Exogenously-supplied trehalose protects thylakoid membranes of winter wheat from heat-induced damage

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Abstract

The effects of trehalose pretreatment on thylakoid membranes of winter wheat were investigated under heat stress. Under normal growth conditions, the winter wheat synthesized 502 μg g^{-1}(f.m.) trehalose, which increased to 1250 μg g^{-1}(f.m.) under heat stress and to 1658 μg g^{-1}(f.m.) in trehalose-pretreated seedlings. Under heat stress, proteins in the thylakoid membranes and the photosynthetic capacity were protected by trehalose pretreatment. Moreover, the electrolyte leakage, contents of malondialdehyde, superoxide anion and hydrogen peroxide, and lipoxygenase activity in trehalose-pretreated seedlings were lower than in the non-pretreated plants.

Additional key words: heat stress, lipoxygenase, malondialdehyde, reactive oxygen species, *Triticum aestivum*.

Introduction

Trehalose (α-D-glucopyranosyl-[1,1]-α-D-glucopyranoside) is a non-reducing disaccharide, which plays an important physiological role as a compatible solute in a large number of organisms, including bacteria, yeast and invertebrates. In most plants, trehalose is hardly detectable, except in certain specialized resurrection species that accumulate the compound quantitatively. Some previous studies have shown that trehalose can stabilize proteins and biological membranes efficiently in microorganisms under stress (Benaroudj *et al*. 2001, Sebollela *et al*. 2004). Others have found that trehalose accumulation in transgenic plants can increase their abiotic stress tolerance (Garg *et al*. 2002, Jang *et al*. 2003), and these results have been widely accepted (Cherian *et al*. 2006). However, in plants the mechanism of improving stress tolerance remains largely unknown.

Heat stress is a prevalent environmental stress that adversely affects organisms by producing reactive oxygen species (ROS), causing membrane integrity loss, damaging proteins, DNA and lipids, and potentially disrupting cell function (Mittler *et al*. 2004). In plants, the links between ROS production and photosynthetic metabolism are particularly important (Nishiyama *et al*. 2004, Couée *et al*. 2006). Chloroplast thylakoid membranes, are highly vulnerable to heat stress. High temperatures led not only to disintegration of the lipid bilayer (Losa *et al*. 2004), but also to damage of the oxygen-evolving complex of photosystem 2 (PS 2; Komayama *et al*. 2007). The thylakoid membranes consist mainly of five lipids: monogalactosyl diacylglycerol (MGDG), digalactosyl diacylglycerol (DGDG), sulfoquinovosyl diacylglycerol (SQDG), phosphatidylglycerol (PG) and phosphatidylcholine (PC). There is an inverse correlation between growth temperature and membrane saturation level. Changes in lipid-protein interactions are thought to play a major role in heat-induced increase in the fluidity of the thylakoid membranes (Larkindale *et al*. 2004). To optimize photosynthesis and other cellular processes, plants often adjust the properties of their cellular membranes, such as membrane fluidity, lipid composition and fatty acid saturation levels, in response to temperature stress (Larkindale *et al*. 2004, Kim *et al*. 2005). By improving the thermal stability of the thylakoid membranes, plants allow the photosynthetic apparatus to preserve its functional potential at high temperatures, thus minimizing the after-effects of heat stress (Haldimann *et al*. 2005).

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Abbreviations: DCBQ - 2,6-dichloro-*p*-benzoquinone; DGDG - digalactosyl diacylglycerol; IUFA - unsaturation indexes of fatty acids; LOX - lipoxygenase; MDA - malondialdehyde; MGDG - monogalactosyl diacylglycerol; $O_2^{\bullet -}$ - superoxid anion; PC - phosphatidylcholine; PG - phosphatidylglycerol; PS 2 - photosystem 2; ROS - reactive oxygen species; SQDG - sulfoquinovosyl diacylglycerol; T6P - trehalose-6-phosphate.
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It has been shown that trehalose accumulation during heat stress protects cells and cellular proteins of *Saccharomyces cerevisiae* from damage by oxygen radicals (Benaroudj *et al*. 2001). However, in plants, whether trehalose possesses this function or not is largely unknown. As far as we know, protective effects of trehalose on the photosynthetic machinery have not been reported. Our recent study has shown that trehalose scavenges ROS directly during heat shock (Luo *et al*. 2008). Therefore, a primary aim of this study is to determine whether this function of trehalose helps to stabilize thylakoid membranes during heat stress.

Materials and methods

Plants and treatments: Seeds of wheat (*Triticum aestivum* L.) were germinated on filter paper moistened with water for 24 h after being sterilized with 0.2 % sodium hypochlorite. Then, the germinating seeds were placed on nylon gauze at an appropriate density and cultured in half-strength Hoagland solution in trays (25 × 18 × 5 cm) that were placed in a growth chamber at temperature of 25 ºC, a 16-h photoperiod (irradiance of 100 μmol m^{-2} s^{-1}) and relative humidity of 70 %. The Hoagland solution was changed every other day. After about 15 d, when the second leaves were fully expanded, wheat seedlings were treated with Hoagland solution containing 1.5 mM trehalose for 3 d. Then they were exposed to a heat treatment of 40 ºC for another 24 h in a growth chamber that had the other conditions as mentioned above. After that, the young fully-expanded leaves were selected for the following assays. All experiments were conducted at least three times.

Detecting trehalose: Before trehalose extraction, samples were ground in liquid nitrogen. Then, 10 g of ground samples were extracted for 30 min at 75 °C with 10 cm^3 85 % ethanol three times. The extract was centrifuged for 10 min at 1 600 *g*, and the supernatant was mixed by a magnetic stirrer at 80 ºC to evaporate the ethanol. Sugars were dissolved in the 1-cm^3 mobile phase (aceto-nitrile:water; 70:30) and filtered through a 0.45-μm filter unit. Quantitative trehalose analysis was carried out by HPLC (*Waters 600*, Milford, MA, USA) with a *Hypersil* NH_2 column (5 μm, 4.6 × 250 mm) and evaporative light scattering detector *2424*. The sample injection volume was 0.01 cm^3, using commercially available trehalose (*Sigma*, St. Louis, USA) as the standard.

Ultrastructure of chloroplast: According to the method described by Gielwanowska *et al.* (2005), fragments 2 - 3 mm in length were sectioned from the second leaves in control and treated wheat seedlings. After the fixation, dehydration and embedment, ultra-thin sections were examined with a *JEOL Jem 100S* (Musashino, Japan) transmission electron microscope. Five or six fragments of different leaves were employed in the study of the ultrastructure for each of the different treatments.

Lipid extraction and analysis: The lipids were extracted from the thylakoid membranes following the procedures described by Bligh *et al*. (1959) and then separated by two-dimensional thin-layer chromatography. After a second extraction using benzene:petroleum ether (1:1, v/v), the combined extracts were esterified with 0.4 M NaOH. The fatty acid methyl esters were determined using a gas chromatogram analyzer (*Shimadzu GC-9A,* Texas, USA), using a methyl esterified arachidic acid as an internal standard. The conditions were the same as described previously (Zhao *et al*. 2007).

Thylakoid membranes: After treatments mentioned above, the thylakoid membranes from young fully expanded leaves were prepared as described previously (Yang *et al*. 2008), were suspended in a buffer containing 20 mM MES 35 mM NaCl, 0.4 M sucrose (pH 6.5), and were stored at -80 °C for further use. Chlorophyll contents in thylakoid suspension were estimated using 80 % acetone following the equation of Lichtenthaler (1987).

Thylakoid polypeptides were separated by urea and SDS-PAGE according to Parida *et al*. (2003) with minor modification to the 15 % separating gel and were detected after staining and de-staining as described in Parida *et al*. (2003). In total, 20 μg of chlorophyll was loaded per lane.

Electron transport activities were measured with a Clark-type oxygen electrode (*Chlorolab 2*, *Hansatech*, King's Lynn, Norfolk, UK) in the thylakoid membranes suspended in the medium [50 mM Tricine, 10 mM NaCl, 2 mM $MgCl_2$, 400 mM sorbitol, 10 mM $K_3Fe(CN)_6$, 1 mM 2,6-dichloro-*p*-benzoquinone (DCBQ), pH 7.6] and under irradiance of 800 μmol m^{-2} s^{-1}.

Determination of lipoxygenase (LOX) activity: The plant material was deep-frozen in liquid nitrogen and ground in 25 mM Tris-hydrochloric acid buffer (pH 7.5). The homogenate was centrifuged at 4 000 *g* for 5 min, and the supernatant was used for determination of LOX activity according to the method of Peever and Higgins (1989), with an oxygen electrode (*Chlorolab 2*, *Hansatech instruments*).

Electrolyte leakage and lipid peroxidation: Electrolyte leakage in different treated leaves was assessed as described by Dionisio-Sese and Tobita (1998) and lipid peroxidation was measured in terms of malondialdehyde (MDA) contents, a product of lipid peroxidation, according to the method of Heath and Packer (1968).

ROS content: $O_2^{\bullet -}$ and H_2O_2 contents in different treated leaves were determined according to the method of Liu *et al*. (2005). Sodium nitrite was used as the standard solution to calculate the level of $O_2^{\bullet -}$, and the H_2O_2 solution was used to calculate H_2O_2 content.

Statistics: Statistical analysis was conducted using the procedures of DPS (Zhejiang University, China). All parameters were analysed using two-way *ANOVA*, completely randomized with the two main factors being the experiment conducted in the presence or absence of trehalose, and different temperatures. All pairwise comparisons were analysed using Duncan's test.

Results

Untreated wheat plants used in this study accumulated about 500 μg g^{-1}(f.m.) trehalose, which increased up to 700 μg g^{-1}(f.m.) in trehalose-pretreated seedlings. During heat stress, trehalose content was enhanced to 1250 μg g^{-1}(f.m.) in control plants and to 1658 μg g^{-1} (f.m.) in trehalose-pretreated probably due to trehalose absorbed by the wheat roots (Table 1).

Under normal growth conditions, the chloroplasts in winter wheat leaves showed an ellipsoid shape with an intact membrane, in which the thylakoid segments were arranged clearly and regularly (Fig. 1*A*,*B*). However, heat damaged the chloroplast ultrastructure, making the grana lamellae anamorphic and irregular. The grana lamellae began to separate and almost no folds of thylakoids were seen. Meanwhile, the chloroplast envelope was seriously damaged (Fig. 1*C*), which might be the result of augmentation of membrane fluidity. If the seedlings were pretreated with trehalose for 3 d, this damage caused by heat was alleviated to some extent, with the more gently irregular grana lamellae and more lightly damaged

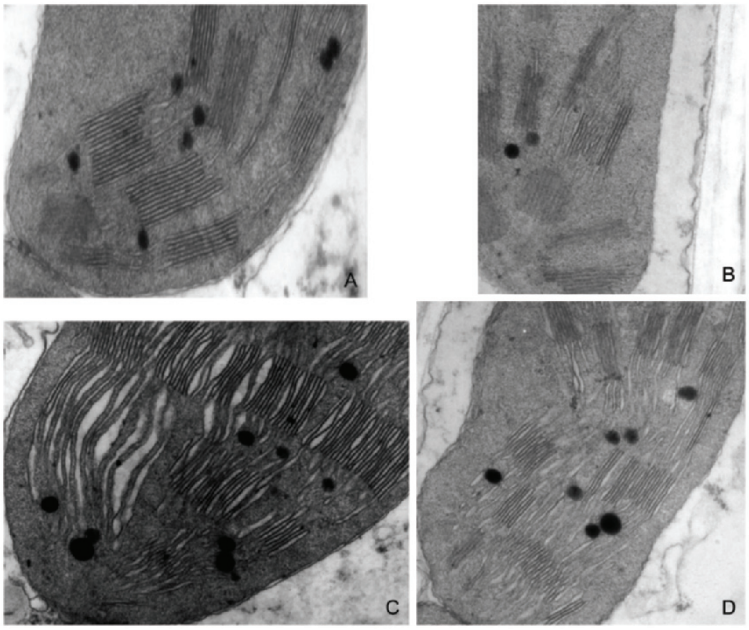

Fig. 1 Transmission electron micrographs of chloroplasts (×25) from winter wheat leaves (fragments of 2 - 3 mm in length). Five or six fragments of different leaves were employed for each treatment.

Table 1. Effect of heat stress and trehalose pretreatment on trehalose content, activity of PS 2 electron transport, unsaturation index of fatty acids (IUFA) in the thylakoid membrane, LOX activity, electrolyte leakage, MDA content, $O_2^{\cdot-}$ generating rate and H_2O_2 content in wheat leaves. Means ± SD of at least three independent samples. IUFA = 2 × [(18:1)mol% + (18:2)mol%] + 3 × (18:3)mol%.

Parameters	25 ºC	25 ºC + trehalose	40 ºC	40 ºC + trehalose
Trehalose content [μg g^{-1}(f.m.)]	502.67 ± 38.2	700.01 ± 50.1	1250.76 ± 48.4	1657.97 ± 44.3
PS 2 activity [μmol(O_2) mg^{-1}(Chl) min^{-1}]	8.21 ± 0.50	10.35 ± 0.40	2.99 ± 0.30	9.71 ± 0.20
IUFA	941.30 ± 25.2	856.00 ± 34.1	1051.50 ± 40.2	875.40 ± 23.5
LOX activity [nmol(O_2) g^{-1}(f.m.) min^{-1}]	29.63 ± 1.21	25.92 ± 0.81	37.02 ± 1.02	30.01 ± 1.91
Electrolyte leakage [%]	13.73 ± 1.28	13.08 ± 1.45	17.71 ± 1.85	12.35 ± 0.63
MDA content [nmol g^{-1}(f.m.)]	3.66 ± 0.41	3.21 ± 0.26	4.19 ± 0.29	3.51 ± 0.21
$O_2^{\cdot-}$ generating rate [μmol g^{-1}(f.m.) min^{-1}]	3.75 ± 0.16	2.81 ± 0.27	18.19 ± 2.81	7.14 ± 0.29
H_2O_2 content [μmol g^{-1}(f.m.)]	275.75 ± 26.1	254.51 ± 16.4	368.64 ± 30.8	278.01 ± 27.3

Table 2. Effect of heat stress and trehalose pretreatment on relative contents of lipids [%] in thylakoid membrane. Means of three independent experiments ± SD.

Treatments	MGDG	SQDG	DGDG	PC	PG
25 ºC	71.0 ± 2.3	3.0 ± 0.2	14.7 ± 0.7	9.1 ± 1.2	2.2 ± 0.1
25 ºC + trehalose	79.7 ± 1.8	1.8 ± 0.4	10.8 ± 1.5	5.9 ± 0.6	1.8 ± 0.1
40 ºC	51.1 ± 2.6	1.2 ± 0.3	35.3 ± 2.4	1.3 ± 0.5	11.1 ± 0.9
40 ºC + trehalose	61.0 ± 3.1	0.0 ± 0.6	29.0 ± 0.9	4.6 ± 0.3	5.4 ± 0.3

chloroplast envelope (Fig. 1*D*).

Contents of some polypeptides of M_r 20 - 97 kD were decreased by heating compared with the control, however, the decrease was less in trehalose-pretreated plants (Fig. 2). PS 2 electron transport activity was also improved by trehalose under heat stress (Table 1).

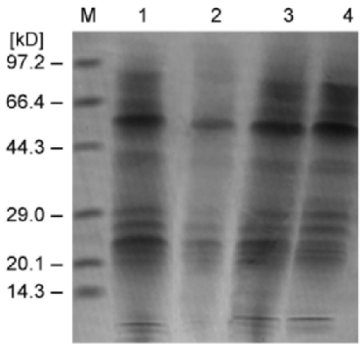

Fig. 2. Effects of trehalose pretreatment on thylakoid polypeptides. M - molecular mass marker; *Lanes* 1, 2, 3 and 4 represent thylakoid polypeptides isolated from the following treatments: control (no trehalose and no heat), heat but no trehalose, trehalose treatment alone and 1.5 mM trehalose pretreatment then heat, respectively.

Heat largely affected the relative contents of MGDG, DGDG, PC and PG. However, in trehalose-pretreated seedlings these lipids were less affected (Table 2). As far as unsaturation indexes of fatty acids (IUFA) were considered, under heat stress trehalose pre-treated seedlings had lower unsaturation indexes of fatty acids (IUFA) than non-treated ones (Table 1), indicating trehalose-pretreated seedlings more easily survive heat damage by maintaining membrane composition.

We investigated LOX activity, which mediates lipid peroxidation and photooxidation, to test whether its activity correlated with trehalose-induced changes in fatty acid composition of thylakoid membranes during heat stress. Under normal growth conditions, LOX activity was 29.6 nmol(O_2) g^{-1}(f.m.) min^{-1} and increased up to 27 % by heat stress. However, LOX activity was increased only to 30 nmol O_2(O_2) g^{-1}(f.m.) min^{-1} when trehalose-pretreated seedlings were exposed to heat stress (Table 1).

To investigate whether trehalose-induced membrane protection is accomplished by preventing lipid peroxidation, we measured electrolyte leakage and MDA content, a product of unsaturated fatty acid peroxidation. Heat-treated seedlings had higher electrolyte leakage and MDA content than controls (Table 1). However, if seedlings were pretreated with trehalose for 3 d, both electrolyte leakage and MDA content were less increased, which showed that trehalose pretreatment could protect the membranes from damage caused by heat.

The production of $O_2^{\bullet-}$ and H_2O_2 was enhanced to a certain extent under heat stress, and trehalose pretreatment depressed the production of $O_2^{\bullet-}$ and H_2O_2 (Table 1).

Discussion

Similarly to trehalose content found in wheat cultivars (*Triticum aestivum* L.) by El-Bashiti *et al*. (2005), in this study, wheat itself also synthesized minor amounts of trehalose, which showed nearly a 150 % increase after heat stress. However, under both control and heat stress conditions trehalose content was higher in trehalose-pretreated leaves showing that exogenously-supplied trehalose was absorbed by wheat roots.

During heat stress, trehalose pretreatment protects the ultrastructure of chloroplasts (Fig. 1) and some polypeptides in thylakoid membranes (Fig. 2), and also improves the photosynthetic capacity of thylakoids (Table 1), which indicates a protective role of trehalose or its metabolite for the thylakoid membrane.

One phenomenon associated with the acclimation of organisms to changes in ambient temperature is to regulate the fluidity of membrane lipids *via* changes in the composition and extent of unsaturation of their fatty acids. Heat stress led to a remarkable increase in total IUFA compared with the control (Table 1). However, IUFA in trehalose-pretreated seedlings was less increased during heat. Changes in IUFA might be ascribed to the alterations of lipolytic enzymes, including phospholipase, non-specific acylhydrolase, lipoxygenase and/or galactolipases, which were activated differently to hydrolyze different lipid species (Welti *et al*. 2002). It is possible that alterations to these enzymes were induced by signals from ROS or heat-induced changes in trehalose or its metabolites.

Heat led to the damage or degradation of some proteins,

most likely including the D1 protein in the thylakoid membrane (Fig. 2), since PS 2 photochemistry was decreased markedly when streptomycin was utilized to inhibit D1 protein synthesis under heat stress (data not shown). PC was found to bind to the surface of the protein and interact predominantly with hydrophobic amino acids that are in close contact with the protein's cofactors (Camara-Artigas *et al*. 2002), and increased PC content in trehalose-pretreatment might promote bilayer phase formation and maintain membrane competency (Welti *et al*. 2002). PG was essential for the function of the PS 2 reaction center (Páli *et al*. 2003). Altered fatty acid compositions of PG could directly influenced susceptibility to photoinhibition (Okazaki *et al*. 2006). The lipid-protein interface around light harvesting complexes probably plays a significant role in determining the structural flexibility of the macroaggregates (Páli *et al*. 2003). Combined, it is quite probable that changes in lipid contents and in the extent of unsaturation of fatty acids modify the PS 2 reaction center complex, thereby affecting the turnover of Dl protein in the PS 2 complex. However, it is equally possible that the main role of lipids is to provide a structurally flexible matrix that transduces the local heat effect to the protein, where it can cause longer-lasting effects. Our results showed that trehalose pretreatment could prevent some thylakoid proteins (potentially including the D1 protein) from heat-induced hydrolyzation (Fig. 2), since PS 2 photochemistry in trehalose-pretreated seedlings was higher than in the absence of trehalose under heat stress when streptomycin was utilized to inhibit D1 protein synthesis (data not shown). Of course, newly synthesized proteins are not ruled out. The lipid-protein interface between PC, PG and these proteins seals proteins in the membrane and couples their motion with the dynamic bulk lipid environment required for the function of the complex photosynthetic machinery (Páli *et al*. 2003). Recently, trehalose has been found to interact directly with the membrane, partially replacing water molecules in the formation of hydrogen bonds with the lipid headgroups (Pereira *et al*. 2008). However, the specific steps remain to be identified.

Thus, it can be concluded that trehalose pretreatment caused changes in lipid contents in thylakoid membranes that may stabilize the photosynthetic apparatus against heat-induced photoinhibition by accelerating the recovery of the photosystem 2 protein complex.

SDS-PAGE of thylakoid membranes revealed that the contents of many polypeptides ranging from 20 to 97 kD significantly increased in trehalose pretreated samples as compared with controls, when these seedlings were exposed to heat. This suggests that the target site of heat may be the dissociation of certain thylakoid polypeptides, and that trehalose pretreatment can prevent some of 20 - 97 kD polypeptides from dissociation. Our results supports the previous finding that moderate heat reduced content of 41 kD polypeptide of Rubisco activase (Haldimann *et al*. 2005), showing that trehalose pretreatment may protect these proteins from heat-induced damage.

Heat shock is connected with oxidative stress, when ROS production exceeds the quenching capacity of protective systems. LOX catalyzes the reaction of O_2 with free, polyunsaturated fatty acids to form conjugated lipid hydroperoxides. Lipid peroxidation by LOX in leaves is considered the best criterion for damage caused by increasing ROS production, which generates both singlet oxygen and superoxide anion radicals and disrupts the structure, composition and function of membranes, leading to their leakage and loss of selective permeability (El-Shintinawy *et al*. 2004). In our study, LOX activity, electrolyte leakage, MDA content, and the generation of $O_2^{\bullet -}$ and H_2O_2 (Table 1) increased under heat stress conditions when compared with controls. These results showed that LOX-mediated peroxidation of membrane lipids likely made a significant contribution to the oxidative damage occurring in heat-stressed leaves. However, when seedlings pretreated with trehalose for 3 d were exposed to heat, they exhibited less ROS production and electrolyte leakage, and lower LOX activity and MDA content (Table 1), which indicated that trehalose pre-treatment might alleviate the damage caused by LOX-mediated lipid peroxidation. This lower LOX activity caused by trehalose pretreatment during periods of heat might be associated with a decrease in LOX synthesis (Bae *et al*. 2005).

Exposure of *Saccharomyces cerevisiae* to a mild heat shock (38 ºC) induced trehalose accumulation and markedly increased the viability of the cells upon exposure to an H_2O_2-generating system, showing trehalose accumulation in stressed cells plays a major role in protecting cellular constituents from oxidative damage by acting as a free radical scavenger (Benaroudj *et al*. 2001). We also previously observed that trehalose could directly scavenge ROS including $O_2^{\bullet -}$ and H_2O_2 in the same wheat cultivar as in this paper (Luo *et al*. 2008). Therefore, the decrease in ROS and the concomitant physiological responses such as depressed LOX activity and less MDA content induced by trehalose pretreatment should most likely be attributed to its ability to scavenge ROS, in addition to its possible protective role for membranes and macromolecules. Furthermore, a role for sugars as signalling molecules has been widely documented (Moore *et al*. 2003, Rolland *et al*. 2006). Alternatively, it is quite possible that trehalose or its intermediate trehalose-6-phosphate (T6P) acts as a potent sugar signal that putatively coordinates metabolism with development in response to carbon availability and stress (Paul *et al*. 2008), since altering the trehalose pathway has numerous effects on metabolism and development (Ramon and Rolland 2007) and the regulation of metabolism appears to be an important function in plants (Paul *et al*. 2008). More recently, exogenous trehalose treatment has been shown to up-regulate a specific combination of genes known from biotic stress responses, and trehalose induces gene expression responses related to ROS and secondary metabolism activation (Aghdasi *et al*. 2008).

References

Aghdasi, M., Smeekens, S., Schluepman, H.: Microarray analysis of gene expression patterns in *Arabidopsis* seedlings under trehalose, sucrose and sorbitol treatment. - Int. J. Plant Prod. **2**: 309-320, 2008.

Bae, H., Herman, E., Bailey, B., Bae, H.J., Sicher, R.: Exogenous trehalose alters *Arabidopsis* transcripts involved in cell wall modification, abiotic stress, nitrogen metabolism, and plant defense. - Physiol. Plant. **125**: 114-126, 2005.

Benaroudj, N., Lee, D.H., Goldberg, A.L.: Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. - J. biol. Chem. **276**: 24261-24267, 2001.

Bligh, E.G., Dyer, W.J.: A rapid method of total lipid extraction and purification. - Can. J. Physiol. Pharmacol. **37**: 911-917, 1959.

Camara-Artigas, A., Brune, D., Allen, J.P.: Interactions between lipids and bacterial reaction centers determined by protein crystallography. - Proc. nat. Acad. Sci. USA **99**: 11055-11060, 2002.

Cherian, S., Reddy, M.P., Ferreira, R.B.: Transgenic plants with improved dehydration-stress tolerance: progress and future prospects. - Biol. Plant. **50**: 481-495, 2006.

Couée, I., Sulmon, C., Gouesbet, G., El Amrani, A.: Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. - J. exp. Bot. **57**: 449-459, 2006.

Dionisio-Sese, M.L., Tobita, S.: Antioxidant response of rice seedlings to salinity stress. - Plant Sci. **135**: 1-9, 1998.

El-Bashiti, T., Hamamci, H., Öktem H.A., Yücel, M.: Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. - Plant Sci. **169**: 47-54, 2005.

El-Shintinawy, F., Ebrahim, M.K.H., Sewelam, N., El-Shourbagy, M.N.: Activity of photosystem 2, lipid peroxidation, and the enzymatic antioxidant protective system in heat shocked barley seedlings. - Photosynthetica **42**: 15-21, 2004.

Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V., Wu, R.J.: Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. - Proc. nat. Acad. Sci. USA **99**:15898-15903, 2002.

Gielwanowska, I., Szczuka, E., Bednara, J., Górecki, R.: Anatomical features and ultrastructure of *Deschampsia antarctica* (Poaceae) leaves from different growing habitats. - Ann. Bot. **96**: 1109 -1119, 2005.

Haldimann, P., Feller, U.: Growth at moderately elevated temperature alters the physiological response of the photosynthetic apparatus to heat stress in pea (*Pisum sativum* L.) leaves. - Plant Cell Environ. **28**: 302-317, 2005.

Heath, R.L., Packer, L.: Photoperoxidation in isolated chloroplasts. 1. Kinetics and stoichiometry of fatty acid peroxidation. - Arch. Biochem. Biophys. **125**: 189-198, 1968.

Jang, I.C., Oh, S.J., Seo, J.S., Choi, W.B., Song, S., Kim, C.H., Kim, Y.S., Seo, H.S., Choi, Y.D., Nahm, B.H., Kim, J.K.: Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. - Plant Physiol. **131**: 516-524, 2003.

Kim, K., Portis, A.R., Jr.: Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications in Rubisco activase and membrane fluidity. - Plant Cell Physiol. **46**: 522-530, 2005.

Komayama, K., Khatoon, M., Takenaka, D., Horie, J., Yamashita, A., Yoshioka, M., Nakayama, Y., Yoshida, M., Ohira, S., Morita, N., Velitchkova, M., Enami, I., Yamamoto, Y.: Quality control of photosystem II: cleavage and aggregation of heat-damaged D1 protein in spinach thylakoids. - Biochim. biophys. Acta **1767**: 838-846, 2007.

Larkindale, J., Huang, B.: Changes of lipid composition and saturation level in leaves and roots for heat-stressed and heat acclimated creeping bentgrass (*Agrostis stolonifera*). - Environ. exp. Bot. **51**: 57-67, 2004.

Lichtenthaler, H.K.: Chlorophylls and carotenoids – pigments of photosynthetic biomembranes. - In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Pp. 350-382. Academic Press, San Diego - New York - Berkeley - Boston - London - Sydney - Tokyo - Toronto 1987.

Liu, Y., Hao, Y.Y., Liu, Y.Y., Huang, W.D.: Effects of wounding and exogenous jasmonic acid on the peroxidation of membrane lipid in pea seedlings leaves. - Agr. Sci. Chin. **38**: 614-620, 2005.

Losa, D.A., Murata, N.: Membrane fluidity and its roles in the perception of environmental signals. - Biochim. biophys. Acta **1666**:142-157, 2004.

Luo, Y.**,** Li, W.M., Yang, X.H., Wang, W.: Trehalose: protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress? - Environ. exp. Bot. **63**: 378-384, 2008.

Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F.: Reactive oxygen gene network of plants. - Trends Plant Sci. **9**: 490-498, 2004.

Moore, B., Zhou, L., Rolland, F., Hall, Q., Cheng, W.H., Liu, Y.X., Hwang, I., Jones, T., Sheen, J.: Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light and hormonal signalling. - Science **300**: 332-336, 2003.

Nishiyama, Y., Allakhverdiev, S.I., Yamamoto, H., Hayashi, H., Murata, N.: Singlet oxygen inhibits the repair of photosystem II by suppressing the translation elongation of the D1 protein in *Synechocystis* sp. PCC 6803. - Biochemistry **43**: 11321-11330, 2004.

Okazaki, K., Sato, N., Tsuji, N., Tsuzuki, M., Nishida, I.: The significance of C16 fatty acids in the sn-2 positions of glycerolipids in the photosynthetic growth of *Synechocystis* sp. PCC6803. - Plant Physiol. **141**: 546-556, 2006.

Parida, A.K., Das, A.B., Mittra, B.: Effects of NaCl stress on the structure, pigment complex composition, and photosynthetic activity of mangrove *Bruguiera parviflora* chloroplasts. - Photosynthetica **41**: 191-200, 2003.

Páli, T., Garab, G., Horváth, L.I., Kóta, Z.: Functional significance of the lipid-protein interface in photosynthetic membranes. - Cell. Mol. Life Sci. **60**: 1591-1606, 2003.

Paul, M.J., Parimavesi, L.F., Jhurreea, D., Zhang, Y.H.: Trehalose metabolism and signaling. - Annu. Rev. Plant Biol. **59**: 417-441, 2008.

Peever, T.L., Higgins, V.J.: Electrolyte leakage, lipoxygenase, and lipid peroxidation induced in tomato leaf tissue by specific and nonspecific elicitors from *Cladosporium fulvum*. - Plant Physiol. **90**: 867-875, 1989.

Pereira, C.S., Hünenberger, P.H.: The effect of trehalose on a phospholipid membrane under mechanical stress. - Biophys. J. **95**: 3525-3534, 2008.

Ramon, M., Rolland, F.: Plant development: introducing trehalose metabolism. - Trends Plant Sci. **12**: 185-188, 2007.

Rolland, F., Baena-Gonzalez, E., Sheen, J.: Sugar sensing and signalling in plants: conserved and novel mechanisms. - Annu. Rev. Plant Biol. **57**: 675-709, 2006.

Sebollela, A., Louzada, P.R., Sola-Penna, M., Sarone-Williams, V., Coelho-Sampaio, T., Ferreira, S.T.: Inhibition of yeast glutathione reductase by trehalose: possible implications in yeast survival and recovery from stress. - Int. J. Biochem. Cell Biol. **36**: 900-908, 2004.

Welti, R., Li, W.Q., Li, M.Y., Sang, Y.M., Biesiada, H., Zhou, H.E., Rajashekar, C.B., Williams, T.D., Wang, X.: Profiling membrane lipids in plant stress responses. Role of phospholipase Dα in freezing lipid changes in *Arabidopsis*. - J. biol. Chem. **277**: 31994-32002, 2002.

Yang, X.H., Liang, Z., Wen, X.G.: Genetic engineering of the biosynthesis of glycinebetaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. - Plant mol. Biol. **66**: 73-86, 2008.

Zhao, X.X., Ma, Q.Q., Liang, C., Fang, Y., Wang, Y.Q., Wang, W.: Effect of glycinebetaine on function of thylakoid membranes in wheat flag leaves under drought stress. - Biol. Plant. **51**: 584-588, 2007.