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Ionic and osmotic effects of salinity on single-leaf photosynthesis in two wheat cultivars with different drought tolerance

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Abstract

The effects of iso-osmotic salinity and drought stresses on leaf net photosynthetic rate (P_N) in two wheat (*Triticum aestivum* L.) cultivars BR 8 and Norin 61, differing in drought tolerance, were compared. In drought-sensitive Norin 61, the decline of P_N was larger than that in drought-tolerant BR 8. Under NaCl treatment, P_N decreased in two phases similarly in both cultivars. In the first phase, photosynthetic depression was gradual without any photochemical changes. In the second phase, photosynthetic depression was rapid and accompanied with a decline of the energy conversion efficiency in photosystem 2 (Φ_{PS2}). Our observations suggest that the osmotic factor may induce a gradual depression of photosynthesis due to stomatal closure under both stress treatments. However, under NaCl treatment, a ionic factor (uptake and accumulation of excess Na^+) may have direct effects on electron transport and cause more severe photosynthetic depression. The drought tolerance mechanism of BR 8 was insufficient to maintain single-leaf photosynthesis under salinity.

Additional key words: chlorophyll fluorescence; cultivar differences; O_2 evolution; photochemical activity; salt tolerance; SPAD; *Triticum*.

Introduction

Salinity is a major environmental factor that limits plant growth and productivity. In wheat, the growth rate and photosynthetic efficiency are generally reduced by salinity even at low salt concentration (Greenway and Munns 1980). In general, salinity involving both osmotic and ionic factors leads to several physiological changes that inhibit the whole plant growth. The changes in photosynthetic parameters could potentially be used as a screening indicator of stress tolerance in plants, because the more tolerant cultivars are expected to exhibit fewer disturbances in the photosynthetic processes (Belkhodja *et al.* 1999). In wheat, the photosynthetic depression under salinity and drought is due to both stomatal and non-stomatal factors (Kingsbury 1982, Gummuluru *et al.* 1989, Renou *et al.* 1990, Xu *et al.* 1990, Kicheva *et al.* 1994, Chernyad'ev and Monakhova 1998, Delfine *et al.* 1998, Reddy *et al.* 1998, Shabala *et al.* 1998, Belkhodja *et al.* 1999). However, photosynthetic depression under drought is mainly caused by the osmotic factor of drought, though the association of both osmotic and ionic factors may lead to several physiological disturbances. Although a similar photosynthesis under the two stress conditions suggests that a common mechanism causes the photosynthetic depression, direct comparison has not been made.

For the breeding of salt-tolerant cultivars, it is important to separate the effects of osmotic and ionic factors on photosynthesis. To evaluate the effect of osmotic factors under two stress conditions, we used two wheat cultivars, which differed in their ability to maintain photosynthesis under drought (Wada *et al.* 1994, Xu and Ishii 1996). The objectives of this study were to analyse, in comparison with the iso-osmotic effect of drought, (*a*) how ionic and osmotic factors of salinity affect leaf photosynthesis, and (*b*) whether the drought tolerance mechanisms in BR 8 can operate for maintaining single-leaf photosynthesis under salinity.

Materials and methods

Plants and treatments: Seeds of common wheat (*Triticum aestivum* L.) cv. Norin 61 were obtained from the Agricultural and Forestry Research Centre, University of Tsukuba and cv. BR 8, accession No. 00027002, from the Ministry of Agriculture, Forestry, and Fisheries Genebank. Seeds were germinated on a filter paper in Petri

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dishes with distilled water for 2 d. Germinated seeds were grown with an aerated modified Kasugaishi nutrient solution (pH 7.0) consisting of: 0.72 mM NH_4NO_3, 0.22 mM KH_2PO_4, 0.58 mM KCl, 0.71 mM $Ca(NO_3)_2$, 0.99 mM $MgSO_4$, 0.08 mM $FeC_6H_5O_7$, 46 μM H_3BO_3, 9.1 μM $MnCl_2$, 0.3 μM $CuSO_4$, 0.5 μM Na_2MoO_4, and 0.8 μM $ZnSO_4$ added to distilled water. At 10 d after sowing, four seedlings were transferred to plastic pots 12 cm in diameter and 15 cm in depth containing the aerated 1 000 cm^3 nutrient solution. All the treatments and measurements were conducted in a growth chamber (*KG-50HLA*, Koito, Japan). Temperature was maintained at 22/20 °C day/night, respectively. Relative humidity was about 70 %. The photoperiod was 12 h and the irradiance at plant level was about PPFD of 500 μmol m^{-2} s^{-1}.

PEG and NaCl treatments were started when the plants were transferred to plastic pots, by the addition of 100 mM NaCl and 12.1 % (m/v) polyethylene glycol (PEG 6000, *Nakarai Chemicals*, Japan) to the nutrient solution, respectively. The water potentials of both the NaCl and PEG treatment solutions were approximately –0.58 MPa. Water loss in each pot due to evapotranspiration was compensated by daily apply of distilled water and all the treatment solutions were exchanged every 4 d. To eliminate the effects of different growth stages and leaf elongation rate between the cultivars, all the measurements of photosynthetic parameters were conducted on the 2nd leaf blade, which had just fully expanded when the stress treatments started.

Gas exchange: P_N and stomatal conductance (g_s) were measured on the 2nd leaf blade of each plant, using a portable photosynthesis system (*LI-6400*, *LI-COR*, USA). All the gas exchange measurements were conducted at 21-24 °C and PPFD of 1 000 μmol m^{-2} s^{-1} on the leaf surface, and air humidity was 40-60 %. The CO_2 response of P_N was determined by decreasing the ambient CO_2 partial pressure (C_a) to 0 μmol mol^{-1} and then increasing it to 800 μmol mol^{-1}. The CO_2 response curves were measured at least 3 times at 4, 8, 10, and 12 d after treatment.

Oxygen evolution rate was measured using an electrode unit for gas-phase measurement of oxygen evolution (*LD-2*, *Hansatech*, England). The leaf blade was detached from the plant and cut into 2-3 pieces of approximately 4 cm^2 leaf area, then immediately placed into the chamber of oxygen electrode. Inside the chamber, irradiance of 900 μmol m^{-2} s^{-1} was provided by a LED light housing (*LH36U*, *Hansatech*, England), and the temperature was controlled to 25 °C. The air, which contained 5 % CO_2 and saturated water vapour, was supplied to the chamber at 2.5 cm^3 s^{-1} for 1 min before each measurement. Then, the chamber was closed and the O_2 evolution rate was recorded for 3 min.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) content: Proteins were separated by SDS-PAGE according to the method of Laemmli (1970). The leaf blade was removed from the sampled plant and cut into a piece of approximately 0.1 g(f.m.). After rapid determination of the exact fresh mass, leaf blades were immediately frozen in liquid N_2 and then stored at –80 °C. The leaf blade was cut into pieces and ground in liquid N_2. The ground and frozen pieces of the leaf blade were dissolved in a sample buffer solution that contained 125 mM Tris-HCl (pH 6.8), 4 % (m/v) SDS, 0.002 % (m/v) bromophenol blue, 20 % (m/v) glycerol, and 10 % (m/v) 2-mercaptoethanol, as 0.1 cm^3 sample buffer per 0.1 g(f.m.) leaf blade. The homogenate was heated at 90 °C for 10 min and centrifuged (17 000×*g*) for 15 min at room temperature. The supernatant was loaded onto a 15 % acrylamide slab gel and electrophoresis was carried out at a constant voltage of 150 mV. After electrophoresis, polypeptides on the gels were detected by staining with 0.1 % Coomassie Brilliant Blue in 50 % methanol and 10 % acetic acid. The intensity of the protein bands on the gel was measured using image analysing software (*NIH Image*, NIH, USA). Determinations of the protein pattern were replicated 4 times and the intensities of the RuBPCO large and small subunits were compared with the intensity of standard markers on the same gel.

Chlorophyll (Chl) *a* fluorescence and SPAD value: Chl *a* fluorescence was measured with a portable Chl fluorometer (*mini-PAM*, *Walz*, Germany) in the middle of the leaf blade under constant artificial irradiance (PPFD 400 μmol m^{-2} s^{-1}). The efficiency of energy conversion in photosystem 2 (PS2) at different PFDs (Φ_{PS2}; Genty *et al.* 1990) was calculated as

$$\Phi_{PS2} = (F_m' - F_s)/F_m'$$

(F_s = stationary level of fluorescence emission, F_m' = maximum fluorescence during irradiation).

SPAD value was determined with a Chl meter (*SPAD-502*, *Minolta*, Japan) in the middle of the leaf blade to estimate the leaf Chl content. Measurements of the SPAD value were conducted 3 times within a leaf blade and an average of 3 values was used.

Growth measurements and ion concentrations: Shoots were harvested and fresh masses were determined every 4 d after the treatment. Samples, dried at 80 °C for 48 h, were weighed and then ground into a fine powder. Appropriate amounts were digested with 1 cm^3 of distilled water, 2 cm^3 sulfuric acid, and 6 cm^3 H_2O_2 (30 %) at 420 °C, and the extracts were diluted to 100 cm^3. Na^+, K^+, Mg^{2+}, and Ca^{2+} concentrations were determined by atomic absorption spectrophotometry (*AA6400*, *Shimadzu*, Japan).

Results

Within 2 d after the onset of the two stress treatments, plants subjected to PEG and NaCl treatments similarly showed a rapid decline and recovery of P_N and g_s (Fig. 1). During the 2- to 12-d period after the PEG treatment, plants showed only a gradual decline of P_N, while the decline of P_N in Norin 61 was larger than that in BR 8. In both cultivars, g_s declined under the PEG treatment. However, no significant differences in g_s were detected between the two cultivars. In contrast, plants subjected to the NaCl treatment showed a decline in P_N in two phases. During the 2- to 6-d period after the NaCl treatment, P_N of the plants decreased gradually in the same way as that of plants subjected to the PEG treatment. However, during the 6- to 12-d period after the NaCl treatment, both cultivars similarly showed a rapid reduction of P_N. And g_s also decreased.

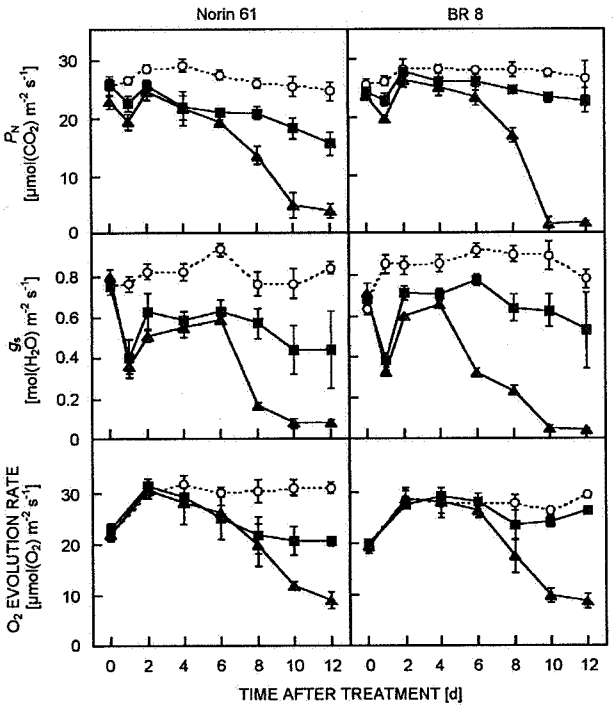

Fig. 1. Changes in net photosynthetic rate (P_N), stomatal conductance (g_s), and O_2 evolution rate of Norin 61 and BR 8 under control (○), PEG (■), and NaCl (▲) treatments. P_N was determined at PPFD of 1 000 μmol m^{-2} s^{-1}. O_2 evolution rate was determined at 5 % ambient CO_2 concentration. Means ± SE ($n = 4$).

During the 8- to 12-d period after the PEG treatment, the O_2 evolution rate (P_{Nmax}) of Norin 61 decreased gradually, though BR 8 did not show such a change (Fig. 1). In both cultivars, the Φ_{PS2} and RuBPCO protein content did not change under the PEG treatment (Figs. 3 and 4). In Norin 61 that was subjected to the PEG treatment, the P_N-C_i (C_i being the intercellular CO_2 concentration) curve showed small changes and the initial slope decreased slightly at the end of the treatment (Fig. 2). However, BR 8 did not show any change in the P_N-C_i

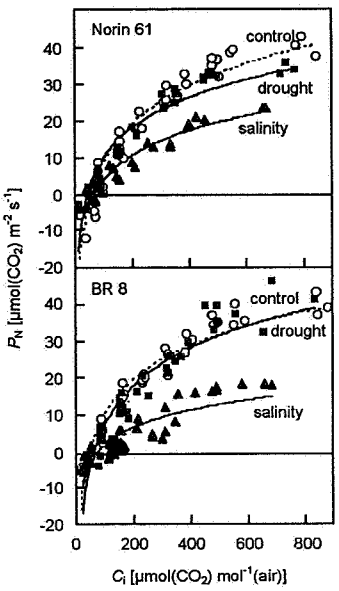

Fig. 2. Relations between net photosynthetic rate (P_N) and intercellular CO_2 concentrations (C_i) in Norin 61 and BR 8 under control (○), PEG (■), and NaCl (▲) treatments at 12 d after the treatments started. Measurements were performed on three individuals for each treatment. Plots show all values of each treatment.

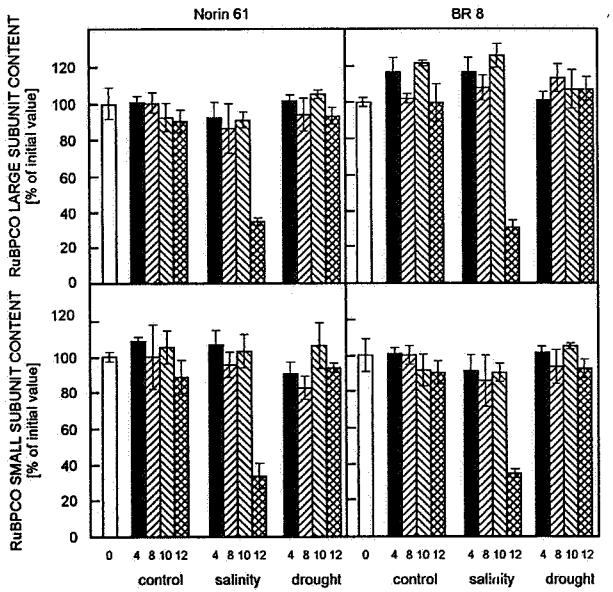

Fig. 3. Changes in contents of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) large and small subunits of Norin 61 and BR 8 under control, PEG, and NaCl treatments. The RuBPCO large and small subunit contents were expressed as % of the initial (0 d) value. Means ± SE ($n = 4$).

curve and initial slope under the PEG treatment. The SPAD value of BR 8 subjected to the PEG treatment was 30 % higher than that of the control throughout the treatment period, though Norin 61 subjected to the PEG treatment did not show such a difference compared to the control (Fig. 4).

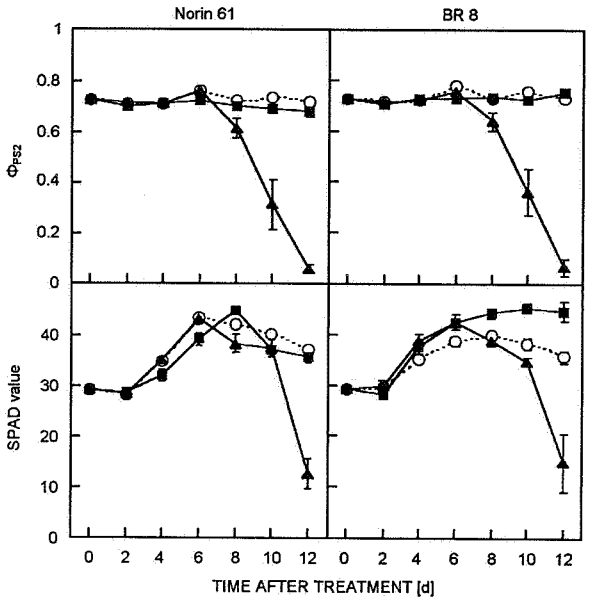

Fig. 4. Changes in the photosystem 2 (PS2) energy conversion efficiency (Φ_{PS2}) and SPAD value of Norin 61 and BR 8 under control (○), PEG (■), and NaCl (▲) treatments. Means ± SE ($n = 5$).

Under the NaCl treatment, the O_2 evolution rate decreased rapidly in both cultivars during the 8- to 12-d period and finally reached a value of 30 % of that in the control (Fig. 1). The Φ_{PS2} of the 2nd leaf also decreased rapidly in both cultivars under the NaCl treatment, though no such change was found in the Φ_{PS2} under the PEG treatment (Fig. 4). The onset of this decline in Φ_{PS2} was observed after 8 d of the NaCl treatment and it corresponded to the decline in the O_2 evolution rate (Figs. 1 and 4). The changes in the P_N-C_i curve were also observed under the NaCl treatment and the RuBPCO content in both large and small subunits was reduced by more than 60 % at the end of the treatment (Figs. 2 and 3). However, these changes were observed only at the end of the NaCl treatment and no difference was found between the two cultivars. The SPAD values of the leaves subjected to the NaCl treatment in both cultivars also showed a rapid decline after 12 d of the NaCl treatment (Fig. 4).

The shoot Na^+ content in both cultivars increased gradually under the NaCl treatment, but the Na^+ uptake rate of BR 8 was slightly higher than that of Norin 61 (Fig. 5). However, the shoot K^+ concentrations in both cultivars did not change under the two stress treatments (Fig. 5). Shoot Ca^{2+} and Mg^{2+} concentrations also failed to show changes in both stress treatments during 12 d (values not shown).

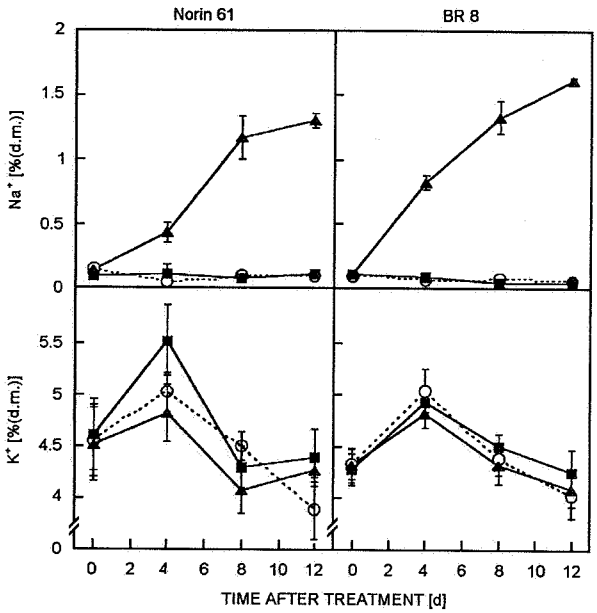

Fig. 5. Changes in the shoot Na^+ and K^+ contents of Norin 61 and BR 8 under control (○), PEG (■), and NaCl (▲) treatments. Means ± SE ($n = 4$).

In the two cultivars subjected to PEG and NaCl treatments, the fresh and dry masses similarly decreased to 45-50 and 50-60 % of the control values, respectively, at the end of treatments (Table 1). Water content in the two wheat cultivars also decreased to 80-90 % of the values in the controls by the two stress treatments (Table 1). In either cultivar, no significant differences in the fresh mass, dry mass, and water content were detected between the two stress treatments.

Discussion

The present study demonstrated that PEG and NaCl treatments influenced leaf photosynthesis differently (Fig. 1). The salinity-induced photosynthetic depression involved a first gradual phase and a second rapid phase, although the PEG treatment only induced a gradual photosynthetic depression. Under salinity, osmotic and ionic factors may cause several physiological changes independently or in association. Thus the effects of these two factors should be analysed separately. To simulate the osmotic effect of salinity, we used a 12.1 % PEG solution that had a similar osmotic potential (–0.58 MPa) as 100 mM NaCl solution.

During first 2-4 d after PEG and NaCl treatments, g_s showed similar fluctuations in both BR 8 and Norin 61

(Fig. 2). Rodríguez *et al.* (1997) reported that maize root subjected to salinity showed a recovery of the osmotic potential due to the accumulation of intracellular organic solutes and inorganic ions within 48 h. Therefore, similar osmotic adjustment by organic solutes or inorganic ions may lead to the recovery of g_s and further photosynthesis after the onset of both stress treatments.

The O_2 evolution rate at high ambient CO_2 concentration enables to estimate the carbon assimilation ability, excluding the effect of stomatal closure (Wakabayashi *et al.* 1996). During the 2- to 6-d period after the PEG and NaCl treatments, plants showed a gradual decline of P_N accompanied by the decline of g_s, while no change in the O_2 evolution rate was detected. Therefore, the gradual photosynthetic depression in this phase may be caused by deficiency of CO_2 supply due to stomatal closure. These observations suggest that the initial event, which leads to the photosynthetic depression after the onset of the NaCl treatment, might be the stomatal closure induced by the osmotic factor of salinity. On the contrary, the rapid phase of photosynthetic depression under the NaCl treatment might have been due to ionic factors of salinity, since it was not observed under PEG treatment. This decline in the O_2 evolution rate, which was observed during the 8- to 12-d period after NaCl treatment, indicates that the rapid photosynthetic depression may be due to both stomatal closure and inactivation of mesophyll factors (Fig. 1).

Table 1. Fresh mass, dry mass, and water content in Norin 61 and BR 8 under stress treatments. Means (n = 4) followed by the same letter in a column are not significantly different at 5 % level by the Tukey-Kramer method.

Cultivar	Treatment	Fresh mass [g plant^{-1}]			Dry mass [g plant^{-1}]			Water content [% of f.m.]		
		4 d	8 d	12 d	4 d	8 d	12 d	4 d	8 d	12 d
Norin 61	control	0.67 a	2.57 a	4.90 a	0.07	0.27 a	0.61 a	89.9 a	89.3 a	87.6 a
	NaCl	0.49 b	1.34 b	2.37 b	0.06	0.19 b	0.36 b	87.5 b	85.7 b	85.0 b
	PEG	0.48 b	1.04 b	2.30 b	0.06	0.14 b	0.33 b	86.8 b	86.8 ab	85.0 b
BR 8	control	0.91 a	3.04 a	5.64 a	0.10	0.36 a	0.75 a	89.1 a	88.2 a	86.8
	NaCl	0.67 b	1.57 b	2.59 b	0.09	0.23 b	0.40 b	86.8 b	85.6 b	84.4
	PEG	0.73 b	1.66 b	2.56 b	0.09	0.22 b	0.40 b	88.0 ab	86.5 b	84.4

The inactivation of mesophyll factors under salinity may be attributed to biochemical changes such as enzymatic inactivity and inhibition of electron transport (Bethke and Drew 1992, Delfine *et al.* 1998, Reddy *et al.* 1998, Belkhodja *et al.* 1999). In our study, the rapid decline in the O_2 evolution rate under the NaCl treatment was also accompanied with some changes of biochemical factors, such as RuBPCO activity, electron transport, and Chl content, although these changes were not found under the PEG treatment (Figs. 1-4). However, the SPAD value decreased only at the end of the NaCl treatment when the electron transport displayed by the Φ_{PS2} had been completely inactivated (Fig. 4). A close relationship between the Chl content and SPAD value was reported in several crop species (Marquard and Tipton 1987, Monje and Bugbee 1992). The analysis of the P_N-C_i curve and RuBPCO protein content also suggested that the decrease of the RuBPCO activity occurs after the decline of the Φ_{PS2} (Figs. 2-4). Delfine *et al.* (1999) also reported that the inactivation of PS2 occurred before the decrease of other photochemical parameters, such as Chl content and RuBPCO activity, in spinach leaves under salinity. Therefore, they concluded that the fraction of open PS2 centres appears to be the most sensitive photochemical component to salt stress and the Φ_{PS2} could be used as a rapid indicator to estimate the photochemical activity in salt-stressed leaves. However, the contribution of osmotic and ionic stress to the decline in Φ_{PS2} under NaCl treatment is still not clear.

The decline in Φ_{PS2} was reported under heat, high irradiance, salinity, and mineral stress (Belkhodja *et al.* 1994, Godde and Hefer 1994, Jimenez *et al.* 1997, Königer *et al.* 1998). However, PS2 is highly drought resistant as found in investigations on the impact of various environmental stresses (drought, heat, strong irradiance), applied separately or in combination (Havaux 1992). Flagella *et al.* (1998) also showed that quantum yield of PS2 is reduced only under drastic water deficit. In this study, the PEG treatment was not sufficient to cause the decline of Φ_{PS2}, though the rapid decline of Φ_{PS2} was detected under iso-osmotic NaCl treatment. These different behaviours under the two stress treatments suggest that the ionic factor of salinity may affect photosynthetic electron transport and cause the decline of Φ_{PS2}.

Allakhverdiev *et al.* (2000a,b) suggested that the accumulation of Na^+ or other ions might lead to the dissociation of extrinsic proteins from PS1 and PS2 complex, causing a decrease in the rates of PS1- and PS2-mediated electron transport in a cyanobacterium. In our study, plants subjected to the NaCl treatment showed the increase of the shoot Na^+ concentration (Fig. 5), though the shoot K^+, Mg^{2+}, and Ca^{2+} concentrations did not change (values not shown). Under the PEG treatment, no changes in the shoot ion concentrations were detected. These findings suggested that, under the NaCl treatment, excess ions such as Na^+ that was taken up and accumulated to the leaves might have induced the decline of Φ_{PS2} and that the inactivated electron transport initially reduced O_2

evolution.

Mozafar and Goodin (1986) reported that a drought-tolerant wheat cultivar showed a greater salt tolerance than a drought-sensitive one during seed germination, suggesting the existence of some relations between drought and salt tolerance. In contrast, no differences between the two cultivars in the ability to maintain photosynthesis under salinity were detected, whereas P_N in drought-tolerant BR 8 was higher than that in drought-sensitive Norin 61 under drought (Fig. 1). This discrepancy in the changes of P_N under the two stress treatments suggests the existence of a subtle relationship between drought and salt tolerance. Under the PEG treatment, the ability to maintain photosynthesis in BR 8 may be attributed to many favourable traits such as higher CO_2 supply, better osmotic adjustment, and rapid detoxification of oxidative damage. However, these traits did not enable drought-tolerant BR 8 to maintain a high photosynthetic activity under the NaCl treatment. The results of the surveys indicate that the ionic effect of salinity was sufficiently intense to inactivate photosynthesis so that the drought tolerance mechanisms of BR 8 were insufficient to maintain P_N of leaves subjected to the NaCl treatment.

As in the case of the decline in leaf area, the decline in single-leaf photosynthesis should reduce the whole plant productivity under salinity, so that the whole plant productivity could be improved by maintaining higher single-leaf photosynthesis. Our study suggests that the ionic factor of salinity is more effective to decrease the photosynthetic activity than the osmotic factor. Thus ion uptake and partitioning traits may be considerably important to maintain the photosynthetic activity under salinity. In fact, no difference in the ion uptake was found between the two wheat cultivars (Fig. 5). Therefore, further investigations should be conducted to determine whether cultivar differences in ion uptake or partitioning traits are related to the ability to maintain photosynthesis under salinity.

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