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BRIEF COMMUNICATION

Application of trehalose ameliorates heat stress and promotes recovery of winter wheat seedlings

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Abstract

Trehalose was supplied to wheat (*Triticum aestivum* L.) seedlings just before a high temperature (40 °C) treatment and some physiological parameters were measured during the heat stress and recovery. The application of trehalose decreased the net photosynthetic rate (P_N) of wheat seedlings under the heat stress, but to a small extent increased the dry mass (DM) and leaf water content (LWC) after recovery from the heat stress. The trehalose-induced decrease in P_N under the heat stress was not associated with a stomatal response. The heat stress slightly decreased the maximal efficiency of photosystem II (PS II) photochemistry (the variable to maximum chlorophyll *a* fluorescence ratio, F_v/F_m) similarly in the trehalose treated or non-treated plants. Under the heat stress, the actual efficiency of PS II photochemistry (Φ_{PSII}) and the efficiency of excitation energy capture by open reaction centers (F_v'/F_m') were lower in the trehalose-pretreated seedlings, whereas they were higher after the recovery. The patterns of changes in non-photochemical quenching (NPQ) were contrary to those of $\Phi_{PS\ II}$ and F_v'/F_m'. The chlorophyll content was lower, whereas the β-carotene content and the degree of de-epoxidation (DEPS) of xanthophyll cycle pigments were higher in the trehalose-pretreated wheat seedlings under the heat stress. These results suggest that exogenous trehalose partially promotes recovery of wheat by the increase of NPQ, β-carotene content, and DEPS.

Additional key words: β-carotene, chlorophyll, fluorescence, net photosynthetic rate, stomatal conductance, xanthophyll cycle.

High temperature stress, likely to become an increasingly important factor with the change of climate, can restrict crop growth and productivity (Boyer 1982). Photosynthesis is particularly sensitive to heat stress. When plants are confronted with heat stress and the photosynthetic apparatus absorbs irradiance in excess of that required for the saturation of photosynthesis, photosystem II (PS II) is subjected to photooxidative damage (Havaux 1993, Melis 1999). Irradiance dependent xanthophyll cycle de-epoxidation is believed to be one of the mechanisms to avoid or minimize this damage (Jin *et al*. 2003).

The accumulation of trehalose is observed during adaptations to various stresses including heat, chilling, drought, and salinity (Kaplan *et al.* 2004, Iordachescu and Imai 2008). Many studies have shown that the application of trehalose can improve the stress tolerance (Garg *et al*. 2002, Bae *et al*. 2005, Karim *et al*. 2007). However, the exact mechanisms of protection remain unclear. Based on our previous study that exogenous trehalose protects

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Abbreviations: A - antheraxanthin; Car - β-carotene; Chl - chlorophyll; DEPS - degree of deepoxidation [(A+Z)/(V+A+Z)]; F_v/F_m - variable to maximum Chl fluorescence ratio in dark adapted leaves (the maximal efficiency of PS II photochemistry); F_v'/F_m' - variable to maximum Chl fluorescence ratio under steady-state conditions (the efficiency of excitation energy capture by open reaction centers); g_s - stomatal conductance; LWC - leaf water content; NPQ - non-photochemical quenching; P_N - net photosynthetic rate; PS - photosystem; q_p - photochemical quenching; V - violaxanthin; Z - zeaxanthin; $\Phi_{PS\ II}$ - the actual efficiency of PS II photochemistry.
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thylakoid membranes of winter wheat from heat-induced damage (Luo *et al*. 2010), we propose here a hypothesis that the physiological basis for the enhanced tolerance of seedlings to heat stress induced by the accumulation of trehalose *in vivo* may be associated with the increased tolerance of photosystem (PS) II reaction center to heat stress.

As described in our previous paper (Luo *et al*. 2010), the seedlings of winter wheat (*Triticum aestivum* L.) cv. Hanfeng 9703 were grown in a half-strength Hoagland solution at a constant temperature of 25 ºC, a 16-h photoperiod, an irradiance of 100 μmol m^{-2} s^{-1}, and a relative humidity of 70 %. About 15 d later when the second leaves were fully expanded, the wheat seedlings were treated with 1.5 mM trehalose added to solution for 3 d. Then they were exposed to a heat treatment of 40 ºC for another 24 h (heat treatment). After that, the plants were moved to 25 ºC for another 12 h for recovery (post-heat treatment). All experiments were conducted at least three times and the young fully expanded leaves of different treatments were selected for the following assays.

Ten seedlings from the different treatments mentioned above were weighed (the determination of fresh mass, FM) and then dried in an oven at 105 °C for 10 min and at 80 °C for 24 h (the determination of dry mass, DM). Leaf water content (LWC) was calculated as (FM - DM)/FM × 100. Chloroplast pigments were extracted in acetone and analyzed by high-performance liquid chromatography (HPLC, *SCL-10AVP*, *Shimadzu*, Kyoto, Japan) as described in detail by Zhao *et al*. (1995). The degree of de-epoxidation (DEPS) of xanthophyll cycle pigments was expressed as (A+Z)/(V+A+Z), in which A, Z, and V were antheraxanthin, zeaxanthin, and violaxanthin, respectively. Photosynthetic gas exchange measurements were performed using an open infrared gas analyser (IRGA) system (*TPS-1*, *PP-Systems*, Hitchin, UK). Net photosynthetic rate (P_N) and stomatal conductance (g_s) were determined at an irradiance of 600 μmol(photon) m^{-2} s^{-1}. In all cases, the samples were irradiated for 30 - 45 min prior to start of the measurements. To determine the state of PS II, chlorophyll (Chl) *a* fluorescence was measured using a portable pulse-modulated fluorometer (*FMS-2*, *Hansatech*, King's Lynn, Norfolk, UK) on the same leaves as were previously used for gas exchange measurements. The following parameters were calculated as described by Maxwell and Johnson (2000): *1*) the maximum efficiency of PS II photochemistry = the variable to maximum chlorophyll fluorescence ratio of dark adapted leaves, F_v/F_m; *2*) the non-photochemical quenching coefficient, NPQ = the ratio of a change in the maximum fluorescence in the light to its final value; *3*) the actual PS II efficiency, Φ_{PSII} = the proportion of the light absorbed by chlorophyll associated with PS II and used in photochemistry; *4*) the efficiency of excitation energy capture by open reaction centers – variable to maximum chlorophyll fluorescence ratio under irradiance of 100 μmol m^{-2} s^{-1}, F_v'/F_m'; and *5*) the coefficient of photochemical quenching, q_p = the proportion of PS II reaction centres that are open.

Statistical analysis was conducted using the software *DPS* (Zhejiang University, China) and the Duncan's test as described in detail in our previous study (Luo *et al*. 2010).

Supplied trehalose is absorbed by wheat roots (Luo *et al*. 2010). After recovery from the heat treatment, the accumulated trehalose slightly promoted the growth of the wheat plants and increased LWC (Table 1). This higher LWC may be good for the recovery of other parameters. Similar results were observed by Nounjana *et al*. (2012) where exogenous trehalose promoted recovery of rice seedlings from salt stress. This might be associated not only with osmoregulation but also with other effects, *e.g.*, protection of membranes (López-Gómez and Lluch 2012).

Under the heat stress, P_N decreased by 58 % in the control plants and by 66 % in the trehalose-pretreated seedlings. However, after recovery, a similar increase was observed in both plant types (Table 1). The heat stress also resulted in a significant decrease in g_s in both the control plants and trehalose-pretreated plants, however, there were no significant differences in g_s between them (Table 1). These results indicate that the higher decrease in P_N in the trehalose-pretreated plants under the heat stress was not associated with the changes in g_s.

The Chl *a* fluorescence parameter F_v/F_m is often considered as the indicator of photoinhibition or other kind of injury caused to PS II. Trehalose treatment does not damage photosynthesis reaction centers under natural growth conditions (Gao *et al*. 2013). The heat stress resulted in a slight decrease in F_v/F_m in both the control and trehalose-pretreated seedlings (Table 1) suggesting that a heat stress induced slight damage to PS II could cause a decrease in CO_2 assimilation. After the recovery from the heat, F_v/F_m increased to a certain extent (Table 1) indicating that such a damage was reversible. In addition, no difference between F_v/F_m of samples with or without exogenous trehalose under the heat stress was observed (Table 1), which means that trehalose did not protect PS II. However, in our previous study, we found that exogenous trehalose protected the ultrastructure of chloroplast and thylakoid membranes of winter wheat from heat-induced damage to a certain extent (Luo *et al*. 2010). Maybe a minor protection of PS II by trehalose could not be detected in this study as the parameter F_v/F_m is extremely stable (Björkman and Demmig 1987, Brestic and Zivcak 2013).

Under the heat stress, the values of Φ_{PSII} were higher in the control than in the trehalose-pretreated seedlings, however, they were higher in the trehalose-pretreated seedlings after the recovery (Table 1). Such a change is associated with changes in F_v'/F_m' and/or q_p, since $\Phi_{PS\,II} = q_p \times F_v'/F_m'$ (Genty *et al.* 1989). At the high temperature, the fact that there was a considerable decrease in F_v'/F_m' in trehalose-pretreated plants (Table 1), but no changes

Table 1. Dry mass (DM), leaf water content (LWC), net photosynthetic rate (P_N), stomatal conductance (g_s), maximal efficiency of PS II photochemistry (F_v/F_m), actual PS II efficiency (Φ_{PSII}), efficiency of excitation energy capture by open reaction centers (F_v'/F_m'), coefficient of photochemical quenching (q_P), coefficient of non-photochemical quenching (NPQ), chlorophyll content (Chl), β-carotene content (Car), and degree of de-epoxidation (DEPS) of xanthophyll cycle pigments [(A+Z)/(V+A+Z)] of young control and trehalose (1.5 mM) pre-treated leaves measured under 25 ºC (pre-heat), after a heat treatment (40 °C for 24 h), and after a recovery (25 ºC for 12 h; post-heat). Means ± SD of three replications for DM, LWC, Chl, β-carotene, and DEPS, five repli-cations for P_N, g_s, and parameters of fluorescence. Different letters indicate significant differences between treatments at $P \leq 0.05$.

Parameters	Pre-heat control	trehalose	Heat control	trehalose	Post-heat control	trehalose
DM [g]	0.22 ±0.01b	0.22 ±0.01b	0.23 ±0.02b	0.22 ±0.02b	0.23 ±0.01b	0.25 ±0.01a
LWC [%]	86.92 ±0.25a	86.98 ±0.69a	83.39 ±0.21a	84.19 ±1.29a	71.89 ±0.27c	78.24 ±0.22b
P_N [μmol m^{-2} s^{-1}]	6.27 ±0.40a	5.97 ±0.29a	2.63 ±0.25b	2.05 ±0.15c	6.0 1±0.15a	5.60 ±0.50a
g_s [mmol m^{-2} s^{-1}]	115.5 ±17.7a	125.0 ±29.8a	35.5 ±16.1b	42.9 ±13.3b	71.3 ±13.4b	49.8 ±8.9b
F_v/F_m	0.843±0.004a	0.847±0.007a	0.814±0.001b	0.812±0.004b	0.825±0.009a	0.834±0.003a
Φ_{PSII}	0.374±0.002a	0.370±0.004a	0.355±0.003b	0.340±0.004c	0.320±0.002c	0.360±0.004b
F_v'/F_m'	0.775±0.018a	0.776±0.009a	0.700±0.005b	0.650±0.012c	0.660±0.013c	0.689±0.014b
q_P	0.540±0.021a	0.530±0.012a	0.440±0.010b	0.428±0.015b	0.390±0.013c	0.435±0.018b
NPQ	0.396±0.043b	0.446±0.045b	0.430±0.010b	0.513±0.037a	0.501±0.017a	0.400±0.013b
Chl [μg g^{-1}(FM)]	2409.24 ±8.14a	2312.87 ±44.23a	2336.96 ±40.56a	2209.16 ±40.98b	2256.98 ±14.73a	2265.42 ±24.58a
Car [μg g^{-1}(FM)]	311.12 ±7.50b	311.40 ±13.92b	312.20 ±13.04b	338.08 ±12.13a	237.56 ±13.16d	295.48 ±13.59c
DEPS	0.043±0.003d	0.085±0.002c	0.090±0.002c	0.113±0.003b	0.173±0.008a	0.125±0.009b

in q_p (Table 1) in the control and the trehalose-pretreated seedlings indicate that the decrease in Φ_{PSII} in the trehalose-pretreated plants could be the result of a decrease in F_v'/F_m' which could be associated with an increase in energy dissipation in the PS II antennae (Gilmore, 1997). Indeed, NPQ in the trehalose-pretreated and then heat-stressed plants was higher than that under the heat stress alone (Table 1). On the other hand, after the recovery from the heat stress, the increase in Φ_{PSII} in the trehalose-pretreated plants could be the result of a decrease in NPQ and an increase in q_p (Table 1). NPQ has been closely associated with the onset of harmless dissipation of excess energy (Gilmore 1997). Thus, the decrease in P_N under the heat stress was associated with an increase in energy dissipation in the PS II antennae. However, after cessation of the heat stress, maybe more energy in the trehalose-pretreated seedlings was used for photosynthesis to maintain plant growth. In this way, the response of NPQ to the heat and recovery in the trehalose-pretreated plants seems to be, to some extent, in favor of the survival and recovery of wheat plants.

Moreover, the Chl content was lower in the trehalose-pretreated than in the control seedlings under the heat stress (Table 1). This might contribute to the reduction in P_N. However, the Car content was higher in the trehalose-pretreated than in the control seedlings after the recovery. In addition, the heat stress increased DEPS and such an increase was much greater in the presence of trehalose than that in the control seedlings. However, after recovery, the DEPS was higher in control seedlings. The xanthophyll cycle has been believed to play a photoprotective role *via* dissipation of excessive radiation energy as heat (Jin *et al*. 2003). Zeaxanthin together with β-carotene are well-conserved mechanisms of photo-protection, either by dissipating excess excitation energy as heat or by scavenging reactive oxygen species (ROS) and suppressing lipid peroxidation (Peñuelas and Munné-Bosch 2005). Therefore, increases in the content of β-carotene and zeaxanthin in the trehalose-pretreated plants at the high temperature might help to protect the photosynthetic apparatus and to enhance their heat tolerance. The protection of trehalose for photosynthetic apparatus has been approved by our previous study (Luo *et al*. 2010). After the recovery, a lower degree of de-epoxidation in the trehalose-pretreated plants than in the control ones suggests that more solar energy was used for photochemical processes and less was dissipated as heat.

In conclusion, our results suggest that exogenous trehalose provided the wheat plants with a slightly enhanced ability to recover as compared with the heat stressed plants alone. This may be associated with the increase of NPQ, Car content and degree of de-epoxidation under the heat stress.

References

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.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. - Planta **170**: 489-504,

1987.
Boyer J.: Plant productivity and the environment. - Science **218**: 443-448, 1982.
Brestic, M., Zivca, M.: PSII fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. - In: Rout, G.R., Das, A.B. (ed.): Molecular Stress Physiology of Plants. Pp. 87-131. Springer, Bhubaneswar 2013.
Gao, J., Wang, N., Xu, S.S., L,i Y., Wang, Y., Wang, G.X.: Exogenous application of trehalose induced H_2O_2 production and stomatal clousure in *Vicia faba*. - Biol. Plant. **57**: 380-384, 2013.
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.
Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **99**: 87-92, 1989.
Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. - Physiol. Plant. **99**: 197-209, 1997.
Havaux, M.: Characterization of thermal damage to the photosynthetic electron transport system in potato leaves. - Plant Sci. **94**: 19-33, 1993.
Iordachescu, M., Imai, R.: Trehalose biosynthesis in response to abiotic stresses. - J. integr. Plant Biol. **50**: 1223-1229, 2008.
Jin, E.S., Yokthongwattana, K., Polle, J.E.W., Melis, A.: Role of the reversible xanthophyll cycle in the photosystem II damage and repair cycle in *Dunaliella salina.* - Plant Physiol. **132**: 352-364, 2003.
Kaplan, F., Kopka, J., Haskell, D.W., Zhao, W., Schiller, K.C., Gatzke, N., Sung, D.Y., Guy, C.L.: Exploring the temperature-stress metabolome of *Arabidopsis*. -Plant Physiol. **136**: 4159-4168, 2004.
Karim, S., Aronsson, H., Ericson, H., Pirhonen, M., Leyman, B., Welin, B., Mäntylä, E.,Palva, E.T., Dijck, P.V., Holmström K.O.: Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. - Plant mol. Biol. **64**: 371-386, 2007.
López-Gómez, M., Lluch, C.: Trehalose and abiotic stress tolerance. - In: Ahmad, P., Prasad, M.N.V. (ed.): Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. Pp. 253-265. Springer-Verlag, New York 2012.
Luo, Y., Li, F., Wang, G.P., Yang, X.H., Wang, W.: Exogenously-supplied trehalose protects thylakoid membranes of winter wheat from heat-induced damage. - Biol. Plant. **54**: 495-501, 2010.
Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence – a practical guide. - J. exp. B. **51**: 659-668, 2000.
Melis, A.: Photosystem II damage and repair cycle in chloroplast: what modulates the rate of photodamage *in vivo*? - Trends Plant Sci. **4**: 130-135, 1999.
Nounjana, N., Nghiab, P.T., Theerakulpisuta, P.: Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. - J. Plant Physiol. **169**: 596-604, 2012.
Peñuelas, J., Munné-Bosch, S.: Isoprenoids: an evolutionary pool for photoprotection. - Trends Plant Sci. **10**: 166-169, 2005.
Zhao, S.J., Meng, Q.W., Xu, C.C., Han, H.Y., Zou, Q.: Analysis of the xanthophyll cycle components in plant tissues by high performance liquid chromatography. - Plant Physiol. Commun. **31**: 438-442, 1995.