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Effects of addition of external nitric oxide on the allocation of photosynthetic electron flux in *Rumex* K-1 leaves under osmotic shock

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Abstract

Photosynthetic electron flux allocation, stomatal conductance, and the activities of key enzymes involved in photosynthesis were investigated in *Rumex* K-1 leaves to better understand the role of nitric oxide (NO) in photoprotection under osmotic stress caused by polyethylene glycol. Gas exchange and chlorophyll fluorescence were measured simultaneously with a portable photosynthesis system integrated with a pulse modulated fluorometer to calculate allocation of photosynthetic electron fluxes. Osmotic stress decreased stomatal conductance, photosynthetic carbon assimilation, and nitrate assimilation, increased Mehler reaction, and resulted in photoinhibition. Addition of external NO enhanced the stomatal conductance, photosynthetic rate, activities of glutamine synthetase and nitrate reductase, and reduced Mehler reaction and photoinhibition. These results demonstrated that osmotic stress reduced CO_2 assimilation, decreasing the use of excited energy *via* CO_2 assimilation which caused significant photoinhibition. Improving stomatal conductance by the addition of external NO enhanced the use of excited energy *via* CO_2 assimilation. As a result, less excited energy was allocated to Mehler reaction, which reduced production of reactive oxygen species *via* this pathway. We suppose that Mehler reaction is not promoted unless photosynthesis and nitrogen metabolism are prominently inhibited.

Additional key words: osmotic stress; photoenergy allocation.

Introduction

Plants maintain their survival by absorbing light and converting light energy to chemical energy. Though it may be partially dissipated through nonphotochemical quenching (Demmig-Adams and Adams 1992, Murchie and Niyogi 2011), the absorbed light energy is consumed mainly through photochemical reactions, such as photosynthesis, photorespiration, nitrogen assimilation, and Mehler reaction under most conditions. The allocation of the photosynthetic electron flux to each of the pathways changes according to the growth conditions of plants. Kumagai *et al.* (2010) found that N-deficiency results in an increase of alternative electron flux. Zhou *et al.* (2004)

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Abbreviations: APX – ascorbate peroxidase; C_a – atmospheric CO_2 concentration; C_i – intercellular CO_2 concentration; F_m – maximal fluorescence yield of the dark-adapted state; F_m' – maximal fluorescence in the light-adapted state; F_0 – minimal fluorescence of the dark-adapted state; F_0' – minimal fluorescence of the light-adapted state; F_s – steady-state fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; GS – glutamine synthetase; GSNO – S-nitrosoglutathione; $Ja_{O2\text{-}dep}$ – electron flux used for Mehler reaction; $Ja_{O2\text{-}indep}$ – O_2-independent alternative electron flux; Je_{PCO} – electron flux used in photorespiratory carbon oxidation; Je_{PCR} – electron flux used in photosynthetic carbon reduction; Je_{PSII} – total electron flux in PSII; NO – nitric oxide; NR – nitrate reductase; PEG – polyethylene glycol; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; PSII – photosystem II; q_P – photochemical quenching coefficient; $1 - q_P$ – measure of closed PSII reaction centers; ROS – reactive oxygen species; Rubisco – ribulose-1,5-bisphosphate carboxylase/oxygenase; RWC – relative water content; SOD – superoxide dismutase; V_c – rate of Rubisco carboxylation; V_o – rate of Rubisco oxidation; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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reported that Mehler reaction (named also water-water cycle) operates at high rate, when photosynthesis is restricted in cucumber leaves subjected to chilling and low light conditions. In our previous studies, we also observed that more photosynthetic electron flux is allocated to Mehler reaction in leaves of *Rumex* K-1 under salt stress (Chen *et al.* 2004).

Drought is one of the most devastating stresses and it causes a half of reduction of crop production (Fischer and Tuerner 1978). Illustrating the allocation of photosynthetic electron flux is important in understanding plant response to drought stress, which could help in improving drought resistance of plant in future. Nevertheless, available information on this matter is limited and incompatible. Wingler *et al.* (1999) found that photorespiration is enhanced, when photosynthesis decreases in leaves of barley under drought stress. However, Biehler and Fock (1996) noticed that Mehler reaction contributes to dissipating excess excitation energy, when photorespiration decreases in wheat leaves under drought stress. In addition, there is not a practical way to quantify the photosynthetic electron flux allocated to nitrogen metabolism. The role of nitrogen metabolism in photosynthetic electron allocation under osmotic stress is still unclear. Therefore, it is necessary to gain insight into the dynamics of photosynthetic electron flux allocation among these processes under osmotic stress conditions.

It has been found that NO, a kind of signaling molecule (Durner and Klessig 1999, Wendehenne *et al.* 2001, Neill *et al.* 2003, Cui *et al.* 2011), increases plant resistance to biotic and abiotic stresses, such as senescence (Guo and Crawford 2005), salt (Zhao *et al.* 2007), low or high temperature (Xuan *et al.* 2010, Cantrel *et al.* 2011), and UV-B irradiation (Tossi *et al.* 2009). NO is also involved in stomata closure (García-Mata and Lamattina 2001, Desikan *et al.* 2002, Li *et al.* 2009). García-Mata and Lamattina (2001) suggested that NO induces stomata closure and enhances drought tolerance in wheat. However, it remains unclear if external NO affects photoenergy allocation in *Rumex* K-1 leaves under osmotic stress. If so, what a role does NO play in regulation of the photosynthetic electron allocation? Among various photochemical reactions, which one is preferably enhanced to consume excessive excited energy under osmotic stress with or without addition of NO? To clarify these questions, we analyzed photosynthetic electron flux allocation to photosynthesis, photorespiration, nitrogen metabolism, and Mehler reaction as well as the activities of key enzymes involved in these processes in *Rumex* K-1 leaves under osmotic shock with and without addition of external NO.

Materials and methods

Plant materials and growth conditions: Seedlings of *Rumex* K-1 were grown in pots (20 cm in diameter and 30 cm in height) in the field. When the third leaves fully expanded, the seedlings were transferred to brown bottles (10 cm in diameter and 20 cm in height) containing Hoagland nutrient solution, which were then kept in outdoor conditions (0–1,200 μmol m^{-2} s^{-1} PPFD, 20–28ºC). After the acclimation to the Hoagland solution cultivation (about 10 d), the seedlings were used for osmotic-stress treatments.

Osmotic shock treatments and the addition of external NO: S-nitrosoglutathione (GSNO) was used as an external NO donor, because this compound has no side effect on ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase and on photosynthesis (Lum *et al.* 2005). We performed a prior experiment to determine an appropriate concentration of GSNO by adding various concentrations of GSNO (1, 2, 3, 4, 5, 6, 7, and 8 mg L^{-1}) into Hoagland solution with 20% polyethylene glycol (PEG) to *Rumex* K-1 seedlings. As the most effective concentration to reverse osmotic stress, 5 mg L^{-1} of GSNO was selected and used further in this study.

After the plant leaves were irradiated with a saturation light of 800 μmol m^{-2} s^{-1} for 30 min at 25ºC, the Hoagland solution was rapidly replaced by 20% PEG 6000 (dissolved in Hoagland solution) or 20% PEG + GSNO (5 mg L^{-1}, dissolved in Hoagland solution) for osmotic shock treatments. Seedlings in the pure Hoagland solution were taken as a control and their solution was also refreshed at the beginning of the treatment.

Chlorophyll (Chl) fluorescence and gas-exchange measurements, and photosynthetic electron flux calculation: Chl fluorescence and gas exchange were measured simultaneously using a *FMS-2* pulse-modulated fluorometer (*Hansatech*, Norfolk, UK) integrated with a *CIRAS-2* photosynthesis system (*PP Systems*, Hitchin, UK). The fiber-optic of *FMS-2* was integrated into an automatic leaf cuvette of *CIRAS-2* for the simultaneous measurements. The far-red light of *FMS-2* was turned off during the whole measurements except for the last point measurements. The measurements were performed at 380 μmol mol^{-1} CO_2, 800 μmol m^{-2} s^{-1} PFD (saturation light), relative humidity of 70%, and 25ºC. Pure nitrogen gas (99.99%) and air were mixed proportionally to produce 2% O_2, which was used to suppress photorespiration and Mehler reaction (Miyake and Yokota 2000). The gas-exchange measurement was performed under ambient CO_2 (380 μmol mol^{-1}) with low (2%) and ambient O_2 (21%) alternately in the same leaves. Specifically, low O_2 with ambient CO_2 was loaded into a tailor-made plastic bag. Under the ambient O_2 and CO_2, the gas-exchange parameters and effective quantum yield of photosystem II photochemistry (Φ_{PSII}) were recorded. Then the ambient air was changed to the low O_2 air, and about 5 min later,

the steady gas-exchange parameters and Φ_{PSII} were recorded. All the measurements were carried out between 8:00 and 12:00 h under controlled conditions.

Maximal quantum yield of PS II photochemistry (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$, photochemical quenching coefficent (q_P) was calculated as $q_P = (F_m' - F_s)/(F_m' - F_0')$, and effective quantum yield of PSII photochemistry (Φ_{PSII}) was calculated as $\Phi_{PSII} = (F_m' - F_s)/F_m'$ (Genty *et al.* 1989). Total photosynthetic electron flux (Je_{PSII}) was calculated according to $Je_{PSII} = \alpha \times \Phi_{PSII} \times$ PPFD (Harley *et al.* 1992). Velocity of Rubisco oxidation (V_o) was estimated following von Caemmerer and Farquhar (1981). Velocity of Rubisco carboxylation (V_c) was estimated according to Miyake and Yokota (2000). The electron flux used in photosynthetic carbon reduction (Je_{PCR}) was calculated as $Je_{PCR} = 4 \times V_c$, whereas electron flux used in photorespiratory carbon oxidation (Je_{PCO}) was calculated as $Je_{PCO} = 4 \times V_o$ (Krall and Edwards 1992). The alternative electron flux was calculated as $Ja = Je_{PSII} - Je_{PCR} - Je_{PCO}$ (Miyake and Yokota 2000). The electron flux used for Mehler reaction ($Ja_{O2\text{-}dep}$) was calculated as $Ja_{O2\text{-}dep} = Ja\ (21\%O_2) - Ja\ (2\%O_2)$ (Miyake and Yokota 2000) and $Ja_{O2\text{-}indep}$ was calculated as $Ja_{O2\text{-}indep} = Je_{PSII}\ (2\%\ O_2) - Je_{PCR}\ (2\%\ O_2) - Je_{PCO}\ (2\%\ O_2)$ (Miyake and Yokota 2000).

To clarify the limitation factor of the photosynthesis after osmotic stress, the photosynthesis of the leaves with 380 μmol mol^{-1} intercellular CO_2 (C_i) was also determined 150 min after treatment, which was realized by adjusting CO_2 for measurement of photosynthesis *via* the CO_2 control function of the *CIRAS-2* system.

Enzyme extraction and assay: After 240-min treatment, leaf samples were taken from the seedlings and immediately frozen in liquid nitrogen for enzyme activity analysis.

For the extraction of nitrate reductase (NR, EC 1.6.6.1) and glutamine synthetase (GS, EC 6.3.1.2), frozen leaf disks (total area of 2.4 cm^2) were ground with a precooled mortar and pestle in 1.8 ml of extraction medium containing 100 mM phosphate buffer (pH 7.5), 10 mM $MgCl_2$, 2 mM ethylenediaminetetraacetic acid (EDTA), 10 mM dithiothreitol, 1% (v/v) Triton X-100, 1% (w/v) bovine serum albumin (BSA), 10% (v/v) glycerol, and 5% (w/v) insoluble polyvinylpolypyrrolidone (PVPP). For the extraction of superoxide dismutase (SOD, EC 1.15.1.1) and ascorbate peroxidase (APX, EC 1.11.1.11), 100 mM phosphate buffer (pH 7.8) containing 2 mM EDTA and 1% Triton X-100 was used. The homogenate was centrifuged at 13,000 × *g* for 20 min at 4ºC and the supernatant was used for the enzyme assay.

The activity of NR and GS was assayed according to the method of Scheible *et al.* (1997) and González *et al.* (1995), respectively. For NR analysis, 3 ml of the reaction mixture contained 100 mM phosphate buffer (pH 7.5), 10 mM KNO_3, 0.25 mM NADH, and 300 μl of the enzyme extract. Incubated for 30 min at 30°C, the reaction was stopped by adding 1.5 ml of 1% sulfanilamide (dissolved in 3 M HCl), and 1.5 ml of 0.2% 1-naphthylamine. After incubation for 15 min at room temperature, the solution was centrifuged at 13,000 × *g* for 5 min, and the supernatant was used for enzyme analysis at 540 nm. A blank reaction was run without KNO_3. For GS analysis, 1.5 ml of the reaction mixture contained 100 mM phosphate buffer (pH 7.5), 20 mM $MgCl_2$, 10 mM NH_2OH (a mix of 0.2 M $NH_2OH{\cdot}HCl$ with 0.2 M NaOH with equal volume), 30 mM glutamate, 5 mM ATP, and 300 μl of the enzyme extract. The reaction mixture without ATP was used as blank. After incubation at 30ºC for 20 min, the reaction was stopped by adding 2.1 ml of the $FeCl_3$ reagent (2.5% $FeCl_3$, 5% TCA (w/v) in 1.5 M HCl). After centrifugation at 16,000 × *g* for 5 min, the supernatant was used for enzyme analysis at 540 nm.

The activity of SOD and APX was assayed according to the method of Giannopolitis and Ries (1977) and Nakano and Asada (1981), respectively. For SOD analysis, 3 ml of the reaction mixture contained 100 mM phosphate buffer (pH 7.8), 6.5 mM methionine, 50 μM nitroblue tetrazolium (NBT), 20 μM riboflavin, 10 μM EDTA, and 100 μl of the enzyme extract. Absorbance of the reaction mixture was measured at 560 nm. One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm. For APX analysis, 3 ml of the reaction mixture contained 100 mM phosphate buffer (pH 7.6), 0.1 mM EDTA, 0.5 mM ascorbate, 1 mM H_2O_2, and 50 μl of the enzyme extract. The decrease in absorbance at 290 nm was measured.

UV-120 spectrophotometer (*Shimadzu Corp*., Kyoto, Japan) was used for all the measurements.

Determination of leaf relative water content (RWC): RWC was calculated according to the equation RWC [%] = 100 × (FM – DM)/(SM – DM), where FM, DM, and SM were fresh mass, dry mass, and saturated mass, respectively. SM was determined after floating the leaf on distilled water for 24 h at room temperature under 20 μmol m^{-2} s^{-1} PPFD.

Statistical analysis: LSD (least significant difference) was used to analyze differences between the measurements in each treatment by using *SPSS 16* (*SPSS Inc.*, Chicago, USA).

Results

Photoinhibition: Osmotic shock significantly reduced F_v/F_m (Table 1). The ratio of F_v/F_m decreased by 13.5% compared with the control. The addition of external NO alleviated significantly the photoinhibition in leaves of *Rumex* K-1.

Table 1. Fluorescence parameters in leaves of *Rumex* K-1 240 min after osmotic (OS) and osmotic + nitric oxide (OS + NO) shock treatments. F_v/F_m – maximal quantum yield of PSII photochemistry; $1 - q_P$ – measure of closed PSII reaction centers. Each value was mean ± SE (n = 5). Data in the same line followed by the *different letter* are significantly different (p<0.05) between different treatment.

	Control	OS	OS+NO
F_v/F_m	0.823 ± 0.007[a]	0.712 ± 0.023[b]	0.803 ± 0.018[a]
$1-q_P$	0.255 ± 0.014[a]	0.426 ± 0.020[b]	0.281 ± 0.019[a]

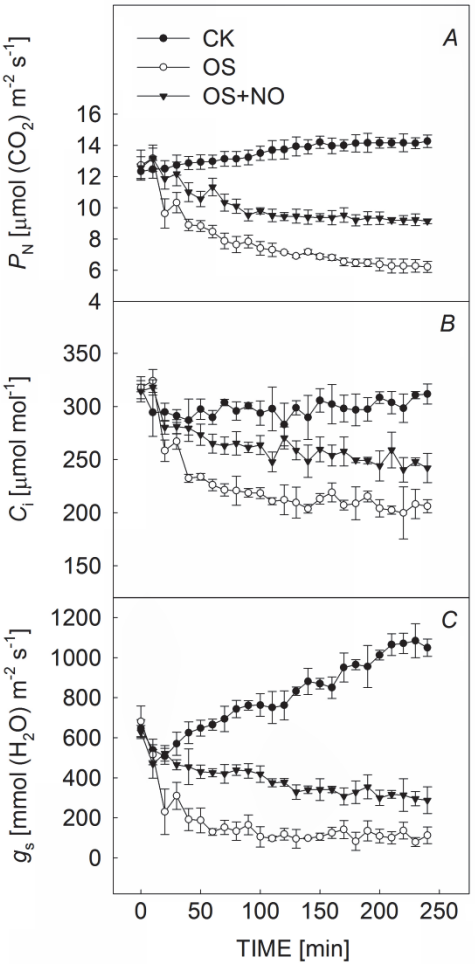

Fig. 1. Time-course of net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) in leaves of *Rumex* K-1 seedlings in response to osmotic (OS) and osmotic + nitric oxide (OS+NO) shock treatments. Each value was mean ± SE (n = 3).

Photosynthetic rate (P_N), C_i, and stomatal conductance (g_s) were significantly reduced by osmotic shock (Fig. 1). P_N decreased sharply and stabilized after 220 min; P_N, g_s, and C_i decreased by about 50%, 90%, and 30%, respectively, after 240 min of the treatment. To clarify the limitation factor of the decrease in photosynthesis caused by osmotic stress, the photosynthesis of the leaves with different C_i was determined. The decrease in P_N caused by the osmotic shock improved significantly by increasing C_i to 380 μmol mol^{-1} (Fig. 2). Under ambient CO_2, P_N in treated leaves was about 44% of that in the control leaves, however, when C_i was enhanced to 380 μmol mol^{-1}, P_N increased to about 84% of that in the control leaves. The above results indicated that the decrease in photosynthesis under osmotic stress was caused mainly by the decrease of g_s. The addition of external NO inhibited markedly the decline in P_N, g_s, and C_i.

Allocation of photosynthetic electron flux: Osmotic shock reduced significantly the electron flux used for Je_{PCR}, while $Ja_{O2\text{-}indep}$, and Je_{PCO} changed only a little; meanwhile, $Ja_{O2\text{-}dep}$ was enhanced significantly (Fig. 3). After 240-min treatment, Je_{PCR} and $Ja_{O2\text{-}indep}$ decreased to about 48% and 40% of those in the control, respectively, and $Ja_{O2\text{-}dep}$ increased to 500% of that in the control. Refreshment of Hoagland solution did not affect the above photosynthetic electron flux in the control, which indicated that the changes in the photosynthetic electron flux in leaves treated with osmotic shock were caused by osmotic shock rather than by the refreshment of the solution. The addition of external NO reversed the effects of osmotic stress on photosynthetic carbon assimilation, Mehler reaction, and energy allocated to O_2-independent alternative pathway. These results indicated that NO was involved in the regulation of photoenergy allocation.

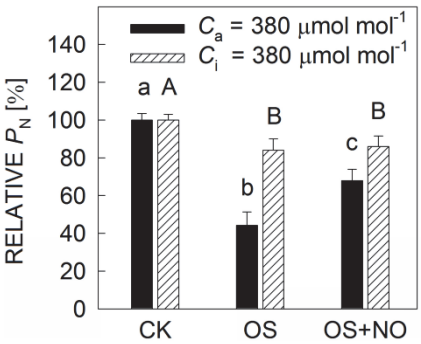

Fig. 2. Relative net photosynthetic rate (P_N) in leaves of *Rumex* K-1 seedlings after treatment with osmotic shock (OS) and osmotic + nitric oxide (OS+NO) for 150 min. Measurements were performed at ambient CO_2 (380 μmol mol^{-1}) and with intercellular CO_2 concentration (C_i) in leaves controlled as 380 μmol mol^{-1}, respectively. The photosynthetic values in control leaves (CK) after 150-min treatment were taken as 100%. Each value was mean ± SE (n = 3).

Changes in enzyme activities: The activities of NR and GS were lowered, but activities of SOD and APX were enhanced by osmotic shock treatment (Fig. 4). After 240 min of the treatment, the activities of NR and GS decreased by 54% and 52%, those of SOD and APX increased by 46% and 44%, respectively. Nevertheless, addition of external NO reversed the influence of PEG shock on the activities of these enzymes (Fig. 4).

RWC: After 240 min of the treatment, RWC decreased by 15%. The addition of NO decreased RWC significantly about 60 min after the treatment; although RWC recovered a little after 120 min, it was still lower than that in the treated seedlings (Fig. 5). The addition of external NO increased significantly the transpiration (data not shown) *via* enhancement of g_s (Fig. 1*C*), which might be the cause of the greater decrease in leaf RWC under osmotic stress.

Discussion

Under osmotic shock, the decrease in P_N was accompanied by a decrease in g_s and C_i (Fig. 1), indicating that the inhibition of photosynthesis was caused mainly by stomatal limitation. Together with the criterion proposed by Farquhar and Sharkey (1982), the fact that increasing C_i in leaves of the osmotically stressed seedlings improved significantly P_N confirmed that the decrease in g_s was the limiting factor for the decrease in P_N caused by the osmotic shock. The addition of external NO increased g_s and C_i under osmotic stress, which resulted in higher electron fluxes allocated to carbon assimilation. It has been found that NO plays a key role in regulation of stomata closure (García-Mata and Lamattina 2001, Li *et al.* 2009). García-Mata and Lamattina (2001) reported that NO induces stomata closure in wheat and enhances the adaptive responses against drought stress. Nevertheless, in *Rumex* K-1 leaves, the reduced g_s caused by osmotic shock recovered markedly due to external NO (Fig. 1*C*). It might be the increase of electron fluxes allocated to photosynthesis that raised g_s in order to get more CO_2 (Fig. 1*B*), because g_s can be feedback regulated by C_i (Wong *et al.* 1979, Farquhar and Sharkey 1982).

Besides CO_2 assimilation, there are other pathways such as nitrate assimilation and Mehler reaction, which consume absorbed light energy in plant leaves (Miyake and Yokota 2000, Chen *et al.* 2004, Zhou *et al.* 2004, Li *et al.* 2007, Johnson *et al.* 2010). These pathways are alternative photosynthetic electron sinks, which are divided into O_2-independent and O_2-dependent sinks (Miyake and Yokota 2000). Miyake and Yokota (2000) proved that O_2-dependent alternative electron sink is Mehler reaction. They also suggested nitrate assimilation and cyclic flow of electrons as candidates of O_2-independent alternative electron sink (Miyake and Yokota 2000, 2001). We observed that the $Ja_{O2\text{-}indep}$ (Fig. 3*C*) changed in a similar way to the changes of the activities of NR and GS (Fig. 4*A*,*B*), which indicated that the $Ja_{O2\text{-}indep}$ was positively correlated to the electron flux used for nitrogen metabolism. Therefore we used $Ja_{O2\text{-}indep}$ (Fig. 3*C*) to reflect the magnitude of electron flux used for nitrogen metabolism. The decreases in O_2-independent alternative electron flux and in activities of NR and GS (Fig. 4*A*,*B*), the key enzymes for nitrate and NH_4^+ assimilation (Caba *et al.* 1995, Martin *et al.* 2006, Nygren *et al.* 2008), indicated that the photosynthetic electron flux allocated to

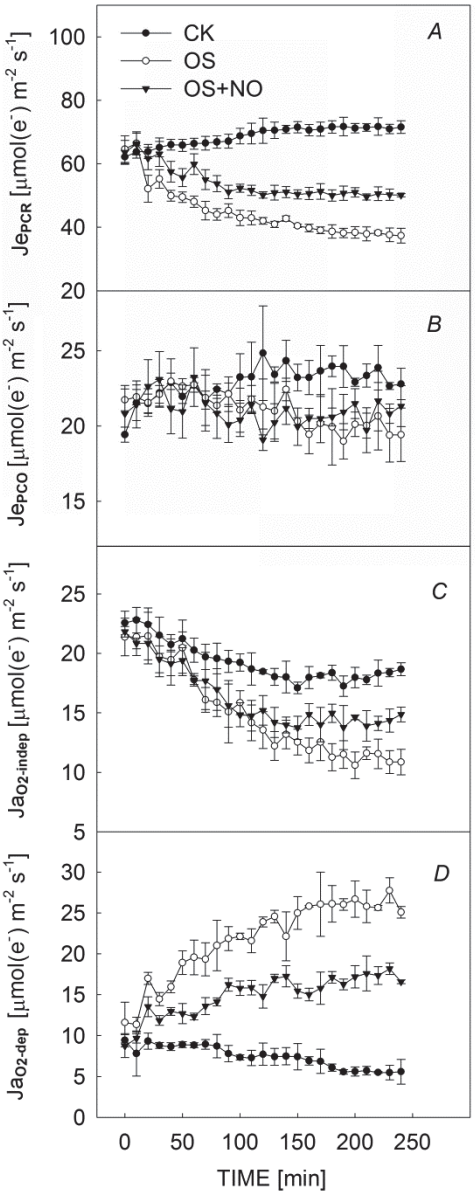

Fig. 3. Time-course of electron flux used in photosynthetic carbon reduction (Je_{PCR}), photorespiratory carbon oxidation (Je_{PCO}), and O_2-independent alternative pathway ($Ja_{O2\text{-}indep}$) or Mehler reaction ($Ja_{O2\text{-}dep}$) in leaves of *Rumex* K-1 seedlings in response to osmotic (OS) and osmotic + nitric oxide (OS+NO) shock treatments. Each value was mean ± SE (n = 3).

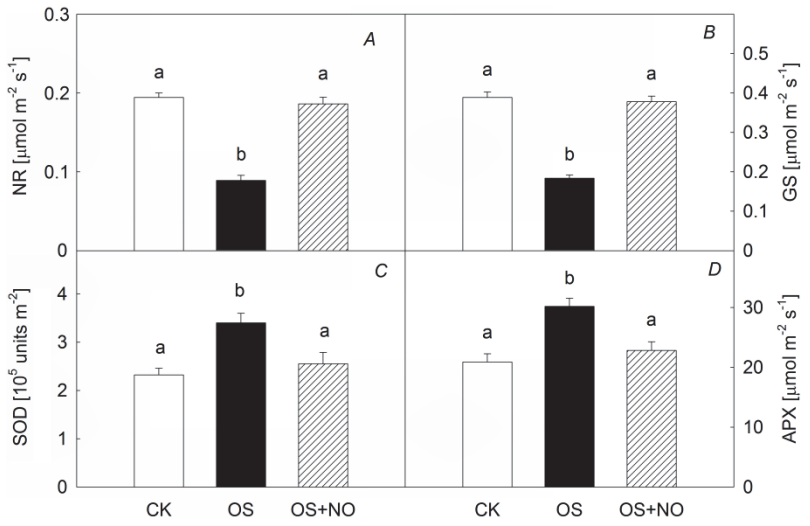

Fig. 4. The activity of nitrate reductase (NR), glutamine synthetase (GS), superoxide dismutase (SOD), and ascorbate peroxidase (APX) in leaves of *Rumex* K-1 without (control) or with 240-min osmotic shock treatments (OS or OS + NO). Each value was mean ± SE (n = 3). *Different letters* above the bar indicate significant difference between different treatments at p<0.05, LSD.

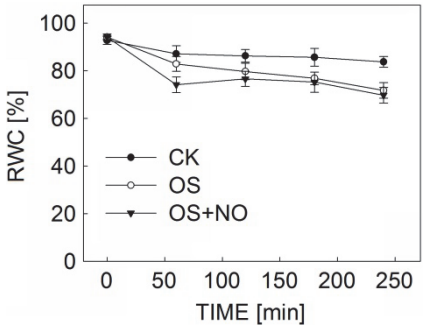

Fig. 5. Time-course of leaf relative water content (RWC) in leaves of *Rumex* K-1 seedlings in response to osmotic (OS) and osmotic + nitric oxide (OS+NO) shock treatments. Each value was mean ± SE (n = 3).

nitrogen metabolism was reduced by osmotic shock. We observed that the photosynthetic electron flux allocated to photorespiration (Fig. 3*B*) decreased a little, while nitrogen metabolism was reduced significantly under osmotic shock (Fig. 4*A*,*B*). Photorespiration stimulates the export of malic acid from chloroplast and regulates the reduction of NO_3^- into NO_2^- through the supply of NADH (Backhausen *et al.* 1998, Igamberdiev *et al.* 2001, Quesada *et al.* 2000). In addition, the amino acids involved in photorespiration, such as glycine, serine, glutamic acid, and glutamine correlate with nitrogen metabolism (Igarashi *et al.* 2006). Besides, GS catalyzes assimilation of NH_4^+ produced in photorespiration (Kozaki and Takeba 1996, Takahashi *et al.* 2007). However, the photorespiration (Fig. 3*B*) and nitrogen metabolism (Fig. 3*C*) changed in different ways under osmotic stress, which indicated a complicated interaction between nitrogen metabolism and photorespiration.

The increase in O_2-dependent alternative electron flux (Fig. 3*D*) suggests that Mehler reaction was enhanced by osmotic shock in *Rumex* K-1 leaves. We think that when the photosynthetic electron flux allocated to CO_2- and nitrogen assimilation decreased, excess of excited energy should inevitably increase. The enhancement of Mehler reaction under this condition could be a beneficial mechanism to dissipate part of the excessive excitation energy. It was argued, however, that Mehler reaction is not a major alternative electron sink for photosynthetic electron flux, when CO_2 assimilation is restricted (Ruuska *et al.* 2000, Driever and Baker 2011). Johnson *et al.* (2010) noticed that the block of electron transfer in a mutant of *Chlamydomonas reinhardtii* lacking Rubisco is compensated partially by an increase in Mehler reaction. In our previous (Chen *et al.* 2004) and other studies (Zhou *et al.* 2004), it was demonstrated that the O_2-dependent alternative electron flux driven by Mehler reaction is upregulated and plays an important role in photoprotection under salt or chilling stress. The function of Mehler reaction under stress may differ in different plants or under different conditions.

Photorespiration is another important mechanism in photoprotection (Barry *et al.* 1997). Takahashi *et al.* (2007) observed that suppression of photorespiration in *Arabidopsis* aggravates photoinhibition. Wingler *et al.* (1999) found that photorespiration increased, when photosynthesis decreased under drought stress in barley. It is interesting to notice that photorespiration in leaves of *Rumex* K-1 decreased a little compared with the control (Fig. 3*B*). However, the proportion of Je_{PCO} to Je_{PCR} increased under osmotic stress. These results indicated that plants maintained photorespiration to alleviate damage of excess energy under osmotic stress.

The observation that addition of external NO alleviated significantly the photoinhibition in *Rumex* K-1 leaves under osmotic shock (Table 1) demonstrated that NO was involved in photoprotection under such conditions. It may be argued that the improvement of RWC might contribute to alleviation of the decrease in photosynthesis with the addition of external NO. In fact, RWC was not improved by the addition of external NO under osmotic shock (Fig. 5), but the photosynthetic rate recovered to 65% of the control. We think that, compared

with the change of RWC, maintaining P_N (Fig. 1*A*) and the activities of NR and GS (Fig. 4*A,B*) with addition of NO, was the key reason to alleviate photoinhibition under osmotic shock.

Although Mehler reaction can reduce excessive excited energy pressure, addition of external NO didn't increase Mehler reaction. In contrast, addition of NO increased photoenergy allocated to photosynthetic carbon and nitrate assimilation (Fig. 3*A*,*C*), consequently, the photoinhibition was alleviated (Table 1). Compared to energy dissipation through Mehler reaction, photosynthetic carbon and nitrate assimilation do not produce damaging reactive oxygen species (ROS) (Asada 1999, Foyer and Noctor 2000). Although ROS can be scavenged by enzymatic and nonenzymatic systems, the best strategy for plants is to decrease the potential of ROS production before they are accumulated excessively.

In general, when CO_2 assimilation was reduced by osmotic shock, more electron flux was allocated to Mehler reaction to dissipate excess of excited energy. The addition of external NO improved significantly g_s and activities of NR and GS under osmotic shock. Consequently, more excited energy was used in carbon- and nitrogen assimilation instead of Mehler reaction to avoid overproduction of damaging ROS *via* Mehler reaction as well as alleviating the photoinhibition. We suppose that Mehler reaction is not stimulated unless photosynthesis and nitrogen metabolism are prominently inhibited. Further research is needed to address the mechanisms by which NO affects g_s and enzyme activities.

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