Chl fluorescence was simultaneously measured with O_2 evolution. Data are means ± SE (n = 4).

activities were less affected after PEG-treatment in those plants.

Photosynthetic Responses to High Temperature

Heating generally leads to lesions in the photosynthetic apparatus, with a concomitant decline in Pmax and F_v/F_m, but causes a rise in F_o values by negatively affecting PSII (Pastenes and Horton, 1996; Yamane et al., 1997). We previously showed that trehalose plants maintain more favorable Chl fluorescence parameters (F_o and F_v/F_m) after heat-treatment (Jun et al., 2001). Here, we more thoroughly studied changes in photosynthetic capacities under the influence of high temperature. Heating lowered photosynthetic activities in all plants, but more rapidly and dramatically in the nontransformants. After 2 h and 4 h of exposure to 45°C in the dark, the Pmax of O_2 evolution declined nearly 80% and to nil, respectively, in nontransformants (Figure 4). In contrast, trehaloseplants of Lines 5 and 6 had Pmax values that were close to 50% of the initial activity even after 4 h of heat-treatment (Figure 4). It is notable that no distinction in protection was observed between Lines 5 and 6 despite a significant difference in their tolerance (Jun et al., 2001).

In LEA plants, Pmax was maintained better in Line 9 plants, but not in Line 2 plants. After 4 h of heat-treatment, the former exhibited about 60% of their initial Pmax while the latter were no better than the nontransformants (Figure 4). Plants in Line 7 had an intermediate response (data not shown). Therefore, protection of the photosynthetic apparatus appeared to be dependent on the amount of LEA protein that accumulated. Changes in F_o and F_v/F_m exhibited a similar trend as for Pmax, remaining more favorable in transgenic plants than in nontransformants after heat treatment at 45°C. In nontransformants, F_o values increased more than two-fold, while F_v/F_m decreased by nearly 70% after 2 h of heat-treatment (Figure 5). In contrast, all transgenic plants, except from Line 2, showed only a slight rise in F_o and a significantly less drop in F_v/F_m (Figure 5). However, LEA plants in Line 2 behaved similarly to the nontransformants, with an abrupt increase in F_o and a decrease in F_v/F_m.

Changes in Chl fluorescence quenching parameters showed a similar pattern. In trehalose plants of both Lines 5 and 6, and LEA plants of Line 9, qP only slightly declined while that of the nontransformants and LEA plants in Line 2 was significantly decreased (Figure 6). NPQ was also diminished in nontransformants and LEA plants of Line 2 although to a much lesser degree, whereas all trehalose plants and LEA plants of Line 9 exhibited constant NPQ values after

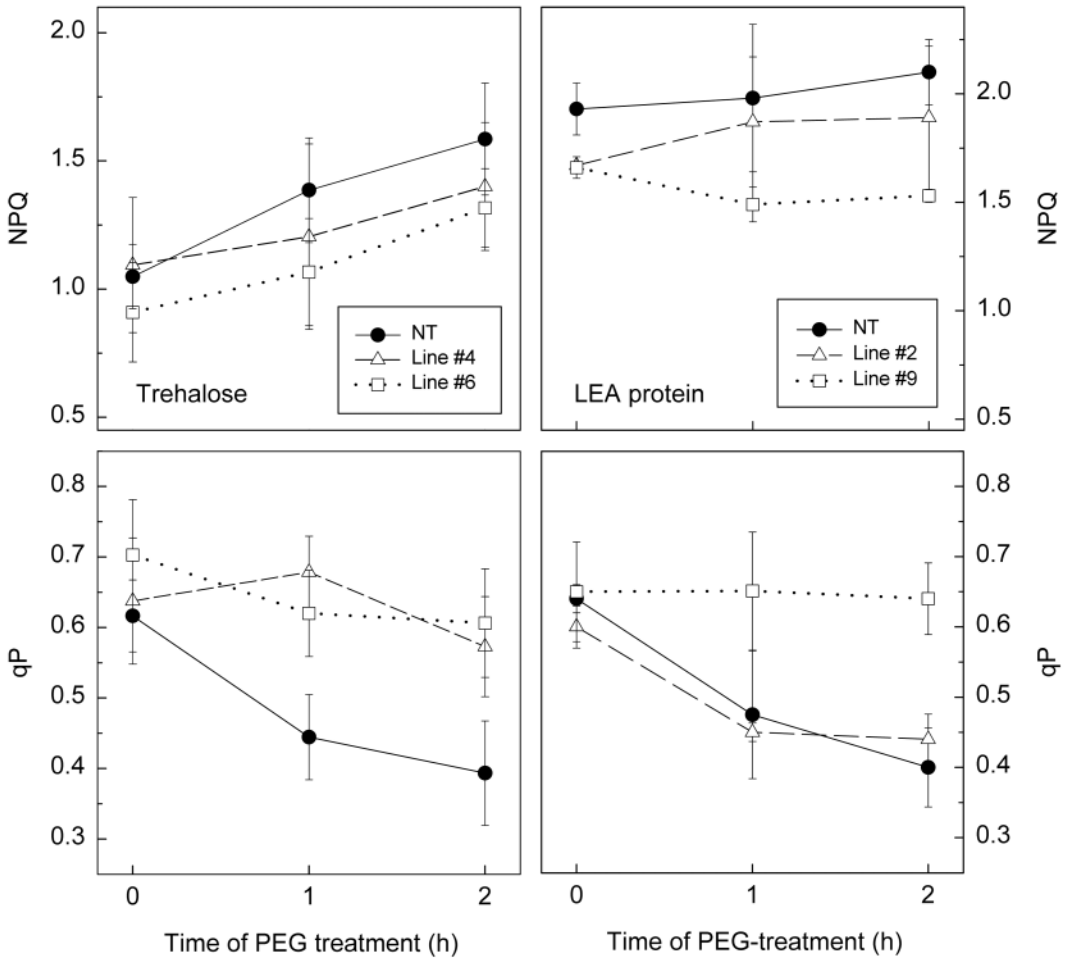

Figure 3. Changes in photochemical quenching (qP) and nonphotochemical quenching (NPQ) after PEG-treatment in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Roots of whole plants were immersed in 10% PEG solution for the indicated time prior to measuring Chl fluorescence. Data are means ± SE (n = 4).

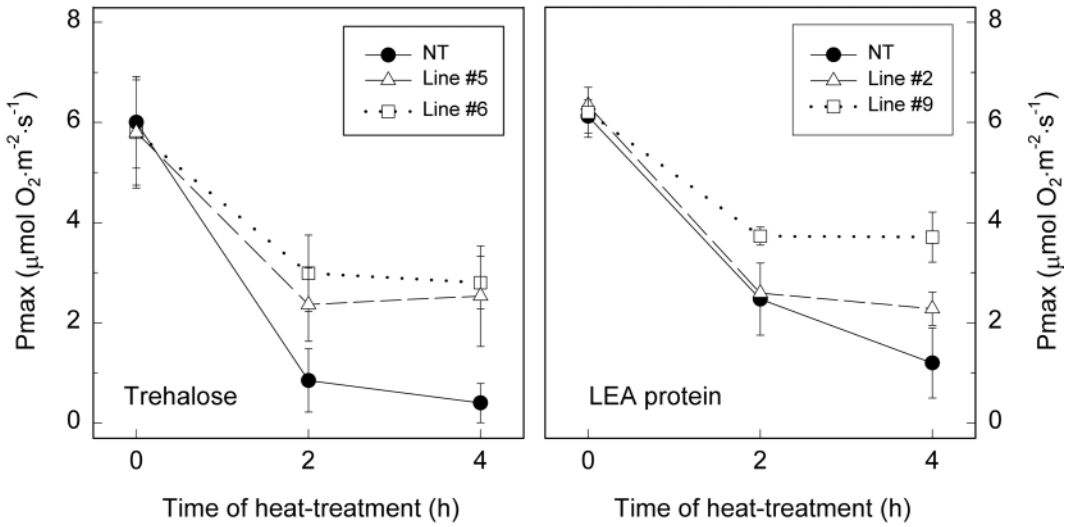

Figure 4. Changes in Pmax of O_2 evolution after heating at 45°C under darkness in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Whole plants were incubated in growth chamber for the indicated time prior to measuring photosynthesis. Data are means ± SE (n = 4).

heat-treatment (Figure 6). When heat-treatment at lower temperatures (40°C and 43°C) were tested, comparable results were yielded but with significantly lesser inhibition overall (data not shown). Therefore, both trehalose and LEA protein can protect the photosynthetic apparatus against the deteriorative effect of high temperatures. However, it

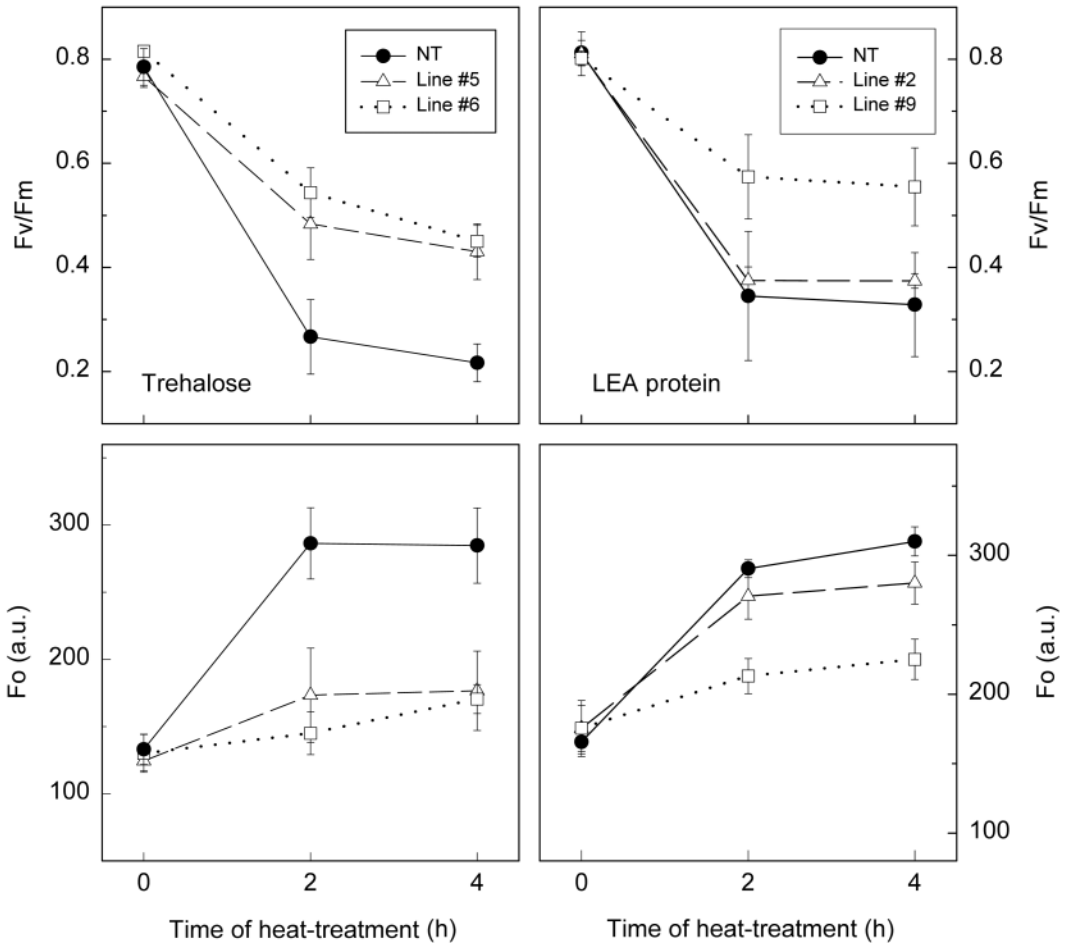

Figure 5. Changes in *Fo* and F_v/F_m after PEG-treatment in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Whole plants were incubated under darkness in growth chamber at 45°C for the indicated time prior to measuring Chl fluorescence. Chl fluorescence was simultaneously measured with O_2 evolution. Data are means ± SE (n = 4).

appears that this protective effect by LEA protein is dose-dependent while that by trehalose is not.

Photosynthetic Responses to High Salinity

High salinity also negatively affects photosynthetic activity by decreasing electron transport, inactivating PSI and PSII, and bringing lesions to the water splitting complex (Greenway and Munns, 1980; Define et al., 1999; Allakhverdiev et al., 2000). We earlier demonstrated that both trehalose and LEA plants maintain higher leaf Chl contents after sustained growth in the presence of NaCl (Jun et al., 2005; Kim et al., 2005). Here, we studied changes in photosynthetic capacities under high salinity. Activities in all plants gradually declined after sustained growth in the presence of 250 mM NaCl. In nontransformants, the Pmax of O_2 evolution decreased almost linearly over the duration of NaCl-treatment, reaching about 70% inhibition after 15 d (Figure 7). The behavior of trehalose plants was almost similar to that of the nontransformants, showing a comparable degree of inhibition in all lines (Figure 7). In contrast, LEA plants responded differently depending on the line tested. Over the 15 d of treatment, LEA plants of Line 9 which had the highest accumulation of LEA protein maintained about 50% of their initial Pmax while those of Line 2, with the lowest LEA protein accumulation, retained only 30% of their initial Pmax, a trend similar to that of the nontransformants (Figure 7). However, Chl fluorescence parameters did not vary much. In all plants, F_v/F_m was little changed while F_o was only slightly increased after 15 d of salt-treatment (Figure 8). In general, Chl fluorescence quenching parameters of the nontransformants and trehalose plants were somewhat affected after salt-treatment, showing some decline in qP and a rise in NPQ. However, for LEA plants of Line 9, these changes were not as extreme, indicating their heightened stability under salinity (Figure 8).

DISCUSSION

Trehalose and LEA protein are relatively well-studied osmoprotectants, and their response to dehydration in plants is clearly evident (Holmström et al., 1996; Xu et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998; Jun et al., 2001, 2005; Garg et al., 2002; Jang et al., 2003; Park et al., 2003; Kim et al., 2005). Their protective role also

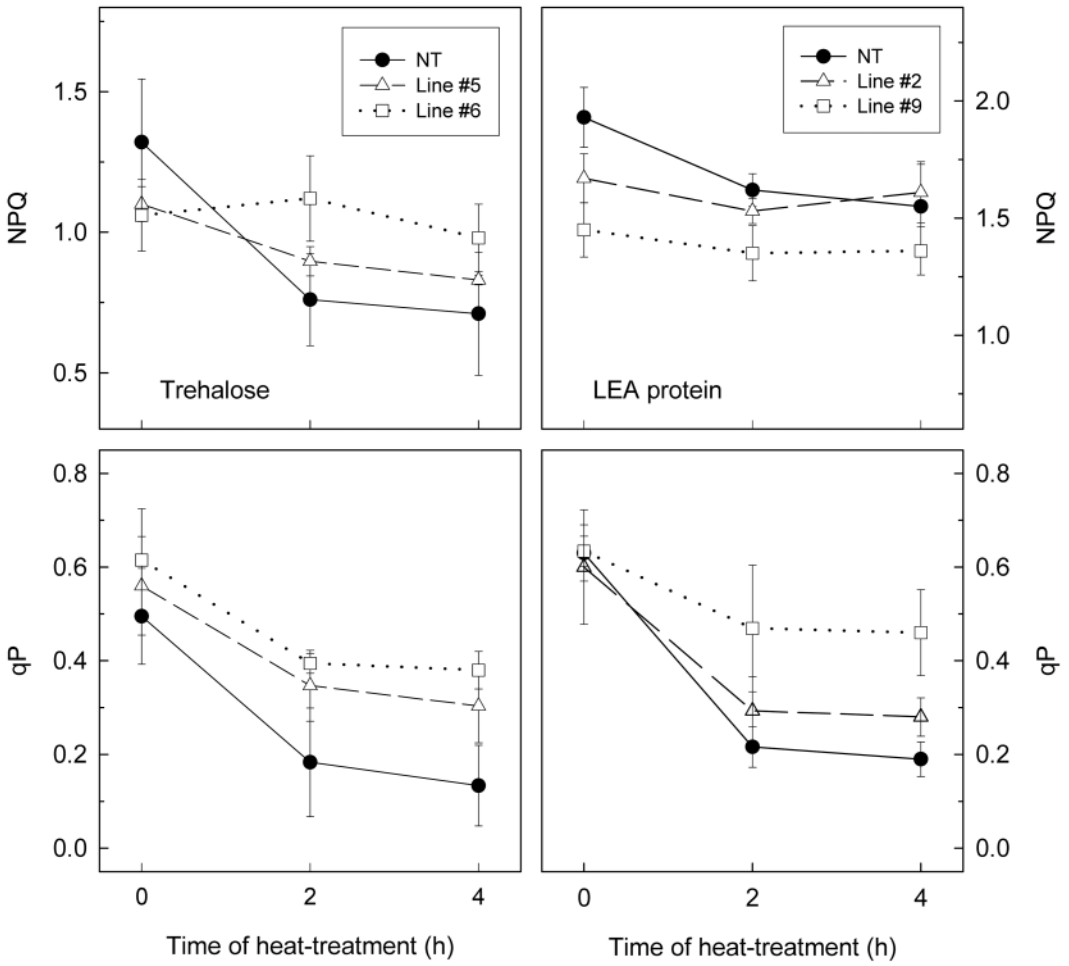

Figure 6. Changes in qP and NPQ after PEG-treatment in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Whole plants were incubated under darkness in growth chamber at 45°C for the indicated time prior to measuring Chl fluorescence. Chl fluorescence was simultaneously measured with O_2 evolution. Data are means ± SE (n = 4).

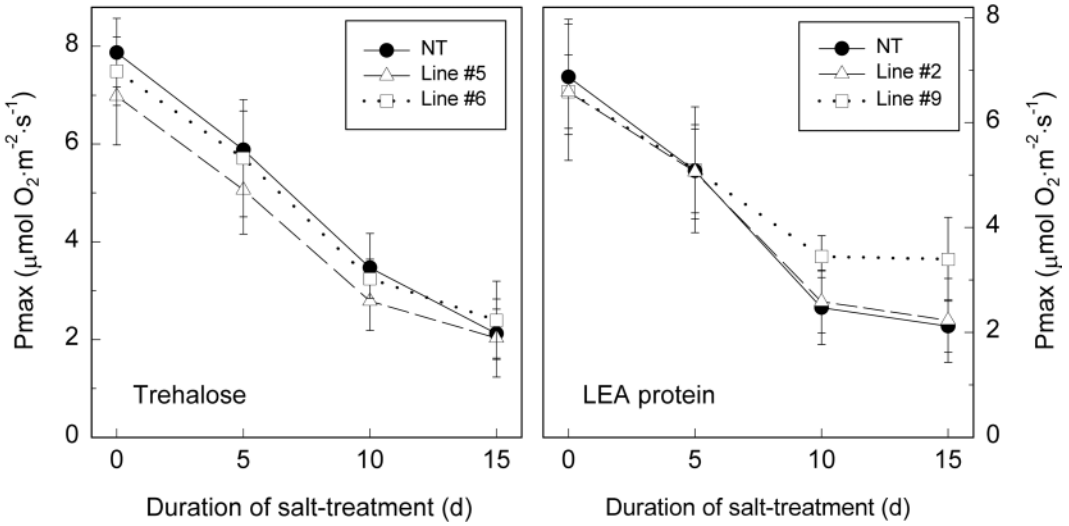

Figure 7. Changes in Pmax of O_2 evolution aftersalt--treatment in nontransformants (NT), trehalose- (Lines 4 and 8), and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Three-week-old whole plants were grown in soil irrigated with 250 mM NaCl for the indicated time prior to measuring photosynthesis. Data are means ± SE (n = 3).

extends toward conditions of high salinity and elevated temperatures (Xu et al., 1996; Jun et al., 2001, 2005; Garg et al., 2002; Jang et al., 2003; Park et al., 2003; Kim et al., 2005). Examinations of transgenic tobacco and Chinese cab-

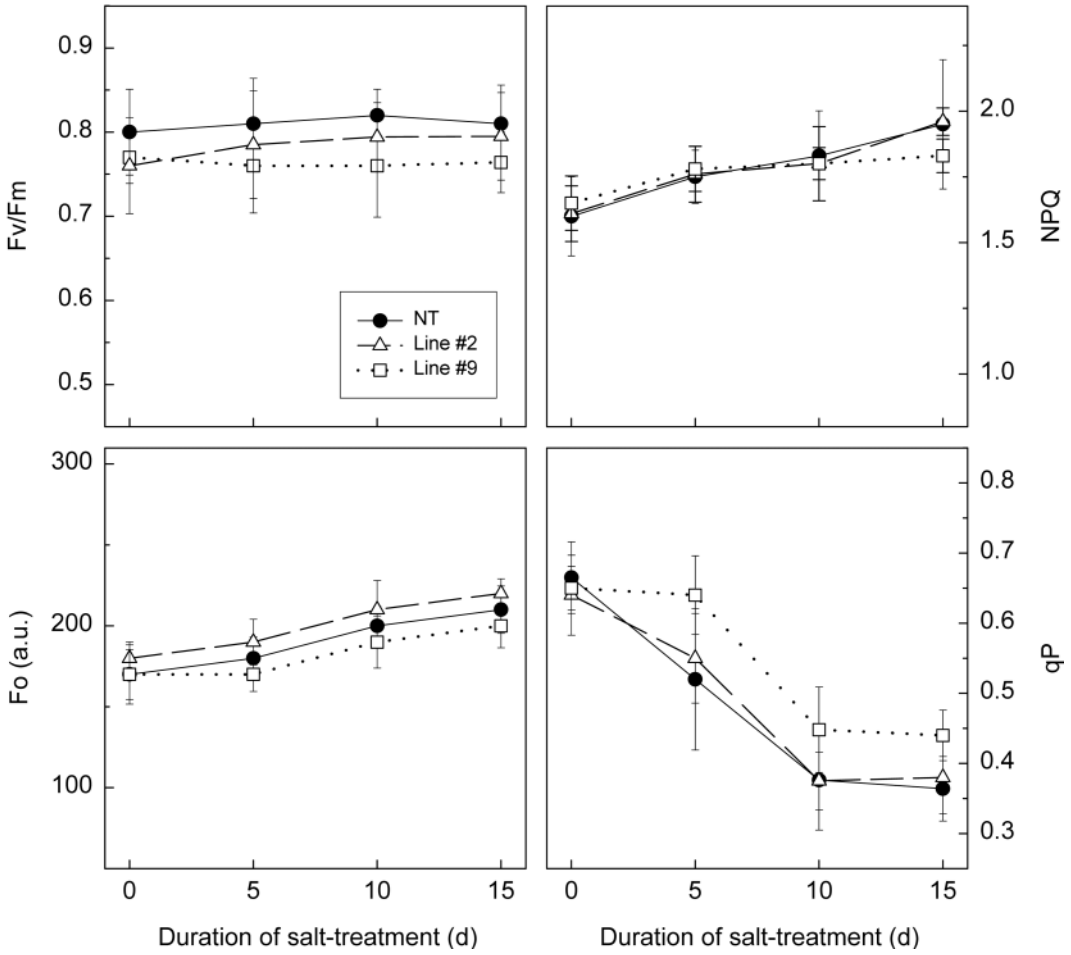

Figure 8. Changes in *Fo*, F_v/F_m, qP, and NPQ after salt-treatment in nontransformants (NT) and LEA protein-producing transgenic tobacco plants (Lines 2 and 9). Three-week-old whole plants were grown in soil irrigated with 250 mM NaCl for the indicated time prior to measuring Chl fluorescence. Chl fluorescence was simultaneously measured with O_2 evolution. Data are means ± SE (n = 3).

bage have shown that both substances are almost equally effective against dehydration, saline, and heat stresses (Park et al., 2003; Kim et al., 2005; our unpublished data). Based on those results, both have been proposed as suitable targets for the metabolic engineering of drought- or salt stress-resistant crop plants (Xu et al., 1996; Garg et al., 2002; Jang et al., 2003; Kim et al., 2005). However, compared with abundant research on their protective effects under abiotic stresses, investigations of their influence on photosynthetic capacities are scarce except for previous reports of the improved photosynthetic capacity by trehalose-producing plants under normal conditions (Pilon-Smits et al., 1998; Pellny et al., 2004). To develop a successful transgenic crop plant that is resistant to environmental stresses, it is necessary not only toincrease its rate of survival but also to improve its productivity in adverse circumstances. Without the maintenance of substantial crop yields under stress, improved survival *per se* is of little use. Thus, because it is essential to monitor photosynthetic behavior, we tested whether trehalose and LEA protein are useful for engineering stress-tolerant transgenic plants. Another objective was to compare these chemically different substances for their osmoprotectant properties in order to learn how they function in conferring enhanced tolerance.

In conferring tolerance against dehydration, both trehalose and LEA protein are equally effective in terms of maintaining leaf turgidity and retaining fresh weights in transgenic tobacco and Chinese cabbage (Park et al., 2003; Jun et al., 2005; Kim et al., 2005). However, we found that they differed significantly in their photosynthetic responses to dehydration. Only LEA plants exhibited considerably higher Pmax than the nontransformants after PEG-treatment while trehalose plants were no better than the nontransformants (see Figure 1). Dehydration, when accompanied by lowered leaf water potential, reduces photosynthetic activity in the chloroplasts, and leaf photosynthesis is inversely related to leaf water potential (Boyer and Bowen, 1970; Mohanty and Boyer, 1976; Lee et al., 1998, 2004). We previously demonstrated that leaf water potential is held constant in LEA plants in contrast to trehalose plants, where its decrease is similar to that of the nontransformants (Jun et al., 2005; Kim et al., 2005). However, the maintenance of leaf water potential is limited just to plants that accumulate a high amount of LEA protein. Therefore, it is no surprise that only LEA plants of Line 9 were better able to maintain their photosynthetic activity under dehydration. Because PSII is not the main site affected by water stress, both F_o and F_v/F_m were little affected in any genotype (see Figure 2). Under

reduced leaf water potential, decline of qP along with increased NPQ values accompanies with Pmax decline (Lee et al., 1998, 2004). Consequently, only LEA plants of Line 9, with the least inhibition of Pmax, showed few changes in qP and NPQ after PEG-treatment (see Figure 3). It is possible that a certain level of LEA protein is required for maintaining cellular water content, thereby alleviating the effect of lowered leaf water potential (Baker et al., 1988; Dure et al., 1989). By comparison, trehalose, regardless of its cellular level, is unable to sustain leaf water potential despite its osmoprotective role.

High temperatures affect many sites, including the water splitting complex, PSII, and Rubisco activation (Schreiber et al., 1994; Pastenes and Horton, 1996; Crafts-Brandner and Salvucci, 2000). Consequently, our heating treatment lowered photosynthetic activities in all plants, although its negative impact was significantly ameliorated in both trehalose and LEA plants. Pmax, Chl fluorescence parameters of F_o and F_v/F_m, and quenching parameters of qP and NPQ all remained favorable, except for LEA plants of Line 2 (see Figures. 4, 5, and 6). Trehalose increases membrane stability through vitrification (Colaco et al., 1995; Argüelles, 2000). We also have shown that membrane peroxidation and PSII-functioning in trehalose plants are less affected after heat-treatment (Jun et al., 2001; our unpublished data). Thus, it is no surprise that trehalose protects the photosynthetic apparatus from heat stress by stabilizing the thylakoid membranes.

In contrast, the protective effect of LEA protein is not widely known. We recently found (data not published) that *CaLEA6* is moderately up-regulated at high temperatures. Furthermore, *CaLEA6*-overexpressing transgenic tobacco plants have enhanced tolerance against heat, the extent to which depends on the amount of LEA protein accumulated (our unpublished data). Therefore, LEA protein appears to have a thermoprotective effect. Furthermore, when present above a certain level, it is likely to protect the thylakoid membrane from heat by increasing membrane stability.

Salt stress usually evokes dehydration in plants. In addition, high salinity alters the ion environment of cells, affecting many metabolic processes and photosynthetic activities (Greenway and Munns, 1980). Therefore, to ameliorate the negative effect of NaCl, an agent must act as an ion sequestrator as well as an osmoprotectant. We have previously shown that both trehalose and LEA protein retard leaf Chl decay under salt stress by stabilizing the thylakoid membrane (Jun et al., 2005; Kim et al., 2005). Only at a high level does LEA protein appears to perform the dual function, thus maintaining high photosynthetic activities under saline conditions. Consequently, LEA plants of Line 9 showed less inhibition in Pmax as well as fewer changes in Chl fluorescence parameters.

In summary, we have demonstrated here that the protective effect of trehalose and LEA protein on the photosynthetic apparatus differs although both are comparatively effective in enhancing tolerance against dehydration, high salinity and heat stress. Our results confirm that, if present at a high level, LEA protein can sustain leaf water potential during a period of dehydration, thereby maintaining photosynthesis. It appears to be a more effective and universal protectant for the photosynthetic apparatus under the abiotic stresses tested here. Therefore, engineering plants that produce LEA protein rather than trehalose would be a more efficient way to develop a multiple stress-tolerant crop plants without compromising its productivity.

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