Exogenously-supplied trehalose protects photosystem II by promoting cyclic electron flow in winter wheat under heat and drought stress

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Abstract

Trehalose protects and stabilizes the reaction center and improves photosystem II (PS II) activity. However, the underlying mechanism remains unknown. Cyclic electron flow is an important mechanism to protect PS II under stress. This study focused on the effects of exogenous trehalose on the activity of PS II, D1 protein content, the plastoquinone (PQ) pool, and ATP synthase activity in wheat seedlings under heat and drought stress to explore the relationship between trehalose and cyclic electron flow (CEF). Our experimental results indicated that heat and drought stress decreased the maximal photochemical efficiency of PS II (Fv/Fm) and the electron transport rate of PS II (EFR(II)), whereas the trehalose pretreatment improved photochemical efficiency and the electron transport rate of PS II. The trehalose pretreatment stimulated CEF under heat and drought stress. Furthermore, the proton gradient (ΔpH) across the thylakoid membrane and ATPase activity increased. The higher ΔpH and ATPase activity played a key role in protecting PS II under stress. Inhibition of the oxidized PQ pool caused by heat and drought stress was alleviated by the trehalose pretreatment. Thus, our results show that photoinhibition of heat and drought-stressed plants was alleviated by the trehalose pretreatment. Our findings further reveal that this effect was mediated by CEF and the PQ pool.

1 INTRODUCTION

Wheat (*Triticum aestivum*) is a significant staple crop, which is widely grown worldwide. Drought and the rising global temperature are important factors affecting wheat production. According to one report, when the temperature increases by 1°C, wheat production is reduced by 1-4% (Wiegand & Cuellar,1981). A single stress, such as heat or drought, does not usually occur alone during natural production (Howarth & Ougham,1993).

PS II is extremely vulnerable to heat and drought stress and is regarded as the original site and principal component of photoinhibition (Dongsansuk, Lütz, & Neuner,2013). The first PS II component to be damaged is the oxygen evolving complex (OEC) (Takahashi, Milward, Fan, Chow & Badger, 2009), which leads to damage to the entire PS II reaction center. A damaged PS II is rapidly repaired at room temperature due to rapid synthesis of the PS II core protein D1, which can be assembled together with undamaged subunits to form a new PS II complex (Huang, Yang, Zhang, Zhang & Cao, 2012).

It has been reported that photoinhibition of PS II due to abiotic stresses can be alleviated by trehalose (Karim et al., 2007). Trehalose changes hydration of the PS II complex, which results in a conformational transition in PS II and more effectively protects the PS II complex (Yanykin, Khorobrykh, Mamedov & Klimov, 2015). Trehalose also significantly stimulates the stabilized oxygen evolution rate in the PS II complex (Mamedov, Petrova, Yanykin, Zaspa & Semenov,2015). However, the specific protective mechanism of trehalose against stress remains unclear. The trehalose level in plants is normally very low. Nevertheless, applying exogenous

trehalose increases the internal level of trehalose and has been suggested as an alternative approach to improve stress tolerance (Chen & Murata,2002).

Activation of cyclic electron flow (CEF) effectively protects PS II against abiotic stressors (Wang et al.,2013). Huang et al. (2010) reported that the PS II complex in a tropical rosewood species rapidly recovers under low light conditions due to excitation of CEF (Huang, Zhang & Cao, 2010) The main feature of this repair process is replacement of the D1 protein in the photodamaged PS II by newly synthesized D1 and reassembly of active PS II (Andersson & Aro, 2001). CEF is necessary to stabilize OEC activity under high intensity light in the tropical tree species *Erythrophleum guineense* and thus prevent PS II from serious photodamage (Huang, Yang, Hu, Zhang & Cao, 2016). CEF generates an extra proton gradient across the thylakoid membrane, which promotes ATP synthesis to meet the demand for more ATP under stress(Zhang, Huang, Zhang & Cao, 2016) Synthesis of the D1 protein is enhanced by synthesis of ATP, which plays a key role in the photodamage and repair process of PS II (Allakhverdiev, Nishiyama, Takahashi, Miyairi, Suzuki & Murata, 2005).

Plants activate protective mechanisms, such as CEF, for PS II under stress. However, it is unknown whether trehalose can protect PS II by promoting this protective mechanism. Therefore, this study focused on the relationship between trehalose and CEF under heat and drought stress. Our results provide new insight into the effects of exogenous trehalose on PS II photoprotective mechanisms in wheat leaves under heat and drought stress.

2 MATERIALS AND METHODS

2.1 Plant materials and growth conditions

Plump seeds of winter wheat (*Triticum aestivum*) were selected and cultured on plastic plate with a wet towel. Three days later, after germinating, seeds were uniformly fixed with special holes in plastic plates, and then watered daily in a growth chamber at 25^{o}C. After the first leaves fully unfolded, the wheat seedlings were cultivated with Hoagland nutrient solution by hydroponics. When the second leaves completely expanded, the first group was cultivated with Hoagland nutrient solution (control), the second group was pretreatment in 1.5 mM trehalose for 72 h (TRE).

Heat stress: after the pretreatment in 1.5 mM trehalose for 72 h, wheat seedlings were subjected to heat treatment (40^{o}C; light intensity: 120 μmol quanta m^{-2} s^{-1}; humidity: 38%) for 24 h (day 13 h; night 11 h) in the climate chamber.

Drought stress: wheat seedlings were subjected to drought stress by immersion in 15% (w/v) polyethylene glycol solutions (PEG-6000). After the pretreatment in 1.5 mM trehalose for 48 h, wheat seedlings were cultivated with 15% (w/v) polyethylene glycol solutions.

Drought plus heat stress: after the pretreatment in 1.5 mM trehalose for 48 h, wheat seedlings were cultivated with 15% (w/v) polyethylene glycol solutions for 24 h. One day later, wheat seedlings of drought stress pretreatment were subjected to heat treatment (40^{o}C; light intensity: 120 μmol quanta m^{-2} s^{-1}; humidity: 38%) for 24 h (day 13 h; night 11 h) in the climate chamber.

Chlorophyll fluorescence and the initial reduction rate of $P700^{+}$

The chlorophyll fluorescence and the P700 redox state were measured using a Dual-PAM-100 fluorometer (Heinz Walz, Effeltrich, Germany). The maximum photochemical quantum yield of PS II (photochemical efficiency) was calculated as $(F_m - F_o)/F_m$ (Genty, Briantais & Baker, 1989). F_o is the minimum chlorophyll fluorescence, and F_m is the maximum fluorescence of dark-adapted (adapted 30 min in darkness) leaves following a saturation pulse (10000 μmol quanta m^{-2} s^{-1}, 300 ms). The electron transfer rate of the PS II (electron transport rate(II)) was calculated as 0.5 × PPFD × Y(II) × 0.84, where 0.5 is the proportion of energy that reaches the PS II, PPFD is the irradiance absorbed by the leaf, considering 0.84 or 84% light intensity, and Y(II) is the effective quantum yield of PS II (Baker,2008). $Y(II) = (F_m' - F_s)/F_m'$ (Genty, Briantais & Baker,1989), where F_m' is the maximum fluorescence of light-adapted leaves following a

saturation pulse (10000 μmol quanta m^{-2} s^{-1}, 300 ms) and F_s is the light-adapted steady-state fluorescence. The initial reduction rate of $P700^+$ after termination of far-red light (>705 nm, 5.2 μmol quanta m^{-2} s^{-1}, 40 s) was monitored by absorbance at 810-830 nm in the P700 Measure Mode of a single channel (Klughammer & Schreiber,1998).

Inhibitors

2.3.1 Streptomycin (SM)

We used the SM to suppress the synthesis of D1 protein. Second detached leaves of wheat seedlings were infiltrated with 3 mM SM for 4 h before stress.

2.3.2 Methyl viologen (MV)

MV (0.5 μl, 2 h) accepts electrons from PSI and limits the electron flow to CEF. Second detached leaves of wheat seedlings were infiltrated with 0.5 μl MV for 2 h before stress.

Measurement of oxygen evolution rate

According to the method described in our previous study (Luo, Li, Wang, Yang & Wang, 2010), the oxygen evolution rate was measured with a Clarke type O_2 electrode unit (Hansatech, King's Lynn, UK) in the thylakoid membranes. The results were the average of 5 independent replicates.

Thylakoid membrane proteins extraction and quantification

Thylakoid membranes were prepared in accordance with the method of Rintamaki et al. (1996). Winter wheat leaves were ground and homogenized with cool isolation buffer. The homogenates were centrifuged at 1500g for 4 min at 4°C. The pellets were washed with 10 mM HEPES-NaOH, pH 7.5, 5 mM sucrose, 5 mM $MgCl_2$, and 10 mM NaF and pelleted at 3000 g for 3 min. Thylakoid pellets were resuspended in a small amount of storage buffer, and then were stored at -80^{o}C before use.

SDS-PAGE and western blot analysis

According to experimental method of Du et al. (1995), thylakoid membrane proteins were separated using 15% polyacrylamide gel. In total, 5 μg of chlorophyll was loaded per lane. The resolved proteins were transferred to NC membrane from gel and detected using a D1 protein antibody (Agrisera AB, 1:5000). Donkey Anti-Rabbit IgG H&L (Alexa Fluor® 680) (Abcam, 1:10000) was used as the secondary antibody. Infrared laser imaging system (Odyssey CLx, USA) was used to detect the membrane and the relative content of D1 protein was obtained by Image J software.

Measurement of PQ Pools

The P700 signal was determined during single turnover flashes (ST, 50ms, PQ pools being oxidized) followed by multiple turnover flashes (MT 50 ms, PQ pools are fully reduced) in the presence of far-red background light (Savitch et al., 2001). The complementary area between the oxidation curve of P700 after single turnover and multiple turnover excitation and the stationary level of $P700^+$ under far-red represents the single turnover - and multiple turnover-areas, respectively. These were used to calculate the functional pools sizes of intersystem electrons relative to P700 as follows: $e^{-/P700}$ = multiple turnover-areas/single turnover -areas (Savitch et al.,2001).

P515/P535 measurements

With the experimental method by Schreiber and Klughammer (Schreiber & Klughammer, 2008) as reference, the dual-beam 550 nm to 515 nm difference signal was monitored simultaneously by using the P515/535 module of the Dual-PAM-100 (HeinzWalz, Effeltrich, Germany). After 10 min of pre-illumination at 531 μmol quanta $m^{-2}s^{-1}$ and 4 min of dark adaptation, P515 changes induced by saturating single turnover flashes were recorded to evaluate ATPase activity. Slow dark-light-dark induction transients of the 550 nm to 515 nm signals reflect changes in the membrane potential (electrochromic pigment absorbance shift). After 30 s, actinic light (AL; 531 μmol quanta m^{-2} s^{-1}) was turned on and off after 330 s.

Statistics

All graphs were made using Origin 8.0 software (Origin Lab, Northampton, MA, USA). Statistical analyses were performed by ANOVA using SPSS version 21.0 (SPSS, Chicago, USA), and comparisons between the mean values were accomplished by the least significant difference test at the 0.05 probability level. Quantitative assessment was conducted on randomly selected samples from five independent biological replicates.

3 RESULTS

Effects of trehalose pretreatment on changes in the initial reduction rate of $P700^+$ under heat and drought stress

A higher initial $P700^+$ reduction rate was observed in trehalose pretreated seedlings under heat and drought stress compared with control plants (Figure 1). After a 24 h recovery from drought stress, the enhancement effect of the initial reduction of $P700^+$ by trehalose pretreatment was retained. However, no significant difference was detected in the effect of the initial $P700^+$ reduction rate by exogenously supplied trehalose between the control group and the stressed group after a 24 h recovery from heat and drought plus heat stress.

Figure 1 shows no differences in the initial $P700^+$reduction rate between trehalose-pretreated and control seedlings during the 24 h recovery (R2 and R3).

Effects of trehalose pretreatment on changes in D1 protein content under heat and drought stress

Western blot was used to determine whether the D1 protein was affected by trehalose under heat and drought stress. D1 protein content increased compared to the control in response to exogenously supplied trehalose during drought and drought plus heat stress (Figure 2B1). When leaves were incubated with SM, a D1 protein synthesis inhibitor, a lower D1 protein content was obtained in seedlings without trehalose than in trehalose pretreated plants under drought stress (Figure 2B2).

Effect of trehalose pretreatment on changes in Fv/Fm under heat and drought stress

Photoinhibition of PS II was measured by comparing the photochemical efficiency values to further study the role of trehalose in the D1 protein and PS II. The photochemical efficiency values in the control and trehalose-pretreated seedlings decreased after the plants were heat and drought stressed (Figure 3). A higher photochemical efficiency value was observed in the trehalose pretreated seedlings than the control plants. Seedlings without trehalose suffered more severe photoinhibition than trehalose-pretreated plants under heat and drought stress when leaves were incubated with SM.

Effect of trehalose pretreatment on changes in the oxygen evolution rate under heat and drought stress

The oxygen evolution rate decreased in the control wheat seedlings under heat and drought stress (Figure 4). A higher oxygen evolution rate was detected in the trehalose pretreated seedlings than that in control plants.

Effect of trehalose pretreatment on changes in the electron transport rate of PS II (EFR(II)) under heat and drought stress

EFR(II) was significantly lower under drought and heat stress compared to that of the control plants (Figure 5). The trehalose pretreatment improved **EFR(II)** significantly under drought and heat stress.

Effect of trehalose pretreatment on changes in thePQ pool under heat and drought stress

The PQ pool decreased in control wheat seedlings under heat and drought stress (Figure 6). The trehalose pretreated seedlings had a higher PQ pool than the control plants.

Effect of trehalose pretreatment on changes in ATPase activity under heat and drought stress

Figure 7 shows the rapid decay of the P515 signal after illumination. Faster decay of the P515 signal represents higher ATPase activity. Higher ATPase activity was observed in trehalose pretreated seedlings than control plants under heat and drought stress. Lower ATPase activity was observed in seedlings without trehalose than in trehalose pretreated plants when leaves were incubated with MV under heat and heat plus drought stress.

Εφφεστς οφ τρεηαλοσε πρετρεατμεντ ον ςηανγες ιν ΔπΗ ασροσς τηε τηψλακοιδ μεμβρανε υνδερ ηεατ ανδ δρουγητ στρεσς

ΔpH component of the proton motive force (ΔpH/pmf) increased significantly in control wheat seedlings under heat and drought stress (Figure 8). A higher ΔpH/pmf was observed in trehalose pretreated seedlings than in control plants. A higher ΔpH/pmf was observed in seedlings with trehalose than control plants when leaves were incubated with MV under heat and heat plus drought stress.

4. DISCUSSION

PS II performance of wheat leaves under heat and drought stress

Our results showed that heat and drought stress caused reversible photoinhibition of PS II (Figure 3). Blocked linear electron transport (Figure 5) resulted in a potential excess of light excitation pressure in the PS II reaction center after heat and drought stress. Excess energy in PS II can lead to the generation of reactive oxygen species, which are deleterious to the function and structure of PS II (Liu, Qi & Li, 2012). In this study, the oxygen evolution rate decreased in the control wheat seedlings under heat and drought stress (Figure 4), indicating that OEC may have been damaged. Destruction of the OEC and D1 protein damage have detrimental effects on the PS II reaction center (Wang, Wang, Hu, Chang, Bi & Hu,2015). Our results also show that D1 protein content was significantly lower than that of the control under heat and drought stress (Figure 2B1), indicating destruction of the PS II reaction center. Furthermore, stress blocked electron transfer (Figure 5) and reduced photochemical efficiency (Figure 3). Subsequently, a low oxygen evolution rate was obtained (Figure 4). These results are evidence of PS II damage.

Fortunately, plants have developed various photo-protective mechanisms, such as CEF, to alleviate damage to PS II. Plants adapt to a variety of environmental stressors by stimulating CEF (Hare, Cress & Van Staden,1998) As CEF generates ΔpH across the thylakoid membrane (Munekage et al.,2002) by transferring electrons from PSI to PQ, it is important to protect PS II by dissipating excess light energy(Takahashi, Milward, Fan, Chow & Badger, 2009). Our results show that CEF was stimulated (Figure 1) and ΔpH increased significantly (Figure 8) under heat and drought stress. In addition, as the functional PQ pool was significantly inhibited by the heat and drought treatment compared to the control (Figure 6), the decrease in PQ was the major factor blocking electron transport (Figure 5). A reduction in the PQ pool decreases PS II excitation, increases CEF, alleviates the ATP deficit, and increases ΔpH thereby downregulating the PS II antenna via the qE mechanism (Yi, Mcchargue, Laborde, Frankel & Bricker,2005). Our results also show that ATPase activity was significantly higher than that of the control under heat and drought stress (Figure 7).

The PS II protective effect of trehalose under heat and drought stress

Some recent studies have demonstrated that exogenous trehalose is effective for protecting the PS II complex under stress conditions. For example, trehalose increases the electron transfer rate of PS II in Mn-depleted PS II membrane fragments of spinach (Yanykin, Khorobrykh, Mamedov & Klimov, 2015). In addition, trehalose significantly stimulates and stabilizes the oxygen evolution rate in the PS II complex (Mamedov, Petrova, Yanykin, Zaspa & Semenov, 2015). Our results show that the trehalose pretreatment increased the PQ pool under heat and drought stress (Figure 6). The increase in the PQ pool may be responsible for the higher linear electron transport observed in the trehalose-pretreated groups compared with the control (Figure 5), in agreement with an earlier study (Zhang, Liu, Ni, Meng, Lu & Li, 2014). In the present study, the trehalose pretreatment significantly promoted CEF under heat and drought stress (Figure 1). The increase in CEF was essential for the higher ΔpH across the thylakoid membrane and ATPase activity

in the trehalose-pretreated seedlings compared with the control plants (Figure 7 and 8). These results show that the trehalose pretreatment improved ΔpH and ATPase activity by promoting CEF under heat and drought stress.

Our results show that the trehalose pretreatment increased D1 protein content and the oxygen evolution rate under heat and drought stress (Figure 2 and 4). ΔpH depends on CEF to play a key role in protecting the OEC in *Dalbergia* under high light intensity (Huang, Yang, Hu, Zhang & Cao, 2016). CEF provides energy to repair the D1 protein in the PS II core complex by establishing ΔpH and synthesizing ATP. Therefore, the increase in D1 protein content and the oxygen evolution rate may have significant associations with the higher CEF observed in the trehalose-pretreated groups than the control plants. Furthermore, inhibition of PS II was relieved by the trehalose pretreatment (Figure 3).

In addition, the absolute rate of *in vivo* D1 protein degradation can be established provided it is not affected by *de novo* D1 synthesis (Schnettger, Critchley, Santore, Graf & Krause,1994) Our results show that the trehalose pretreatment increased D1 protein content by promoting synthesis of the D1 protein under heat and drought plus heat stress, whereas it reduced degradation of the D1 protein under drought stress (Figure 2). Under heat and drought plus heat stress, exogenous trehalose enhanced ΔpH and ATPase activity, which did not entirely depend on CEF (Figure 7 and 8), and other potential mechanisms may exist. The common physiological function of both the water-water cycle and CEF is to supply ATP and ΔpH (Miyake, 2010). Therefore, the other potential mechanism may be the water-water cycle. However, the trehalose pretreatment increased ΔpH and ATPase activity only by stimulating CEF under drought stress (Figure 7 and 8).

5 CONCLUSIONS

In conclusion, the damage to PS II observed after the heat and drought stress was attributed to blocked linear electron transport caused by a decrease in the PQ pool. CEF protects PS II from heat and drought-induced photo-inhibition. Exogenous trehalose alleviated the PS II photo-inhibition caused by heat and drought stress by promoting CEF. The water-water cycle as well as CEF may play a key role protecting PS II under heat and drought plus heat stress by increasing ΔpH and ATPase activity.

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Yin Luo designed the experiments; Dan He and Wang Wang performed most of the experiments; Suifang Yuan cultured the wheat seedlings; Y.Luo wrote the manuscript.

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Data Accessibility Statement

All data involved in this research can be gotten at the following place:

https://pan.baidu.com/s/120-uCX6N_5A6-7kn02cZUQ

REFERENCES

Allakhverdiev, S.I., Nishiyama, Y., Takahashi, S., Miyairi, S., Suzuki, I., & Murata N. (2005). Systematic analysis of the relation of electron transport and ATP synthesis to the photodamage and repair of photosystem II in Synechocystis. *Plant physiology* , 137(1),263-273.

Andersson, B. & Aro, E-M. (2001). Photodamage and D1 protein turnover in photosystem II. In: *Regulation of photosynthesis.*Springer,377-393.

Baker, N.R. (2008). Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annual Review of Plant Biology* , 59:89-113.

Chen, T.H.H. & Murata, N. (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current Opinion in Plant Biology* , 5(3):250-257.

Dongsansuk, A., Lütz, C. & Neuner, G. (2013). Effects of temperature and irradiance on quantum yield of PSII photochemistry and xanthophyll cycle in a tropical and a temperate species. *Photosynthetica ,* 1-9.

Du, L., Sun, X., Pan, Y., Lin, H., & Liang, H. (1995). Influence of calcium ion on photosystem II oxygen evolution. *Science in China (Scienctia Sinica) Series B* , 12(38):1439-1447.

Genty, B., Briantais, J-M., & Baker, N.R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta-General Subjects* , 990(1):87-92.

Hare, P., Cress, W., Van Staden, J. (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell and Environment* , 21(6):535-553

Howarth, C.J. & Ougham, H.J. (1993). Gene expression under temperature stress. *New Phytologist* , 125(1):1-26.

Huang, W., Yang, S.J., Zhang, S.B., Zhang, J.L. & Cao, K.F. (2012). Cyclic electron flow plays an important role in photoprotection for the resurrection plant Paraboearufescens under drought stress. *Planta* , 235(4):819-828.

Huang, W., Yang, Y.J., Hu, H., Zhang, S.B. & Cao, K.F. (2016) Evidence for the role of cyclic electron flow in photoprotection for oxygen-evolving complex. *Journal of Plant Physiology* , 194:54-60.

Huang, W., Zhang, S. B., & Cao, K.F. (2010). Stimulation of cyclic electron flow during recovery after chilling-induced photoinhibition of PSII. *Plant Cell Physiology* , 51(11):1922-1928.

Karim, S., Aronsson, H., Ericson, H., Pirhonen, M., Leyman, B., Welin, B., Mantyla, E., Palva, E.T., Van Dijck, P. & Holmstrom, K.O. (2007). Improved drought tolerance without undesired side effects in transgenic plants producing trehalose.*Plant Molecular Biology* , 64(4):371-386.

Klughammer, C. & Schreiber, U. (1998). Measuring P700 absorbance changes in the near infrared spectral region with a dual wavelength pulse modulation system.*Photosynthesis: mechanisms and effects* , 5:4357-4360.

Liu, Y.F., Qi, M. & F, Li, T.L. (2012). Photosynthesis, photoinhibition, and antioxidant system in tomato leaves stressed by low night temperature and their subsequent recovery. *Plant Science* , 196(11):8-17.

Luo, Y., Li, F., Wang, G., Yang, X. & Wang, W. (2010). Exogenously-supplied trehalose protects thylakoid membranes of winter wheat from heat-induced damage. *Biologia Plantarum* , 54(3):495-501.

Mamedov, M., Petrova, I., Yanykin, D., Zaspa, A. & Semenov, A.Y. (2015). Effect of trehalose on oxygen evolution and electron transfer in photosystem 2 complexes.*Biochemistry (Moscow)* , 80(1):61-66.

Miyake, C. (2010). Alternative electron flows (water-water cycle and cyclic electron flow around PSI) in photosynthesis: molecular mechanisms and physiological functions.*Plant Cell Physiology* , 51(12):1951.

Munekage, Y., Hojo, M., Meurer, J., Endo, T., Tasaka, M. & Shikanai, T. (2002). PGR5 is involved in cyclic electron flow around photosystem I and is essential for photoprotection in Arabidopsis. *Cell* , 110(3):361-371.

Rintamäki, E., Kettunen, R. & Aro, E. M. (1996). Differential D1 Dephosphorylation in Functional and Photodamaged Photosystem II Centers. DEPHOSPHORYLATION IS A PREREQUISITE FOR DEGRADATION OF DAMAGED D1. *Journal of Biological Chemistry* , 271(25):14870-14875.

Savitch, L.V., Barker-Åstrom, J., Ivanov, A.G., Hurry, V., Öquist, G., Huner, N. P. & Gardeström, P. (2001). Cold acclimation of Arabidopsis thaliana results in incomplete recovery of photosynthetic capacity, associated with an increased reduction of the chloroplast stroma. *Planta* , 214(2):295-303.

Schnettger, B., Critchley, C., Santore, U., Graf, M. & Krause, G. (1994). Relationship between photoinhibition of photosynthesis, D1 protein turnover and chloroplast structure: effects of protein synthesis inhibitors. *Plant, Cell and Environment,* 17(1):55-64.

Schreiber, U. & Klughammer, C. (2008). New accessory for the Dual-PAM-100: The P515/535 module and examples of its application. *PAM Application Notes* , 1:1-10.

Takahashi, S., Milward, S.E., Fan, D.Y., Chow, W.S. & Badger, M.R. (2009). How does cyclic electron flow alleviate photoinhibition in Arabidopsis? *Plant Physiology* , 149(3):1560-1567.

Wang, B., Wang, X., Hu, Y., Chang, M., Bi, Y. & Hu, Z. (2015). The combined effects of UV-C radiation and H 2 O 2 on Microcystis aeruginosa, a bloom-forming cyanobacterium. *Chemosphere* , 141:34-43.

Wang, J.H., Li, S.C., Sun, M., Huang, W., Cao, H., Xu, F., Zhou, N.N. & Zhang, S.B. (2013). Differences in the stimulation of cyclic electron flow in two tropical ferns under water stress are related to leaf anatomy. *Physiologia plantarum* , 147(3):283-295.

Wiegand, C. & Cuellar, J. (1981). Duration of grain filling and kernel weight of wheat as affected by temparature. Crop Science, 21(1):95-101.

Yanykin, D., Khorobrykh, A., Mamedov, M. & Klimov, V. (2015). Trehalose stimulation of photoinduced electron transfer and oxygen photoconsumption in Mn-depleted photosystem 2 membrane fragments. *Journal of Photochemistry and Photobiology B: Biology* , 152:279-285.

Yi, X., Mcchargue, M., Laborde, S., Frankel, L.K. & Bricker, T.M. (2005). The manganese-stabilizing protein is required for photosystem II assembly/stability and photoautotrophy in higher plants. Journal of biological chemistry, 280(16):16170-16174.

Zhang, G., Liu, Y., Ni, Y., Meng, Z., Lu, T. & Li, T. (2014). Exogenous calcium alleviates low night temperature stress on the photosynthetic apparatus of tomato leaves. PLoS ONE, 9(5):e97322-e97322.

Zhang, S., Huang, W., Zhang, J. & Cao, K. (2016). Differential responses of photosystems I and II to seasonal drought in two Ficus species. Acta Oecologia, 73:53-60.

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