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A Comparative Study on the Protective Role of Trehalose and LEA Proteins against Abiotic Stresses in Transgenic Chinese Cabbage (*Brassica campestris*) Overexpressing *CaLEA* or *otsA*

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Trehalose and LEA proteins, representative low MW chemicals that are synthesized under dehydration, are known to protect plants from drought stress. To compare their effectiveness on enhancing tolerance against various abiotic stresses, we generated transgenic Chinese cabbage plants overexpressing *E. coli* trehalose-6-phosphate synthase gene (*otsA*) or hot pepper (*Capsicum annuum*) LEA protein gene (*CaLEA*). Both transgenic plants exhibited altered phenotype including stunted growth and aberrant root development. When subjected to drought, salt or heat stress, these plants showed remarkably improved tolerance against those stresses compared with nontransformants. After dehydration treatment, leaf turgidity and fresh weight was better maintained in both transgenic plants. *CaLEA*-plants performed somewhat better under dehydrated condition. When treated with 250 mM NaCl, both *otsA*-plants and *CaLEA*-plants remained equally healthier than nontransformants in maintaining leaf turgidity and delaying necrosis. Furthermore, leaf Chl content and Fv/Fm was maintained considerably higher in both transgenic plants than nontransformants. After heat-treatment at 45°C, both transgenic plants appeared much less damaged in external shape and PSII function, but LEA proteins were more protective. Our results indicate that although both trehalose and LEA proteins are effective in protecting plants against various abiotic stresses, LEA proteins seem to be more promising in generating stress-tolerant transgenic plants.

Key words: dehydration, high temperature, photosynthesis, salinity, trehalose

INTRODUCTION

Various abiotic stresses, such as water deficit, increased soil salinity and extreme temperature are major factors limiting plant growth and productivity. In response to these stresses, plants developed a number of different strategies to cope with unfavorable conditions. The early events of plant adaptation to environmental stresses are the sensing and subsequent signal transduction to activate various physiological and metabolic responses including activation of various stress-responsive genes (Bray et al., 2000). Among various environmental stresses water deficit leading to dehydration is usually the most common of all to which land plants are exposed (Bray, 1997). Water stress affects the growth and development of plants through alternations in metabolism and gene expression (Ingram and Bartels, 1996; Bray, 1997). During periods of water deficit, plants undergo many physiological changes and induce a large number of genes for adaptation. Studies on the molecular responses to water deficit through two-dimensional PAGE and differential screening of cDNA libraries led to the isolation of many genes that are induced under water deficit and identification of multiple changes in gene expression. A typical change in gene expression during water deficit is the induction of the genes involved in the synthesis of various osmolytes and low molecular weight proteins such as dehyrins and late embryogenesis abundant (LEA) proteins (Ingram and Bartels, 1996; Bray et al., 2000; Ramanjulu and Bartels, 2002).

Accumulation of osmolytes such as proline, glycinebetaine and some sugar alcohols is an important mech-

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Chl, chlorophyll; Fm, maximal fluorescence after dark-adaptation; Fo, initial fluorescence; Fv, variable fluorescence; Fv/Fm, maximum photochemical efficiency of PSII; LEA, late embryogenesis-abundant; PEG, polyethylene glycol; PS, photosystem; TPS, trehalose-6-phosphate

anism to adapt osmotic stress (Yancey et al., 1982; Bray et al., 2000). Recently, accumulation of trehalose, a non-reducing disaccharide of two glucose units, is also implicated in this response in plants (Drennan et al., 1993; Zentella et al., 1999). Trehalose is widely distributed in nature and commonly found in many organisms ranging from bacteria to higher organisms including both plants and mammals (Argüelles, 2000). Owing to its unique several physical properties including high hydrophilicity, chemical inertness, and nonhygroscopic glass formation, trehalose works as a protective molecule under abiotic stresses in various organisms including bacteria, yeast, nematode, fungi, and primitive plants, especially against desiccation and high temperature (Thevelein, 1984; Larsen et al., 1987; Drennan et al., 1993; Crowe et al., 1998; Argüelles, 2000). In higher plants, however, its presence not to mention its role has been dubious until the identification of functional homologs for genes involved in trehalose synthesis in *Selaginella* and *Arabidopsis* (Blázquez et al., 1998; Vogel et al., 1998; Zentella et al., 1999). Engineered tobacco or rice plants expressing yeast *TPS* or *E. coli otsA* showed enhanced tolerance against dehydration imposed by air-drying in keeping leaf turgidity and retention of higher fresh weight (Holmström et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998; Garg et al., 2002; Jang et al., 2003). In our recent study, trehalose-producing tobacco plants showed increased tolerance to heat stress in addition to drought and salt stress (Jun et al., 2001). Therefore, it is rather well established that trehalose confers improved tolerance against various abiotic stresses in higher plants as well.

Other typical changes in gene expression during water deficit is the induction of a set of genes encoding special proteins such as dehydrins, osmotins, PIN, and LEA proteins whose nature of function is not identified yet (Close, 1996; Bray et al., 2000). LEA proteins are a large group of plant proteins, which are heavily synthesized and stored during seed maturation (Bray, 1993; Dure, 1993). They were first identified during the desiccation phases of seed development, and were reported to protect specific cellular structure or ameliorate the effect of drought stress by sequestering ions and maintaining a minimum cellular water requirement (Bray et al., 2000; Ramanjulu and Bartels, 2002). LEA proteins are classified into 7 subgroups based on the amino acid sequence homology and the specific motif, which will presumably undertake different function under water deficit (Dure, 1993). Most LEA proteins are cytosolic and hydrophilic containing random coil or a-helices (Bray et al., 2000; Soulages et al., 2002). Some non-typical proteins such as soybean D95-4, cotton Lea 14-A, tomato ER5 and Lemmin9, pcP27-45 of resurrection plants are hydrophobic (Piatkowski et al., 1990; Galau et al., 1993; Maitra and Cushman, 1994; Zegzouti et al., 1997). It was shown that transgenic rice plants overexpressing barley *HVA1*, a gene for group 3 LEA proteins, exhibited enhanced tolerance against both drought and salt stress (Xu et al., 1996). Recently, we also have demonstrated that transgenic tobacco plants overexpressing hot pepper LEA proteins exhibit increased tolerance to heat and salt stress in addition to drought stress (Kim et al., submitted). Thus LEA proteins appear to be involved in protecting cellular structures under various abiotic stresses, but their exact function is yet to be elucidated.

Although both trehalose and LEA proteins are highly effective as stress protectants against various abiotic stresses, their relative effectiveness, let alone the mode of action, is far beyond our understanding. As a first step to address this matter, we generated transgenic Chinese cabbage plants overexpressing either *otsA* or *CaLEA* to induce overproduction of trehalose or LEA proteins, and studied their response toward various abiotic stresses. We show here that both trehalose and LEA proteins confer enhanced tolerance against drought, salt, and heat stress, but their effectiveness varies depending on the nature of abiotic stress, suggesting that their mode of action as a protectant may be significantly different. Furthermore, LEA proteins seem to be a more efficient protectant in overall against abiotic stresses we tested, making them to be a more promising target material in generating stress-tolerant transgenic plants.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Chinese cabbage (*Brassica campestris* L. ssp *napus* var *pekinensis* cv Seoul) plants were grown for several weeks in a growth chamber maintained at 25±1°C with a diurnal cycle of 16 h-light and 8 h-dark under the light intensity of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Plasmid Construct and Plant Transformation

TPS overexpressor was constructed as following. The coding region of *otsA* gene (Kassen et al., 1992) was obtained by PCR using Vent polymerase (New England Biolabs, Beverly, MA, USA) and primers corresponding to its 5- and 3-ends with *Xba*I and *Eco*RI site, respectively. The PCR fragments were cloned into pWP90 vector containing a double 35S *CaMV* promoter and

a *CaMV* poly(A) terminator, and the whole fragment including promoter and terminator was again cloned into pBIN90 vector using *Kpn*I and *Sal*I site. The coding region of *CaLEA* gene was obtained by PCR using Vent polymerase (New England Biolabs, Beverly, MA, USA) and primers corresponding to its 5- and 3-ends with *Eco*RI site to clone into pWP90 vector. The whole fragment was cloned into pBIN90 vector as above. Transgenic Chinese cabbage was generated by the leaf disc transformation procedure (Shin et al., 2003) and selected on MS medium containing kanamycin. Heterozygous plants grown from seeds obtained from transgenic plants in F_1 generation were directly used for the subsequent physiological experiments.

Water Stress, Heat Stress, and Salt Stress Treatment

Water stress was imposed by air-drying detached leaves in the flow or by PEG treatment of whole plants. To do this 6-week old young plants were first carefully pulled out of the pots and washed in running water to remove soil without damaging roots, and then their roots were immersed in the Hoagland solution containing 10% (w/v) PEG-6000. PEG was chosen for its chemical inertness (Michel, 1970). High temperature treatment was done by transferring 6-week old plants to the growth chamber maintained at 45°C under continuous light. For Chl fluorescence measurements, heat stress was given by placing the whole plants in the chamber maintained at 45°C in the dark. Salt stress was imposed by supplementing 250 mM NaCl twice a week at the time of irrigation.

RNA Isolation and RT-PCR

Total RNA was isolated according to the modified method of Chomczynski and Sacci (1981) using TRIzol reagent (Invitrogen, Carlsbad, California, USA). RT-PCR was performed using primers covering the whole coding region of *otsA* or partial C-terminal region covering 400 bp of *CaLEA*.

Measurement of Chl Content and Chl Fluorescence

Chl content was measured by Minolta Chl meter (SPAD-502, Minolta, Japan) and also by conventional extraction method using acetone according to Porra et al. (1989). Chl fluorescence parameters (Fo, Fv, and Fm) were obtained using Plant Efficiency Analyzer (Hansatech, England) under the light intensity of 640 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

RESULTS

Morphological Features of Transgenic Cabbage Plants

Several lines of transgenic Chinese cabbage plants overexpressing *E. coli* TPS gene (*otsA*) or hot pepper LEA gene (*CaLEA*) were generated by leaf disc transformation as described before (Shin et al., 2003). The identity of transgenic plants and their expression level were examined by RT-PCR. Expression of *CaLEA* or *otsA*, quantified by RT-PCR, was at varying degree depending on the transgenic lines (Fig. 1). We selected two transgenic lines from each construct for the subsequent experiments based on the results of RT-PCR; line #9 for the weakly expressed one and line #2 for the strongly expressed one in *CaLEA*-transformants, and line #7 for the weakly expressed one and line #1 for the strongly expressed one in *otsA*-transformants (Fig. 1).

Both transgenic plants irrespective of their transgene expression level showed some distinct phenotypical alteration including stunted growth and aberrant root development: thicker taproots, well-developed lateral roots, and excessive root hairs (Fig. 2). The dwarfism became increasingly evident, as plants grew older. The generation time is considerably lengthened to be nearly twice of that in wild type accompanying with retarded senescence (data not shown). More often than not leaves looked greener due to higher Chl content especially in *CaLEA*-transformants (data not shown).

Response to Water Stress

It is now firmly established that both trehalose and LEA proteins are beneficial under water stress. Trehalose acts as a protectant against desiccation stress in microorganisms, ferns, and resurrection plants (Strøm and

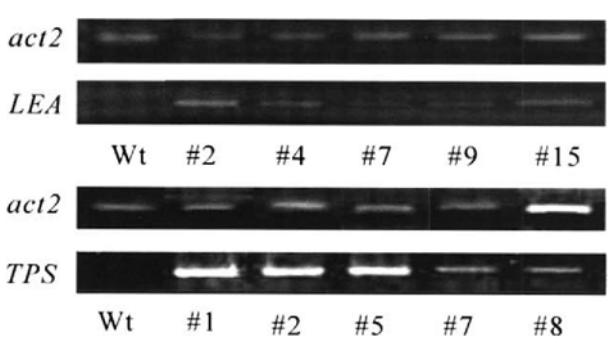
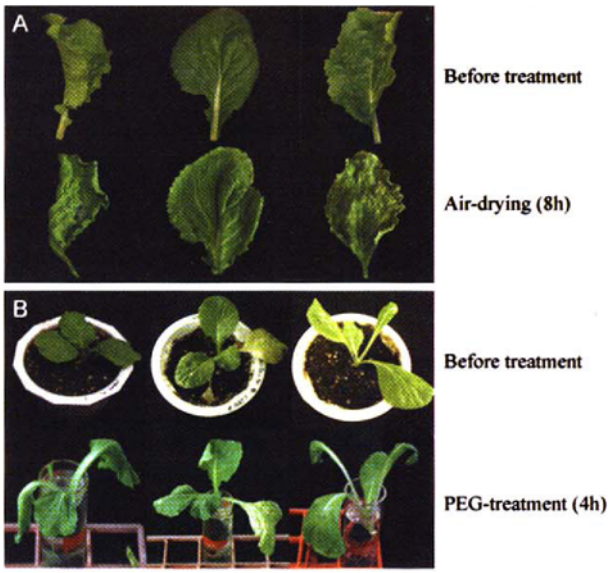


Figure 1. RT-PCR analysis of *TPS* and *CaLEA* expression in various transgenic cabbage plants (Wt; nontransformants).

Wt *CaLEA* plants (#2, #9) *TPS* plants (#1, #7)

Figure 2. Altered root phenotypes in 6-week old *CaLEA*- and *TPS*-transgenic plants due to the aberrant root development. Note that both transgenic plants developed thicker taproots along with well-developed lateral roots and excessive root hairs.

Kaasen, 1993; Argüelles, 2000). Furthermore, trehalose-producing transgenic tobacco and rice plants exhibited enhanced tolerance to drought and maintained higher photosynthetic capacity under water stress (Holmström et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998; Jun et al., 2001; Garg et al., 2002; Jang et al., 2003). *LEA* gene is one of the best-known water stress-responsive genes, and LEA proteins were reported to protect specific cellular structure or ameliorate the effect of drought stress by sequestering ions and maintaining minimal cellular water requirement (Bray, 1997; Ramanjulu and Bartels, 2002). Various LEA proteins including wheat Em, and tomato le-25 conferred increased tolerance to yeast against salt and chilling stress (Ried and Walker-Simmons, 1993; Imai et al., 1996; Swire-Clark and Marcotte, 1999; Zhang et al., 2000). In addition, transgenic plants overexpressing barley LEA proteins or hot pepper LEA proteins exhibited increased tolerance against water and salt stress (Xu et al., 1996; Kim et al., submitted). In view of these, we compared the effectiveness of trehalose and LEA proteins on improving tolerance against water stress in higher plants using transgenic Chinese cabbage plants overexpressing each gene. First, we observed changes in leaf turgidity upon air-drying of detached leaves in the flow. After 8 h of air-drying, nontransformant leaves manifested severe wilting while transgenic leaves remained more or less turgid (Fig. 3A). In general, *CaLEA*-transgenic leaves appeared less wilted than *TPS*-transgenic ones against air-drying. Next, water stress was administered to the whole plants by immersing their roots in 10% (w/v) PEG-solution. Nontransformed cabbage plants began to wilt after 2 h and showed a clear sign of wilting after 4 h of PEG-treatment. In contrast, all transgenic plants showed no sign of wilting after 2 h and significantly less



Wt *CaLEA* plant (#2) *TPS* plant (#1)

Figure 3. Increased tolerance to dehydration in *CaLEA*- or *TPS*-transgenic plants. Detached leaves from 6-week old cabbage plants were air-dried for 8 h (A) or whole plants were subjected to PEG-treatment for 4 h (B). Note the better maintenance of leaf turgidity in transgenic plants compared with withered leaves in nontransformant (Wt). *CaLEA* transgenic plants appeared to perform better under dehydration.

wilting after 4 h (Fig. 3B). There was variation in response among transgenic plants, but no distinct difference was observed between *TPS*- and *CaLEA*-transgenic plants. In general, transgenic plants showing higher expression level of transgene appeared to better withstand dehydration. When dehydration was induced by limiting water supply, similar trend was observed (data not shown). Upon rehydration after 8 h of PEG-treatment, which induced severe leaf wilting, nontransformants recovered poorly while both *CaLEA*- and *TPS*-transgenic plants fully regained leaf turgidity and revived eventually (data not shown). *TPS*-transgenic plants took a longer time for the full recovery than *CaLEA*-transgenic plants.

Changes in Fresh Weight and Chl Fluorescence after Dehydration

To compare the water-retaining ability quantitatively against dehydration, decrease in fresh weight of detached leaves after air-drying and of whole plants after PEG-treatment were also measured. The fresh weight of the detached leaves was decreased upon air-drying but in a different pace: nontransformant lost nearly 80% while *CaLEA*-transgenic one lost only 30% (Fig. 4A).

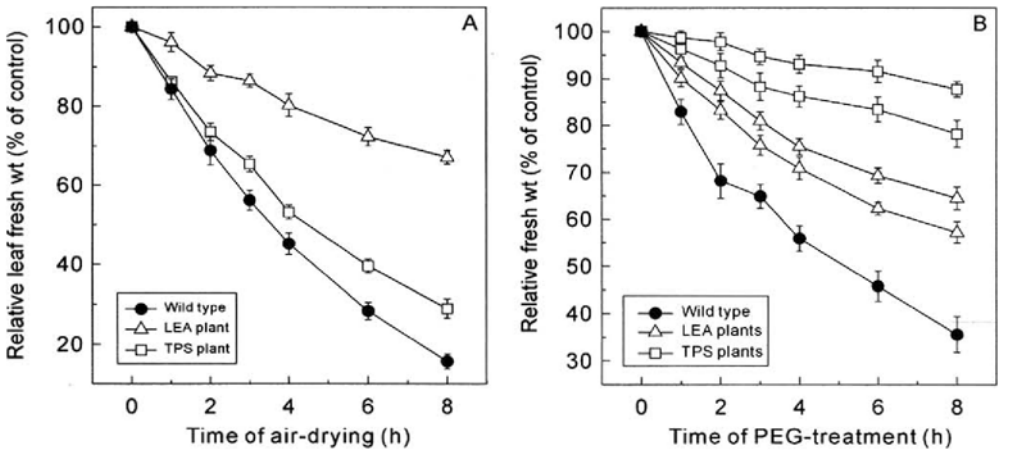


Figure 4. Decrease of fresh weight in the detached leaves (A) and whole plants (B) after dehydration-treatment. Dehydration was induced by air-drying of detached leaves or immersing roots of whole plants in 10% (w/v) PEG-solution. Data presented are mean values ± SE (n = 3).

TPS-transgenic one showed an intermediate response (Fig. 4A). When the roots of whole plants were immersed in 10% (w/v) PEG-solution, nontransformant lost more than 65% of their fresh weight after 8 h of PEG-treatment. In contrast, *TPS*-transgenic plants (line #1 and #7) lost only about 10-20% of their fresh weight and *CaLEA*-transgenic plants (line #2 and #9) lost 35-45% of their fresh weight after same treatment (Fig. 4B). Plants of higher transgene expression were more efficient in retaining fresh weight. The better performance in *TPS*-transgenic plants against PEG-treatment was somewhat contradictory to what was observed in the detached leaves and recovery response where *LEA*-transgenic plants were superior.

Dehydration when accompanied with lowered leaf water potential reduces the photosynthetic activity of chloroplasts (Mohanty and Boyer, 1976; Lee et al., 1998). Therefore, changes in leaf photosynthesis due to dehydration were compared between nontransformants and transgenic plants. Chl fluorescence parameters, the initial Chl fluorescence (Fo) and the maximal photochemical efficiency of PSII (Fv/Fm) are often used to assess PSII function (Schreiber et al., 1994). Although no significant changes in Fo and Fv/Fm were observed after PEG-treatment in both nontransformants and transgenic plants, transgenic plants performed slightly better than nontransformants in maintaining Chl fluorescence parameters (Table 1). The result is in accordance with various earlier reports that water stress did not affect PSII significantly (Ögren and Öquist, 1985; Lee et al., 1998).

Response to High Temperature

High temperature negatively affects most plants leading retarded growth, chlorosis and ultimate death. In our early separate studies using *TPS*- or *CaLEA*-transgenic tobacco plants, both trehalose and LEA proteins were shown to alleviate the negative effect of heat stress (Jun et al., 2001; Kim et al., submitted). Consequently, their effectiveness against heat stress was compared. To evaluate tolerance against high temperature 6 week-old plants grown at 24°C were transferred to a growth chamber at 45°C under continuous light. After 8 h of heat-treatment at 45°C, nontransformants were severely wilted. Subsequently, they began to manifest extensive leaf chlorosis leading to ultimate death. In contrast, both *CaLEA*-and *TPS*-transgenic plants showed lesser sign of wilting and remained healthier after treatment (Fig. 5). Eventually they recovered fully and grew into

Table 1. Changes in Pmax ($\mu mol \cdot m^{-2} \cdot s^{-1}$) of O_2 evolution and maximal photochemical efficiency of PSII (Fv/Fm) after dehydration. Dehydration was induced by PEG-treatment for 2h.

Treatment	Fo (a.u.)			Fv/Fm		
	NT	LEA	TPS	NT	LEA	TPS
Control	4555±38	5034±49	4927±53	0.81±0.02	0.81±0.01	0.80±0.01
Dehydrated	4602±56	5042±55	4891±48	0.79±0.01	0.80±0.01	0.79±0.01

*Data presented are mean values±SD for 3-5 measurements. Standard deviations for Fv/Fm are no shown, but less than 2%.

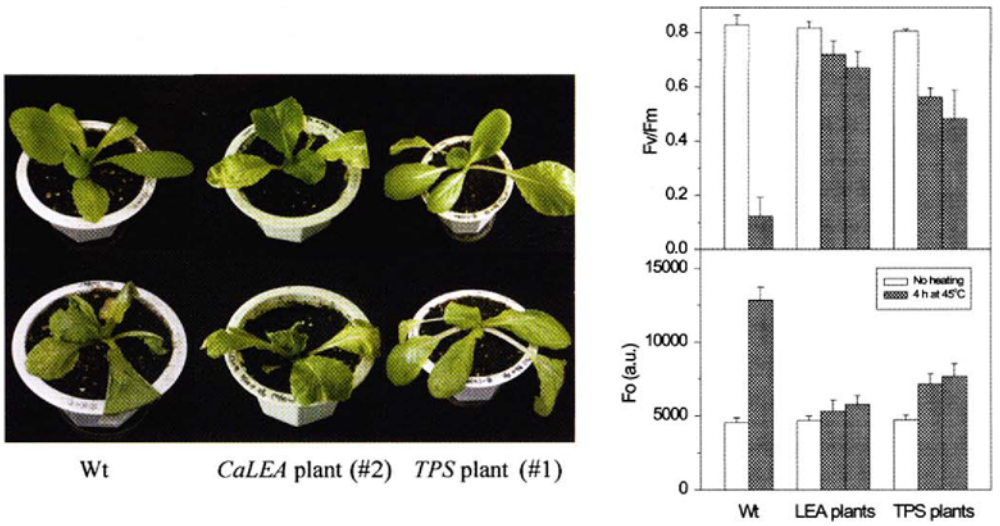


Figure 5. Increased tolerance to high temperature in *CaLEA*- or *TPS*-transgenic plants. Photograph shown on the left side are nontransformant (Wt) and transgenic plants before and after incubation at 45°C for 8 h. Cabbage plants grown at 25°C for 6 weeks were transferred to a growth chamber maintained at 45°C under continuous light. Note that both transgenic plants maintained healthier state under high temperature. On the right side are histograms showing changes in Fo and Fv/Fm after heat treatment at 45°C for 4 h in the dark. *CaLEA*-transgenic plants appeared to photosynthetically perform better after heat-treatment. Data presented are mean values ± SE (n = 5).

mature plants (data not shown). The results confirm that production of either LEA proteins or trehalose provides plants substantial protection against heat stress as previously demonstrated in transgenic tobacco plants (Jun et al., 2001; Kim et al., submitted), but *TPS*-transgenic plants appeared to outperform *CaLEA*-transgenic plants under high temperature in maintaining healthier external shape.

Changes in PSII Function after Heat Treatment

Heating leads to the damage in the photosynthetic apparatus, resulting in the decline of O_2 evolution and CO_2 fixation. Chl fluorescence parameter, Fv/Fm is decreased, but Fo is increased since PSII is known as the main labile site for heat stress (Schreiber et al., 1994). Therefore, as an additional way to assess the tolerance against high temperature, changes in PSII function after heat-treatment were evaluated by measuring leaf Chl fluorescence. After 4 h of heat-treatment at 45°C in the dark, Fo was increased about three times in nontransformants while it was increased only 50% and 30% in *TPS*-plants (line #1 and #7) and *CaLEA*-plants (line #2 and #9), respectively (Fig. 5). Likewise, Fv/Fm remained much more favorable in transgenic plants after heat treatment at 45°C, showing 10-40% decrease in comparison to more than 80% decrease in nontransformants (Fig. 5). As in dehydration treatment, plants of higher expression lines slightly outperformed those of lower expressed lines. *CaLEA*-plants appeared to be more efficient than *TPS*-plants in maintaining PSII function under high temperature. The result is rather contradictory of the earlier observation on external shape, where trehalose appeared to be more protective. Nevertheless it is likely that both trehalose and LEA proteins may stabilize thylakoidal membrane to preserve PSII function under high temperature.

Response to Salt Stress

Salt stress is closely related with water stress in the sense that plants respond similarly to both stresses, but high salinity could be more damaging to plants because it creates hyperionic cellular environment in addition to causing cellular dehydration (Zhu, 2002). To test tolerance against salt stress, 6-week old plants grown under normal conditions in the absence of salinity were continuously grown with supplemented 250 mM NaCl at the time of irrigation. Plant growth was apparently inhibited after supplementation of 250 mM NaCl in all plants, but the negative effect of salinity including growth retardation and leaf necrosis was initiated significantly earlier in nontransformants compared with transgenic plants. After 10 days of growth under supplemented NaCl, leaf chlorosis began to occur exclu-

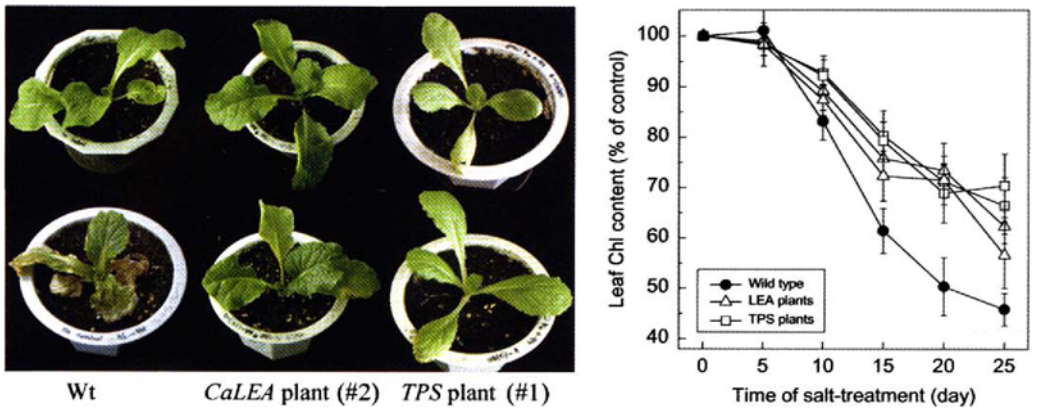


Figure 6. Increased tolerance to salinity in *CaLEA*- or TPS-transgenic plants. Photograph shown on the left side are nontransformant (Wt) and transgenic plants prior to or after salt treatment for 10 days. Cabbage plants grown at 25°C for 6 weeks were continuously grown under the supplementation of 250 mM NaCl. Note that both transgenic plants maintained healthier state anc manifested less leaf chlorosis after salt treatment. Graph on the right side shows the decline of leaf Chl content after salt treatment. Leaves showing no apparent necrosis were chosen for the measurement. Data presented are mean values ± SE (n = 3).

sively in the nontransformants. On the 20th day after salinity treatment nontransformant leaves manifested extensive chlorosis and necrosis while leaves of transgenic plants were about to show slight bleaching (Fig. 6). No distinctive difference was observed between *TPS*- and *CaLEA*-transgenic plants after salt treatment in external shape.

Changes in Chl Content and Chl Fluorescence after Salt Stress

Since Chl bleaching was accompanied with salinity treatment, changes in Chl content in the leaves showing no apparent necrosis were traced. After NaCl supplementation, Chl content in the leaves of nontransformants was maintained for 5 days, but was sharply declined in spite of no visible chlorosis, showing about 50% decrease on the 20th day compared with less than 30% reduction in those of transgenic plants (Fig. 6). The efficiency in maintaining leaf Chl content was independent of transgene expression level, but both trehalose and LEA proteins appear to be equally effective in delaying chlorosis imposed by high salinity. No significant decline in PSII function as indicated by Fv/Fm was observed in all plants by salt treatment of up to 20 days (data not shown). Fo values were not changed significantly either.

DISCUSSION

When plants are subjected to the conditions evoking water deficit, various dehydration-responsive genes are induced. Among them are the genes involved in the synthesis of various osmolytes such as glycinebetaine, proline, trehalose and some sugar alcohols (Yancey et al., 1982; Drennan et al., 1993; Ingram and Bartels, 1996; Bray et al., 2000). In addition, a set of genes encoding special small proteins such as dehydrins, osmotins, PIN, and LEA proteins are also activated (Dure, 1993; Close, 1996; Ingram and Bartels, 1996; Bray, 1997; Ramanjulu and Bartels, 2002). A number of transgenic studies overexpressing some of these genes have shown that they conferred increased tolerance against dehydration, freezing and salinity stress (Tarczynski et al., 1993; Kishor et al., 1995; Pilon-Smits et al., 1995; Holmström et al., 1996; Imai et al., 1996; Xu et al., 1996; Hayashi et al., 1997; Swire-Clark and Marcotte, 1999; Guo et al., 2000; Garg et al., 2002; Jang et al., 2003). In the past several years, we have been conducting a study for functional characterization of water stress responsive genes, primarily focusing on the role of trehalose and LEA proteins under water deficit. Trehalose, a non-reducing disaccharide, is of special interest among them because in spite of its wide distribution and distinctive role as a stress protectant in lower organisms ranging from bacteria to resurrection plants (Thevelein, 1984; Larsen et al., 1987; Drennan et al., 1993; Argüelles, 2000), its existence, not to mention its role, has been dubious in higher plants until recently. However, the identification of functional homologs of *TPS* and *TPP* in *Arabidopsis* strongly vindicated that it is synthesized in *Arabidopsis* (Blázquez et al., 1998; Vogel et al., 1998). Furthermore, recent studies demonstrated that trehalose-producing transgenic human cell, tobacco and rice plants showed

enhanced tolerance against dehydration and salt, further supporting its role as a stress protectant (Holmström et al., 1996; Romero et al., 1997; Pilon-Smits et al., 1998; Guo et al., 2000; Jun et al., 2001; Garg et al., 2002; Jang et al., 2003). In addition, another functional *Arabidopsis TPS* homologue (*AtTPS4*) specifically expressed in silique was recently identified by us, and *AtTPS1* was up-regulated under water deficit in our own study, together suggesting the functional importance of trehalose in higher plants related with dehydration (Jun et al., unpublished data). LEA proteins are a large group of small proteins induced under water deficit and synthesized during seed development (Bray, 1993; Dure, 1993). Transgenic rice plants overexpressing barley LEA protein showed increased resistance to drought and salt stress (Xu et al., 1996). In our recent study, transgenic tobacco plants overexpressing hot pepper LEA proteins, which are heavily up-regulated under dehydration and salt stress, exhibited increased tolerance to drought, heat and salt stress (Kim et al., submitted). Therefore, LEA proteins are also likely to be involved in protecting cellular structures under various abiotic stresses. Although both trehalose and LEA proteins are shown to be highly effective as stress protectant against various abiotic stresses in several transgenic studies, no systematic attempt to assess their relative effectiveness has been tried yet. The comparative study on their effects against various abiotic stresses would be a basis for the further understanding their mode of action in the stress response. In addition, proper evaluation for the relative effectiveness of various stress protectants would be an efficient and economic step to develop stress-tolerant plants of agricultural importance.

As shown in this study using transgenic Chinese cabbage plants, production of either trehalose or LEA proteins is beneficial in improving tolerance against drought, salt and heat stresses. The degree of tolerance conferred by theses compounds was somewhat dependent on the expression level of transgene except in salt stress where no significant difference was observed among different lines (compare Fig. 4, 5 and 6). Both trehalose and LEA proteins had osmoprotective effect in the sense that they allowed to maintain leaf turgidity and relative water content (see Fig. 3 and 4). However, judging from the amount synthesized in other studies, they are not likely to provide osmotic adjustments (Goddijn et al., 1997; Jun et al., 2001). Rather, they appear to protect cellular structures under dehydrated conditions. As demonstrated in transgenic tobacco plants, trehalose and LEA proteins also were protective against heat stress in Chinese cabbage plants presumably by stabilizing membrane (see Fig. 5). Trehalose is known to increase membrane stability by vitrification (Crowe et al., 1998), but how LEA proteins protect membrane structure is not understood at this point. However, it is certain that both of them stabilize thylakoid membrane since PSII was better protected in both transgenic plants in terms of maintaining more favorable Fo and Fv/Fm after heat-treatment at 45°C in the dark (see Fig. 5). As in dehydration treatment, both transgenic plants manifested much lesser negative effect of salinity in leaf wilting and necrosis, and leaf Chl content was better maintained in transgenic plants than nontransformants (see Fig. 6). The protection is likely to result from their membrane-stabilizing effect since high salt destabilizes thylakoid membrane structure by affecting water splitting complex and leads to Chl bleaching. Although both trehalose and LEA proteins conferred improved tolerance against abiotic stresses we tested, their relative effectiveness varied depending on the nature of abiotic stress, suggesting that their mode of action as a protectant may be significantly different. In overall estimation, although there was no distinguishing protective difference against salt stress between trehalose and LEA proteins, LEA proteins slightly outperformed trehalose against dehydration and heat stress, making them a more attractive candidate in generating agronomically useful plants.

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