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Salt pretreatment alleviated salt-induced photoinhibition in sweet sorghum

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Abstract Sweet sorghum is an important energy crop. This study aimed to investigate the effects of salt pretreatment on the interaction between photosystem II (PSII) and photosystem I (PSI) upon salt stress. In this study, sweet sorghum was pretreated with 150 mM NaCl for 10 days, and subsequently, the pretreated plants were subjected to severe salt stress at 300 mM NaCl. PSII and PSI photoinhibition occurred in non-pretreated plants after 4 days of salt stress, as the maximum quantum yield of PSII (Fv/Fm) and the maximal photochemical capacity of PSI ($\triangle MR/MR_0$) significantly decreased, and their normal coordination was destroyed. The significant positive correlation between Fv/Fm and $\triangle MR/MR_0$ under salt stress indicated that PSII photoinhibition was in relation to PSI photoinhibition, and PSI photoinhibition might lead to PSII photoinhibition through inhibiting electron transport at the acceptor side of PSII. Salt stress did not induce PSII photoinhibition in salt-pretreated plants, and thus, salt pretreatment protected PSI against photoinhibition not by aggravating PSII photoinhibition. Salt pretreatment mitigated the decrease in CO_2 assimilation, reduced the feedback inhibition on photosynthetic electron transport and then contributed to suppressing PSI and PSII photoinhibition in sweet sorghum under salt stress. Therefore, the normal coordination between PSII and PSI was maintained in salt-pretreated plants. In conclusion, salt pretreatment ensured normal PSII and PSI coordination by preventing photoinhibition in sweet sorghum under salt stress.

Keywords Photosynthesis · PSII · PSI · Na^+

Abbreviations

Ci	Intercellular CO_2 concentration
ETo/ABS	Quantum yield for electron transport
ETo/TRo	Probability that an electron moves further than primary acceptor of PSII
Fv/Fm	The maximal quantum yield of PSII
g_s	Stomatal conductance
MDA	Malondialdehyde
PSI	Photosystem I
PSII	Photosystem II

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ROS	Reactive oxygen species
△MR/MR_0	The maximal photochemical capacity of PSI
ΦPSII	Actual photochemical efficiency of PSII
1−qP	Excitation pressure of PSII

1 Introduction

Soil salinity is always an agricultural problem due to the inhibition on crop growth (Rozema and Flowers 2008). Besides soil remediation, it is feasible to combat salinity by improving crop salt tolerance (Lea et al. 2004; Schroeder et al. 2013). Eco-physiological methods seem more convenient and easier to be applied in agricultural practice, in contrast to genetic engineering and conventional breeding (Yan et al. 2013c; Guerrero et al. 2014). For example, salt pretreatment with sublethal level of salinity has been evidenced as an available way for enhancing crop salt tolerance (Amzallag et al. 1990; Umezawa et al. 2000; Djanaguiraman et al. 2006; Tajdoost et al. 2007; Saha et al. 2012).

Photosynthesis closely correlates with plant growth and is sensitive to salt stress, and photosynthetic capacity is an important criterion for diagnosing plant adaptability to salinity (Kalaji and Pietkiewicz 1993; Kalaji et al. 2011; Wani et al. 2013; Yan et al. 2015). Salt stress inhibits CO_2 assimilation by inducing stomatal limitation and reducing the activities of key enzymes for CO_2 fixation (Loreto et al. 2003; Feng et al. 2007; Yang et al. 2008; Lu et al. 2009). The inhibited CO_2 assimilation will cause over-reduction of photosynthetic electron transport chain, elevate excitation pressure in chloroplast and may eventually lead to the increase of ROS production as well as photoinhibition (Takahashi and Murata 2008; Oukarroum et al. 2014). Photosystem II (PSII) photoinhibition is a result of the imbalance between PSII photodamage and the repair of such damage (Murata et al. 2007), whereas photosystem I (PSI) photoinhibition is induced by ROS produced at the acceptor side of PSI through Mehler reaction in vivo (Sonoike 2011). The electron flow from PSII is essential for PSI photoinhibition, and the interception of electron flow from PSII can suppress PSI photoinhibition and help PSI recovery after photoinhibition (Sonoike 1996; Zhang et al. 2011).

PSI photoinhibition is more dangerous than PSII photoinhibition because of the difficult recovery process of PSI (Kudoh and Sonoike 2002; Zhang and Scheller 2004). PSII photoinhibition under high temperature or high light stress can protect PSI against photoinhibition by restricting the electron flow to PSI (Yan et al. 2013a, b; Zivcak et al. 2014). Oppositely, PSI photoinhibition often arises under chilling stress with low light because of the limited restriction on electron flow to PSI (Li et al. 2004; Zhang et al. 2011). Therefore, PSII and PSI interaction is crucial for protecting PSI or even the whole photosynthetic apparatus. Under salt stress, chilling-induced PSI photoinhibition was attenuated due to the aggravation of PSII injury in cucumber (Yang et al. 2014). Salt stress also induced PSII photoinhibition and lowered electron flow to PSI in diploid honeysuckle, however, more severe photoinhibition occurred in PSI than PSII (Yan et al. 2015). Thus, the interaction between PSII and PSI under salt stress seems complex.

Salt pretreatment can alleviate salt-induced stomatal limitation on CO_2 fixation by increasing accumulation of osmolytes (Sivritepe et al. 2005). In addition, the decreased leaf Na^+ accumulation by salt pretreatment also may reduce the toxic effects on the enzymes for CO_2 fixation. As a result, salt-induced PSII and PSI photoinhibition may be mitigated by salt pretreatment because of the lowered feedback inhibition on photosynthetic electron transport. Djanaguiraman et al. (2006) reported that salt pretreatment abated PSII photoinhibition in rice. However, it has not been evidenced that salt pretreatment can alleviate salt-induced PSI photoinhibition, and it is not clear about the effects of salt pretreatment on the interaction between PSII and PSI in plants upon salt stress.

Sweet sorghum is an annual C4 crop and has been considered as an important energy crop for exploiting marginal land (Vasilakoglou et al. 2011). Amzallag et al. (1990) reported that salt pretreatment enhanced salt tolerance of sorghum with less Na^+ accumulation in the shoot. In contrast to sorghum, sweet sorghum has greater salt acclimation ability and more fermentable sugars (Almodares et al. 2008). This study aimed to investigate the effects of salt pretreatment on PSII and PSI interaction in sweet sorghum upon salt stress through an analysis of chlorophyll *a* fluorescence transient and 820 nm reflection transient. We hypothesized that salt pretreatment could alleviate PSI

photoinhibition in sweet sorghum under salt stress, and the normal PSII and PSI coordination could be maintained. Our study may present a deep insight into the mechanism of salt pretreatment for improving plant salt tolerance.

2 Materials and methods

2.1 Plant material and treatment

Seeds of sweet sorghum (*Sorghum bicolor* (L.) Moench. cv. YaJin) were immersed in 30 °C water for 2 h, and then placed between two sheets of filter paper in a Petri dish to germinate at 25 °C in the dark. The filter paper was kept wet by spraying Hoagland nutrient solution with pH at 5.7 (Arnon 1950). After 2 days, seeds with similar buds (about 0.6 cm) were transferred to perforated plastic pots (1 l volume) filled with vermiculite (one bud in each pot) and grown in artificial climatic chambers (Huier, China). The photon flux density (PFD) was approximately 200 μmol m^{-2} s^{-1} (12 h per day from 07:00 AM to 07:00 PM), and day/night temperature and humidity were controlled at 25/18 °C and 65 %. The seedlings were daily watered with Hoagland nutrient solution (pH 5.7). After 30 days, plants with uniform growth pattern (about 0.30 m height and 0.8 mm diameter of the stem) were selected as experimental materials and separated to four groups.

In the first group, plants were watered with Hoagland solution without NaCl addition. In the second group, plants were always exposed to 150 mM NaCl. These two groups were used as control. In the third group, plants were pretreated at 150 mM NaCl for 10 days, and then subjected to severe salt stress with 300 mM NaCl for 7 days. In the fourth group, the non-pretreated plants were subjected to 300 mM NaCl for 7 days. In all groups, NaCl was added to nutrient solution to provide the final concentration by 50 mM step per day. The solution in the pots was refreshed every 2 days, and in order to avoid accumulation of ions, nutrient solution was used to leach the culture substrate before refreshing solution. The newest fully expanded leaves were selected for measuring physiological parameters.

2.2 Measurements of gas exchange and chlorophyll fluorescence parameters

Gas exchange and modulated chlorophyll fluorescence parameters were simultaneously detected by using an open photosynthetic system (LI-6400XTR, Li-Cor, Lincoln, NE, USA) equipped with a fluorescence leaf chamber (6400-40 LCF, Li-Cor).

The leaves were exposed to actinic light (800 μmol m^{-2} s^{-1}) in leaf cuvette, and maintained for about 30 min until CO_2 assimilation reached a steady state. The temperature, CO_2 concentration and relative humidity were respectively set at 25 °C, 400 μmol mol^{-1} and 65 % in the leaf cuvette. Photosynthetic rate (Pn), stomatal conductance (g_s) and intercellular CO_2 concentration (Ci) were simultaneously recorded. In addition, steady-state fluorescence yield (Fs) was also recorded. Subsequently, a saturating actinic light pulse of 8000 μmol m^{-2} s^{-1} for 0.7 s was used to produce maximum fluorescence yield (Fm′) by temporarily inhibiting PSII photochemistry, and the minimum fluorescence in the steady state (Fo') was determined during a brief interruption of actinic light irradiation in the presence of far-red light ($\lambda = 740$ nm). At last, actual photochemical efficiency of PSII (ΦPSII) and excitation pressure of PSII (1−qP) were calculated as: $\Phi PSII = (Fm' - Fs)/Fm'$ and $1 - qP = (Fs - Fo')/(Fm' - Fo')$ (Maxwell and Johnson 2000).

2.3 Measurement of chlorophyll *a* fluorescence and modulated 820 nm reflection transients

The measurements were made by using a multifunctional plant efficiency analyzer (MPEA, Hansatech, UK). The operating mechanism of this instrument has been elucidated in detail by (Strasser et al. (2010); Kalaji et al. 2012). The leaves were adapted in dark for 30 min before the measurement (Kalaji et al. 2014b). Thereafter, the leaves were orderly illuminated with 1 s red light (627 nm, 5000 μmol photons m^{-2} s^{-1}), 10 s far red light (735 nm, 200 μmol photons m^{-2} s^{-1}) and 2 s red light (627 nm, 5000 μmol photons m^{-2} s^{-1}). Chlorophyll *a* fluorescence and modulated 820 nm reflection were simultaneously recorded during the illumination.

Chlorophyll *a* fluorescence transients in the first 1 s red illumination were quantified according to JIP test

(Strasser et al. 2000) by using the following original data: (1) fluorescence intensity at 20 μs (Fo, when all reaction centers of PSII are open); (2) the maximum fluorescence intensity (Fm, when all reaction centers of PSII are closed) and (3) fluorescence intensities at 2 ms (F_J, J step). By using these original data, some parameters could be calculated for quantifying PSII behavior. The maximum quantum yield of PSII (Fv/Fm), probability that an electron moves further than primary acceptor of PSII (Q_A) (ETo/TRo) and quantum yield for electron transport (ETo/ABS) were calculated as: Fv/Fm = (Fm−Fo), ETo/TRo = (Fm−F_J)/(Fm−Fo) and ETo/ABS = (Fm−F_J)/Fm (Strasser et al. 2010).

PSI oxidation is known to cause an increase in absorption in 820 nm. Monitoring modulated reflection change near 820 nm is a very convenient way to follow the redox state of PSI. The relative value of the maximal difference of 820 nm reflection during the last 2 s red illumination was used to indicate the maximal photochemical capacity of PSI ($\triangle MR/MR_0$) (Schansker et al. 2003). MR_0 is the value of 820 nm reflection at 0.7 ms (the first reliable MR measurement). △MR is the value of the maximal difference of 820 nm reflection at the last 2 s red light illumination.

2.4 Measurements of leaf dry weight, Na^+ and leaf relative water content

Leaves were sampled, dried at 105 °C for 10 min in an oven, and dried to constant weight at 70 °C. The extraction of Na^+ was performed according to Song et al. (2011). Deionized H_2O was added to 0.1 g dried plant powder and boiled for 2 h. The supernatant was diluted with deionized H_2O for measuring Na^+ content by using an atomic absorption spectrophotometer (TAS-990, China).

Fresh leaves were harvested and weighed (fresh weight, FW), and then were immersed in distilled water for 4 h at room temperature to determine saturated fresh weight (SW). At last, the leaves were dried completely in an oven at 70 °C and weighed (dry weight, DW). Relative water content (RWC) was calculated as: RWC = (FW − DW)/(SW − DW) × 100 %.

2.5 Measurement of malondialdehyde (MDA) content

The lipid peroxidation degree was determined in terms of MDA content by the thiobarbituric acid reaction method. Leaf tissues (0.5 g) were ground under liquid nitrogen and then homogenized in 5 ml of 50 mM potassium phosphate buffer (pH 7.8). After centrifugation at 4 °C and 13000×*g* for 10 min, the supernatant was prepared for the assay of MDA content (Yan et al. 2010).

2.6 Statistical analysis

The experiment was designed to analyze the difference of parameters among four sets of plants. One-way ANOVA was carried out by using SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for all sets of data. The values presented are the mean of measurements with five replicate plants in each set. The total freedom degree, freedom degree among and within groups was 19, 3 and 16. The comparisons of means were determined through LSD test. Difference was considered significant at $P < 0.05$. Pearson correlation analysis was also carried out by using SPSS 16.0.

3 Results

3.1 Leaf dry weight, MDA, Na^+ and leaf relative water contents

After salt stress for 7 days, leaf dry weight and leaf relative water contents were significantly decreased in non-pretreated and salt-pretreated plants compared with the control plants exposed to 0 and 150 mM NaCl, whereas leaf Na^+ content was significantly increased ($P < 0.05$), and the greater change was found in non-pretreated plants than salt-pretreated plants (Table 1). The extent of lipid peroxidation represented by MDA content reflects the state and integrity of membranes in plant cells (Blokhina et al. 2003; Yazici et al. 2007). Leaf MDA content was significantly increased by salt stress ($P < 0.05$), and the greater increase was observed in non-pretreated plants than in salt-pretreated plants (Table 1).

3.2 Gas exchange, PSII actual quantum yield and excitation pressure

After 1 day of salt stress, Pn, g_s, Ci and ΦPSII were decreased in non-pretreated and salt-pretreated plants compared with the control plants exposed to 0 and 150 mM NaCl, whereas 1−qP was increased (Fig. 1).

Table 1 Dry weight, malondialdehyde (MDA), Na^+ and relative water contents in the leaf of sweet sorghum after 7 days of salt stress at 300 mM NaCl. Data in the table indicate the mean of five replicates (±SD)

Treatments	DW per leaf (g)	MDA content (mg g^{-1} DW)	Na^+ content (mg g^{-1} DW)	Leaf relative water content (%)
T1	0.50 ± 0.01a	0.021 ± 0.0035c	0.81 ± 0.15d	89.20 ± 0.77a
T2	0.45 ± 0.10b	0.022 ± 0.0041c	1.71 ± 0.41c	83.15 ± 0.78b
T3	0.31 ± 0.05b	0.028 ± 0.0051b	5.53 ± 0.62b	78.05 ± 0.32c
T4	0.20 ± 0.03c	0.040 ± 0.0047a	8.79 ± 0.59a	72.04 ± 0.46d

Within each column, means followed by the same letters are not significantly different at $P < 0.05$

DW indicates dry weight. T1 indicate plants exposed to 0 mM NaCl; T2 indicates plants exposed to 150 mM NaCl; T3 indicates plants pretreated with 150 mM NaCl for 10 days and then exposed to 300 mM NaCl; T4 indicates non-pretreated plants exposed to 300 mM NaCl

Upon salt stress for 4 and 7 days, Pn decreased respectively by 68.37 and 82.03 % in salt-pretreated plants and 84.6 and 95.61 % in non-pretreated plants compared with the control plants exposed to 0 mM NaCl (Fig. 1a), and the decrease was greater in non-pretreated plants. g_s was significantly lowered in non-pretreated and salt-pretreated plants upon 4 and 7 days of salt stress, and the significant increase in Ci was just noted in non-pretreated plants ($P < 0.05$) (Fig. 1b, c).

3.3 Chlorophyll *a* fluorescence and modulated 820 nm reflection transients

J step appears because accumulation of Q_A^- reaches the maximal level (Strasser et al. 1995; Kalaji et al. 2014a). After 4 days of salt stress at 300 mM NaCl, J step was elevated in non-pretreated plants (Fig. 2a), suggesting the electron transport at PSII acceptor side was inhibited. Upon 7 days of salt stress, J step in salt-pretreated plants was also elevated, but greater elevation was observed in non-pretreated plants (Fig. 2b). The 820 nm reflection signals are presented by MR/MR_0 ratio, where MR_0 is the value at the onset of actinic illumination (at 0.7 ms). Decrease in MR/MR_0 from MR_0 to the minimal value (MR_{min}, at about 29 ms) reflects PSI oxidation process, and the oxidation amplitude in the first 1 s red illumination was expressed as MR_0-MR_{min}. The minimal value of MR is a transitory steady state with equal oxidation and re-reduction rate of PSI. Subsequently, increase in MR/MR_0 indicates PSI re-reduction driven by the electron flow from PSII. Thus, 820 nm reflection transient in the first 1 s red illumination was influenced by both PSII and PSI capacity and could reflect their coordination. Salt stress significantly decreased PSI oxidation amplitude and obviously changed 820 nm reflection transient in non-pretreated plants, indicating the negative effects on PSII and PSI coordination, whereas normal PSII and PSI coordination was maintained in salt-pretreated plants (Fig. 2c, d).

3.4 PSII behaviors and the maximal photochemical capacity of PSI

After 4 and 7 days of salt stress, Fv/Fm and $\triangle MR/MR_0$ were significantly decreased in non-pretreated plants ($P < 0.05$), indicating the occurrence of PSII and PSI photoinhibition, but they were not markedly affected in salt-pretreated plants (Fig. 3a, b). In accordance with the elevated J step (Fig. 2a), significant decrease in ETo/TRo and ETo/ABS was observed in non-pretreated plants and salt-pretreated plants respectively upon 4 and 7 days of salt stress ($P < 0.05$).

3.5 Relationship between PSII and PSI activity

$\triangle MR/MR_0$ was positively correlated with Fv/Fm, ETo/TRo, ETo/ABS and ΦPSII and negatively correlated with 1−qP in sweet sorghum upon salt stress ($P < 0.05$) (Table 2), indicating that PSII photoinhibition was in relation to PSI photoinhibition. Pn was positively correlated with ΦPSII and negatively correlated with 1−qP ($P < 0.05$) (Table 2).

4 Discussion

Salt pretreatment enhanced salt acclimation in sweet sorghum, because salt-pretreated plants accumulated

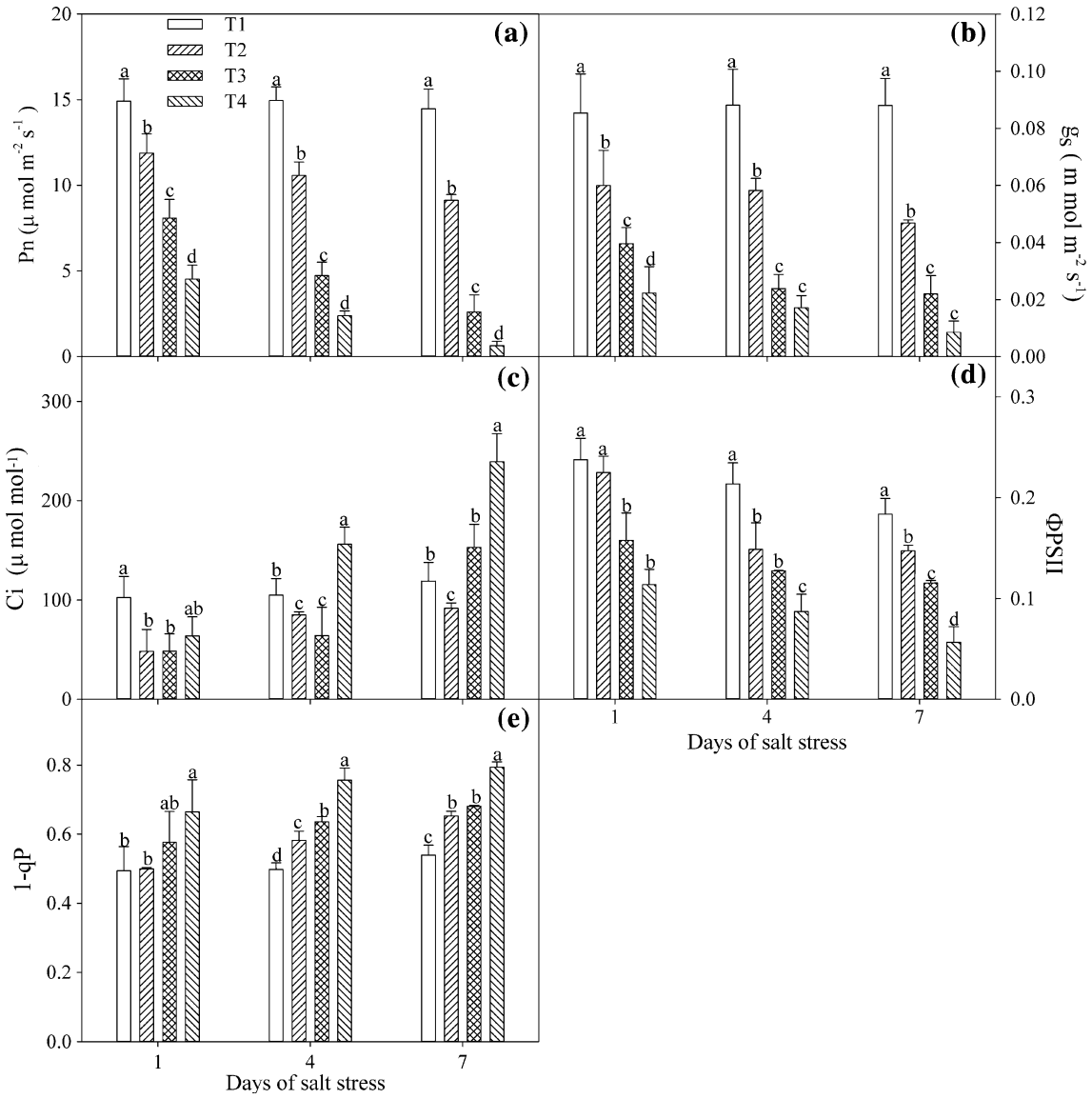

Fig. 1 Photosynthetic rate (Pn, **a**), stomatal conductance (g_s, **b**), intercellular CO_2 concentration (Ci, **c**), actual photochemical efficiency of PSII (ΦPSII, **d**) and excitation pressure (1−qP, **e**) in the leaves of sweet sorghum exposed to 300 mM NaCl. Data in the figure indicate mean of five replicates (±SD). Different letters on error bars indicate significant difference at $P < 0.05$. T1 indicate plants exposed to 0 mM NaCl; T2 indicates plants exposed to 150 mM NaCl; T3 indicates plants pretreated with 150 mM NaCl for 10 days and then exposed to 300 mM NaCl. T4 indicates non-pretreated plants exposed to 300 mM NaCl. These symbols are also used in the following figures

greater biomass and maintained higher photosynthetic activity than non-pretreated plants under salt stress (Table 1; Fig. 1a). Stomatal aperture often declines under salt stress for reducing water loss from transpiration (Chaves et al. 2009; Yan et al. 2012). The lower salt-induced decrease in leaf relative water content suggested the enhanced osmotic resistance in pretreated plants (Table 1). Thus, pretreated plants could maintain higher g_s and mitigate stomatal limitation on photosynthesis under salt stress (Fig. 1b). As the primary toxic component, Na^+ can inhibit CO_2 fixation by inducing negative effects on the enzymes for CO_2 fixation (Yang et al. 2008). Salt pretreatment decreased Na^+ accumulation in the leaf

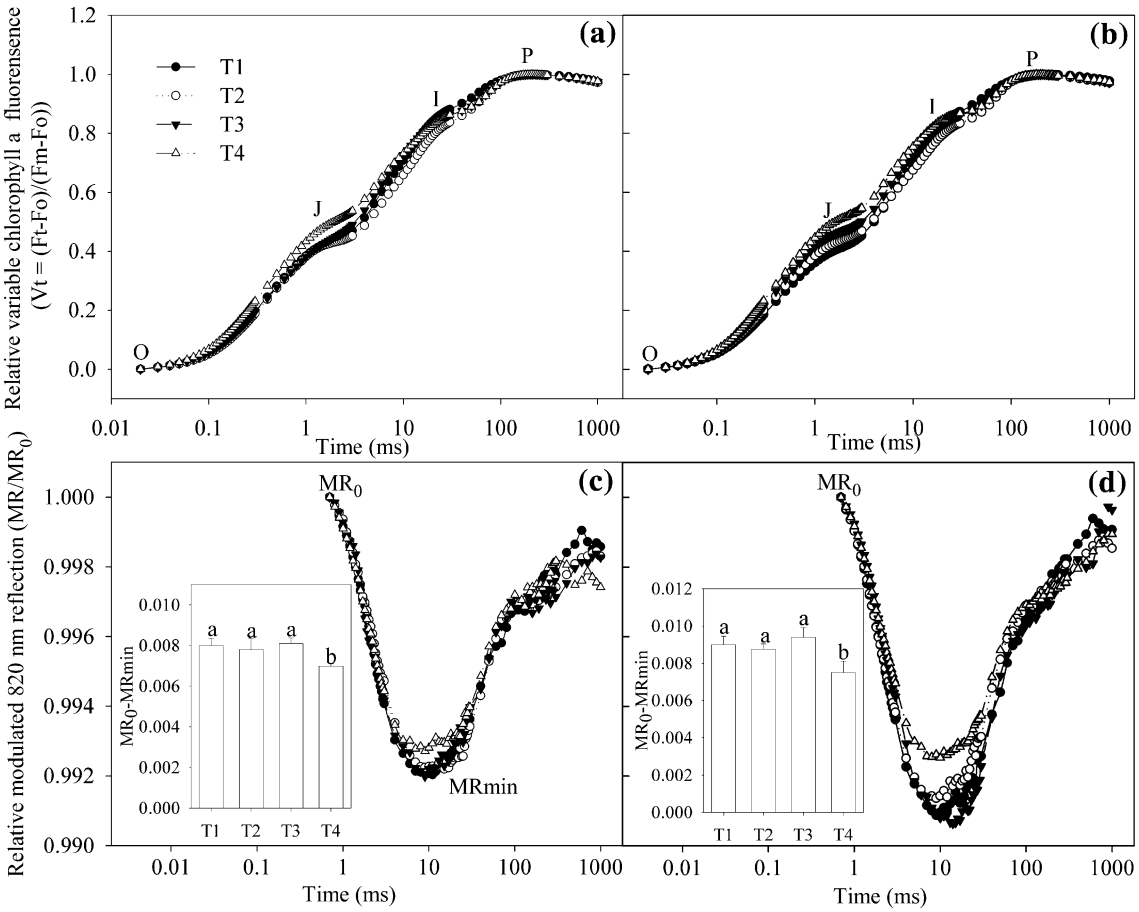

Fig. 2 Chlorophyll *a* fluorescence transient and 820 reflection transient during the first 1 s red illumination in the leaves of sweet sorghum exposed to 300 mM NaCl for 4 days (**a**, **c**) and 7 days (**b**, **d**). O, J, I and P indicates the specific steps in this transient. MR_0 is the value at the onset of actinic illumination (at 0.7 ms), and MR was the reflection signal during the 1 s of red illumination. MR_{min} is the minimal value of MR/MR_0 at about 29 ms. PSI oxidation amplitude was expressed as $MR_0 - MR_{min}$. Data in the figure indicate mean of five replicates (±SD). *Different letters* on *error bars* indicate significant difference at $P < 0.05$

under salt stress and then could reduce ionic toxicity on photosynthetic activity (Table 1).

Inhibition on CO_2 assimilation can reduce the utilization of reducing equivalents, increase ROS production and lead to PSII photoinhibition by inhibiting the recovery of D1 protein (Nishiyamaa et al. 2011; Koyro et al. 2013). Under salt stress, the decrease in Pn might induce the elevation of 1−qP and the decrease in ΦPSII because of their remarkable correlation (Table 2), and could increase the possibility of ROS generation in PSII. As a result, PSII photoinhibition arose in non-pretreated plants (Fig. 3b). As the traditional viewpoint, PSII is more susceptible to abiotic stresses than PSI, and PSII photoinhibition can protect PSI against photoinhibition by reducing the electron transport to PSI (Sonoike 2011). In parallel with PSII photoinhibition, electron transport in PSII acceptor side was depressed in non-pretreated plants (Fig. 2a, b), which restricted electron donation for PSI (Fig. 3c, d). As a consequence, PSI re-reduction should not be effectively driven and PSI oxidation amplitude would be increased in the 1 s red light illumination. On the contrary, PSI re-reduction was not delayed, and PSI oxidation amplitude was even significantly decreased (Fig. 2c, d). This contradict resulted from PSI photoinhibition which inhibited electron transport to its acceptor side and could depress PSI oxidation, and more severe photoinhibition of PSI brought about the

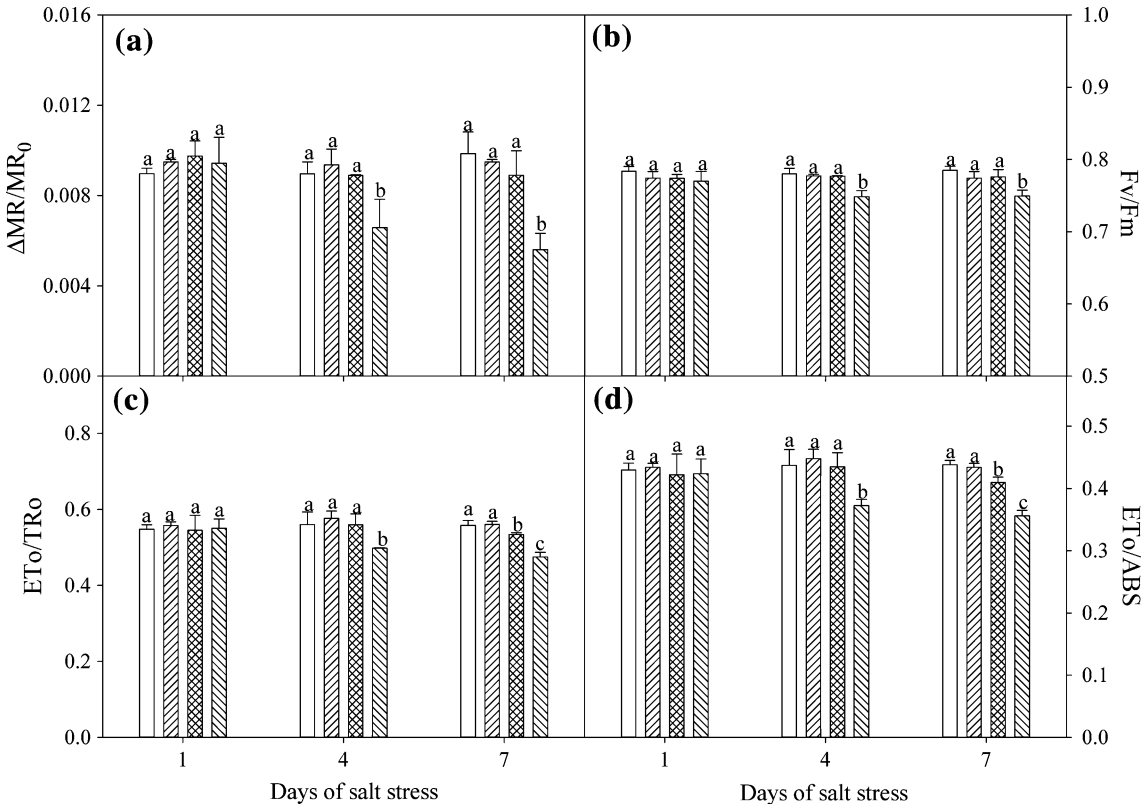

Fig. 3 The maximum PSI redox activity (△MR/MR$_0$, **a**), the maximum quantum yield of PSII (Fv/Fm, **b**), probability that an electron moves further than Q_A (ETo/TRo, **c**) and quantum yield for electron transport (ETo/ABS, **d**) in salt-pretreated plants exposed to 300 mM NaCl. Data in the figure indicate mean of five replicates (±SD). *Different letters* on *error bars* indicate significant difference at $P < 0.05$

Table 2 Correlation among Fv/Fm, △MR/MR$_0$, ETo/TRo, ETo/ABS, ΦPSII, 1−qP and Pn in salt-pretreated and non-pretreated sweet sorghum during salt stress at 300 mM NaCl

	Fv/Fm	△MR/MR$_0$	ETo/TRo	ETo/ABS	ΦPSII	1−qP	Pn
Fv/Fm	1	0.938**	0.923**	0.936**	0.834*	−0.866*	0.710
△MR/MR$_0$		1	0.887*	0.885*	0.857*	−0.853*	0.741
ETo/TRo			1	0.998**	0.868*	−0.893*	0.778
ETo/ABS				1	0.874*	−0.905*	0.788
ΦPSII					1	−0.987**	0.966**
1−qP						1	−0.965**
Pn							1

* $P < 0.05$, ** $P < 0.01$

significant decrease in PSI oxidation amplitude. Thus, PSI seems more susceptible to salt stress than PSII in sweet sorghum. On one hand, PSII photoinhibition can alleviate or prevent PSI photoinhibition by reducing electron flow to PSI, and they do not change in the same way, as severe PSII photoinhibition appears without PSI photoinhibition (Yan et al. 2013a; Yang et al. 2014). On the other hand, PSI photoinhibition can result in PSII photoinhibition due to the feedback inhibition on electron transport at PSII acceptor side, and they share the same change pattern and have positive correlation (Zhang et al. 2012, 2014). In this study, the significant positive correlation among Fv/Fm, △MR/MR$_0$, ETo/TRo and ETo/ABS indicated that salt stress might initially induce PSI photoinhibition, and then inhibited the electron transport at the

acceptor side of PSII (Table 2). As a result, excitation pressure of PSII was elevated with the occurrence of PSII photoinhibition. In agreement with the occurrence of photoinhibition, lipid peroxidation was significantly increased in non-pretreated plants by salt stress (Table 1), confirming the oxidative damage of ROS.

In line with the study on rice, salt pretreatment alleviated PSII photoinhibition in sweet sorghum under salt stress (Fig. 3b), and the actual electron flow from PSII to PSI was in a higher level in salt-pretreated plants than non-pretreated plants (Fig. 1d). Under abiotic stress, more electron donation from PSII is hazardous for PSI (Sonoike 2011; Zhang et al. 2011). However, PSI photoinhibition did not occur in salt-pretreated plants (Fig. 3a), and the normal coordination between PSII and PSI was maintained (Fig. 2c, d). Therefore, salt pretreatment protected PSI against photoinhibition not by aggravating PSII photoinhibition. PSI photoinhibition is induced by ROS produced at its acceptor side, and besides the electron donation from PSII, the amount of ROS generation also depends on the electron use efficiency at PSI acceptor side. CO_2 assimilation is mainly responsible for consuming reducing equivalents and quenching electrons at PSI acceptor side. When CO_2 assimilation is inhibited, the possibility of photoinhibition will increase, because more electrons will be transferred to O_2 to generate ROS (Takahashi and Murata 2008). Thus, salt pretreatment contributed to suppressing PSI photoinhibition and then alleviating PSII photoinhibition in sweet sorghum under salt stress by mitigating the decrease in CO_2 assimilation (Figs. 1a, 3a, b). It is worth to note that the decreased amplitude of Pn in salt-pretreated plants after 7 days of salt stress is nearly equivalent to that in non-pretreated plants after 4 days of salt stress (Fig. 1a), however, PSI photoinhibition did not appear yet in salt-pretreated plants. In addition, excitation pressure of PSII was also remarkably elevated in salt-pretreated plants (Fig. 1e), but PSII photoinhibition was not observed. Therefore, salt pretreatment suppressed salt-induced PSI and PSII photoinhibition not just by alleviating the decrease in CO_2 assimilation. There are protection mechanisms such as xanthophyll cycle, PSI cyclic electron transport and antioxidants to dissipate the excess excitation energy or scavenge ROS in chloroplast (Lu et al. 2008; Azzabi et al. 2012; Zivcak et al. 2013; Kiani-Pouya 2015). We supposed that salt pretreatment might stimulate these protection mechanisms to resist the negative effects of salt stress on photosynthetic apparatus.

To summarize, salt pretreatment protected PSII and PSI against photoinhibition in sweet sorghum under salt stress and ensured their normal coordination. To some extent, the alleviated decrease in CO_2 assimilation by salt pretreatment helped to suppress PSI photoinhibition and then alleviate PSII photoinhibition.

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References

Almodares A, Hadi MR, Ahmadpour H (2008) Sorghum stem yield and soluble carbohydrates under different salinity levels. Afr J Biotechnol 7:4051–4055

Amzallag GN, Lerner HR, Poljakoff-Mayber A (1990) Induction of increased salt tolerance in sorghum bicolor by NaCl pretreatment. J Exp Bot 41:29–34

Arnon DI (1950) Dennis Robert Hoagland: 1884–1949. Science 112(2921):739–742

Azzabi G, Pinnola A, Betterle N, Bassi R, Alboresi A (2012) Enhancement of non-photochemical quenching in the bryophyte *Physcomitrella patens* during acclimation to salt and osmotic stress. Plant Cell Physiol 53(10):1815–1825

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91:179–194

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann Bot 103:551–560

Djanaguiraman M, Sheeba JA, Shanker AK, Devi DD, Bangarusamy U (2006) Rice can acclimate to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. Plant Soil 284:363–373

Feng LL, Han YJ, Liu G, An BG, Yang J, Yang GH, Li YS, Zhu YG (2007) Overexpression of sedoheptulose-1,7-bisphosphatase enhances photosynthesis and growth under salt stress in transgenic rice plants. Funct Plant Biol 34:822–834

Guerrero RY, González LM, Dell'Amico J, Núñez M, Pieters AJ (2014) Reversion of deleterious effects of salt stress by activation of ROS detoxifying enzymes via foliar application of 24-epibrassinolide in rice seedlings. Theor Exp Plant Physiol 27:31–40

Kalaji HM, Pietkiewicz S (1993) Salinity effects on plant growth and other physiological processes. Acta Physiol Plant 15:89–124

Kalaji HM, Govindjee Bosa K, Koscielniak J, Zuk-Golaszewska K (2011) Effects of salt stress on photosystem II efficiency and CO_2 assimilation of two Syrian barley landraces. Environ Exp Bot 73:64–72

Kalaji HM, Goltsev V, Bosa K, Allakhverdiev SI, Strasser RJ, Govindjee (2012) Experimental in vivo measurements of

light emission in plants: a perspective dedicated to David Walker. Photosynth Res 114:69–96

Kalaji HM, Oukarroum A, Alexandrov V, Kouzmanova M, Brestic M, Zivcak M, Samborska IA, Cetner MD, Allakhverdiev SI, Goltsev V (2014a) Identification of nutrient deficiency in maize and tomato plants by in vivo chlorophyll *a* fluorescence measurements. Plant Physiol Biochem 81:16–25

Kalaji HM, Schansker G, Ladle RJ, Goltsev V, Bosa K, Allakhverdiev SI, Brestic M, Bussotti F, Calatayud A, Dabrowski P, Elsheery NI, Ferroni L, Guidi L, Hogewoning SW, Jajoo A, Misra AN, Nebauer SG, Pancaldi S, Penella C, Poli DB, Pollastrini M, Romanowska-Duda ZB, Rutkowska B, Serodio J, Suresh K, Szulc W, Tambussi E, Yanniccari M, Zivcak M (2014b) Frequently asked questions about in vivo chlorophyll fluorescence: practical issues. Photosynth Res 122:121–158

Kiani-Pouya A (2015) Changes in activities of antioxidant enzymes and photosynthetic attributes in triticale (×*Triticosecale* Wittmack) genotypes in response to long-term salt stress at two distinct growth stages. Acta Physiol Plant 37:72

Koyro HW, Hussain T, Huchzermeyer B, Khan MA (2013) Photosynthetic and growth responses of a perennial halophytic grass *Panicum turgidum* to increasing NaCl concentrations. Environ Exp Bot 91:22–29

Kudoh H, Sonoike K (2002) Irreversible damage to photosystem I by chilling in the light: cause of the degradation of chlorophyll after returning to normal growth temperature. Planta 215:541–548

Lea PJ, Parry MAJ, Medrano H (2004) Improving resistance to drought and salinity in plants. Ann Appl Biol 144:249–250

Li XG, Wang XM, Meng QW, Zou Q (2004) Factors limiting photosynthetic recovery in sweet pepper leaves after short-term chilling stress under low irradiance. Photosynthetica 42:257–262

Loreto F, Centritto M, Chartzoulakis K (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. Plant Cell Environ 26:595–601

Lu KX, Yang Y, He Y, Jiang DA (2008) Induction of cyclic electron flow around photosystem 1 and state transition are correlated with salt tolerance in soybean. Photosynthetica 46:10–16

Lu KX, Cao BH, Feng XP, He Y, Jiang DA (2009) Photosynthetic response of salt-tolerant and sensitive soybean varieties. Photosynthetica 47:381–387

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. J Exp Bot 51:659–668

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta 1767:414–421

Nishiyamaa Y, Allakhverdiev SI, Murata N (2011) Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. Physiol Plant 142:35–46

Oukarroum A, Bussotti F, Goltsev V, Kalaji HM (2014) Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* L. plants under salt stress. Environ Exp Bot 109:80–88

Rozema J, Flowers T (2008) Ecology crops for a salinized world. Science 322:1478–1480

Saha P, Kunda P, Biswas AK (2012) Influence of sodium chloride on the regulation of Krebs cycle intermediates and enzymes of respiratory chain in mungbean (*Vigna radiata* L. Wilczek) seedlings. Plant Physiol Biochem 60:214–222

Schansker G, Srivastava A, Govindjee Strasser RJ (2003) Characterization of the 820 nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves. Funct Plant Biol 30:785–796

Schroeder JI, Delhaize E, Frommer WB, Guerinot ML, Harrison MJ, Herrera-Estrella L, Horie T, Kochian LV, Munns R, Nishizawa NK, Tsay YF, Sanders D (2013) Using membrane transporters to improve crops for sustainable food production. Nature 497:60–66

Sivritepe HO, Sivritepe N, Eris A, Turhan E (2005) The effects of NaCl pre-treatments on salt tolerance of melons grown under long-term salinity. Sci Hortic 106:568–581

Song J, Shi GW, Gao B, Fan H, Wang BS (2011) Waterlogging and salinity effects on two Suaeda salsa populations. Physiol Plant 141:343–351

Sonoike K (1996) Degradation of psaB gene product, the reaction center subunit of photosystem I, is caused during photoinhibition of photosystem I: possible involvement of active oxygen species. Plant Sci 115:157–164

Sonoike K (2011) Photoinhibition of photosystem I. Physiol Plant 142:56–64

Strasser RJ, Srivastava A, Govindjee (1995) Polyphasic chlorophyll-alpha fluorescence transient in plants and cyanobacteria. Photochem Photobiol 61:32–42

Strasser RJ, Srivatava A, Tsimilli-Michael M (2000) The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Yunus M, Pathre U, Mohanty P (eds) Probing photosynthesis: mechanism, regulation and adaptation. Taylor & Francis, Bristol, pp 445–483

Strasser RJ, Tsimilli-Michael M, Qiang S, Goltsev V (2010) Simultaneous in vivo recording of prompt and delayed fluorescence and 820 nm reflection changes during drying and after rehydration of the resurrection plant *Haberlea rhodopensis*. Biochim Biophys Acta 1797:1313–1326

Tajdoost S, Farboodnia T, Heidari R (2007) Salt pretreatment enhance salt tolerance in *Zea mays* L. seedlings. Pak J Biol Sci 10:2086–2090

Takahashi S, Murata N (2008) How do environmental stresses accelerate photoinhibition? Trends Plant Sci 13:178–182

Umezawa T, Shimizu K, Kato M, Ueda T (2000) Enhancement of salt tolerance in soybean with NaCl pretreatment. Physiol Plant 110:59–63

Vasilakoglou I, Dhima K, Karagiannidis N, Gatsis T (2011) Sweet sorghum productivity for biofuels under increased soil salinity and reduced irrigation. Field Crop Res 120:38–46

Wani AS, Ahmad A, Hayat S, Fariduddin Q (2013) Salt-induced modulation in growth, photosynthesis and antioxidant system in two varieties of *Brassica juncea*. Saudi J Biol Sci 20:183–193

Yan K, Chen W, He XY, Zhang GY, Xu S, Wang LL (2010) Responses of photosynthesis, lipid peroxidation and antioxidant system in leaves of *Quercus mongolica* to elevated O_3. Environ Exp Bot 69:198–204

Yan K, Chen P, Shao H, Zhao S, Zhang L, Zhang L, Xu G, Sun J (2012) Responses of photosynthesis and photosystem II to

higher temperature and salt stress in sorghum. J Agron Crop Sci 198:218–226

Yan K, Chen P, Shao HB, Shao CY, Zhao SJ, Brestic M (2013a) Dissection of photosynthetic electron transport process in sweet sorghum under heat stress. PLoS One 8:e62100

Yan K, Chen P, Shao HB, Zhao SJ (2013b) Characterization of photosynthetic electron transport chain in bioenergy crop Jerusalem artichoke (*Helianthus tuberosus* L.) under heat stress for sustainable cultivation. Ind Crop Prod 50:809–815

Yan K, Shao HB, Shao CY, Chen P, Zhao SJ, Brestic M, Chen XB (2013c) Physiological adaptive mechanisms of plants grown in saline soil and implications for sustainable saline agriculture in coastal zone. Acta Physiol Plant 35:2867–2878

Yan K, Wu CW, Zhang LH, Chen XB (2015) Contrasting photosynthesis and photoinhibition in tetraploid and its autodiploid honeysuckle (*Lonicera japonica* Thunb.) under salt stress. Front Plant Sci 6:227

Yang XH, Liang Z, Wen XG, Lu CM (2008) 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

Yang C, Zhang ZS, Gao HY, Fan XL, Liu MJ, Li XD (2014) The mechanism by which NaCl treatment alleviates PSI photoinhibition under chilling-light treatment. J Photochem Photobiol B 140:286–291

Yazici I, Tuerkan I, Sekmen AH, Demiral T (2007) Salinity tolerance of purslane (*Portulaca oleracea* L.) is achieved by enhanced antioxidative system, lower level of lipid peroxidation and proline accumulation. Environ Exp Bot 61:49–57

Zhang SP, Scheller HV (2004) Photoinhibition of photosystem I at chilling temperature and subsequent recovery in *Arabidopsis thaliana*. Plant Cell Physiol 45:1595–1602

Zhang ZS, Jia YJ, Gao HY, Zhang LT, Li HD, Meng QW (2011) Characterization of PSI recovery after chilling-induced photoinhibition in cucumber (*Cucumis sativus* L.) leaves. Planta 234:883–889

Zhang LT, Zhang ZS, Gao HY, Xue ZC, Yang C, Meng XL, Meng QW (2012) Mitochondrial alternative oxidase pathway protects plants against photoinhibition by alleviating inhibition of the repair of photodamaged PSII through preventing formation of reactive oxygen species in *Rumex* K-1 leaves. Physiol Plant 143:396–407

Zhang ZS, Yang C, Gao HY, Zhang LT, Fan XL, Liu MJ (2014) The higher sensitivity of PSI to ROS results in lower chilling-light tolerance of photosystems in young leaves of cucumber. J Photochem Photobiol B 137:127–134

Zivcak M, Brestic M, Balatova Z, Drevenakova P, Olsovska K, Kalaji HM, Yang XH, Allakhverdiev SI (2013) Photosynthetic electron transport and specific photoprotective responses in wheat leaves under drought stress. Photosynth Res 117:529–546

Zivcak M, Brestic M, Kalaji MH, Govindjee (2014) Photosynthetic responses of sun- and shade-grown barley leaves to high light: is the lower PSII connectivity in shade leaves associated with protection against excess of light? Photosynth Res 119:339–354