DOI: 10.1007/s11099-016-0241-7 PHOTOSYNTHETICA 55 (2): 317-328, 2017

Photosynthetic responses of a wheat (Asakaze)–barley (Manas) 7H addition line to salt stress

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Abstract

The photosynthetic responses to salt stress were examined in a wheat (*Triticum aestivum* L. cv. Asakaze) – barley (*Hordeum vulgare* L. cv. Manas) 7H addition line having elevated salt tolerance and compared to the parental wheat genotype. For this purpose, increasing NaCl concentrations up to 300 mM were applied and followed by a 7-day recovery period. Up to moderate salt stress (200 mM NaCl), forcible stomatal closure, parallel with a reduction in the net assimilation rate (P_N), was only observed in wheat, but not in the 7H addition line or barley. Since the photosynthetic electron transport processes of wheat were not affected by NaCl, the impairment in P_N could largely be accounted for the salt-induced decline in stomatal conductance (g_s), accompanied by depressed intercellular CO_2 concentration and carboxylation efficiency. Both, P_N and nonstomatal limitation factors (L_{ns}) were practically unaffected by moderate salt stress in barley and in the 7H addition line due to the sustained g_s, which might be an efficient strategy to maintain the efficient photosynthetic activity and biomass production. At 300 mM NaCl, both P_N and g_s decreased significantly in all the genotypes, but the changes in P_N and L_{ns} in the 7H addition line were more favourable similar to those in wheat. The downregulation of photosynthetic electron transport processes around PSII, accompanied by increases in the quantum yield of regulated energy dissipation and of the donor side limitation of PSI without damage to PSII, was observed in the addition line and barley during severe stress. Incomplete recovery of P_N was observed in the 7H addition line as a result of declined PSII activity probably caused by enhanced cyclic electron flow around PSI. These results suggest that the better photosynthetic tolerance to moderate salt stress of barley can be manifested in the 7H addition line which may be a suitable candidate for improving salt tolerance of wheat.

Additional key words: chlorophyll fluorescence induction; leaf gas exchange; recovery; salt tolerance; wheat-barley addition.

Introduction

Salinity is a problem in many parts of the world, not only on irrigated areas (Pitman and Läuchli 2002), but also on non-irrigated fields, reducing the growth and plant yield production through the shortening of lifetime in leaves. Considering that the maintained photosynthetic activity of leaves even under adverse salt-stress conditions may

Received 25 January 2016, *accepted* 5 May 2016, *published as online-first* 16 May 2016.
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Abbreviations: 7H add – wheat-barley 7H addition line; CEF – cyclic electron flow around PSI; C_i – intercellular CO_2 concentration; F – steady-state fluorescence; F_0, F_m – minimum and maximum Chl fluorescence determined in the dark-adapted state, respectively; F_m' – maximal fluorescence in the light-adapted state; F_v – variable fluorescence; F_v/F_m – maximum quantum yield of PSII photochemistry; g_s – stomatal conductance; L_{ns} – nonstomatal limitation; L_s – stomatal limitation; NPQ – nonphotochemical quenching; P_0 – minimal P700 signal; P_m – maximal P700 level; P_m' – maximal P700 signal in a given light state; P_N – net assimilation rate; P_{Nmax} – maximal assimilation rate; RuBP – ribulose-1,5-bisphosphate; RWC – relative water content; ε – carboxylation efficiency; ϕ_{CEF} – quantum yield of cyclic electron flow around PSI; ϕ_{NA} – quantum yield of the acceptor side limitation of PSI; ϕ_{ND} – quantum yield of the donor side limitation of PSI; ϕ_{NO} – quantum yield of nonregulated energy dissipation; ϕ_{NPQ} – quantum yield of regulated energy dissipation; ϕ_{PSI} – effective quantum yield of photochemical energy conversion in PSI; ϕ_{PSII} – effective quantum yield of photochemical energy conversion in PSII.
Acknowledgements: The authors wish to thank J. Prónay and R. Tarnai for their technical assistance. This work was supported by the Hungarian National Research Fund (K75466 and K112226), TÁMOP (4.2.2A-11/1/KONV-2012-0008), WHEALBI EU project, and by a János Bolyai Research Scholarship from the Hungarian Academy of Sciences (for István Molnár).

contribute to the higher yield production (Munns 2002), hence it is necessary to develop crop varieties capable of sustaining photosynthesis even under saline conditions. Although both wheat and barley are considered to be glycophytes (Sanada *et al.* 1995), barley is regarded as being less sensitive to salt stress than cultivated wheat (Colmer *et al.* 2005, 2006) suggesting that they have different strategies for salt tolerance (Munns *et al.* 2006, Munns and Tester 2008).

Salinization is caused predominantly by NaCl. Under saline conditions water availability decreases causing osmotic stress, but ionic stress also occurs if the ion components of NaCl, especially Na^+ ions, reach a toxic concentration as reviewed by da Silva *et al.* (2011).

Photosynthesis is particularly sensitive to salinity (Sudhir and Murthy 2004, Ashraf and Harris 2013). The limitation of photosynthetic capacity takes place in two stages: (*1*) limitation associated with increased stomatal resistance, known as stomatal limitation (Centritto *et al.* 2003); (*2*) limitation due to nonstomatal disturbance mainly at high salt concentrations (James *et al.* 2002, Centritto *et al.* 2003, Munns *et al.* 2006, Stępień and Kłbus 2006). The regulation of stomatal conductance (g_s) is an important physiological process leading to reduced water loss, and appears to be dominant at intermediate salinity levels (Everard *et al.* 1994). Closed stomata have both positive and negative effects on photosynthesis. Reduced g_s may contribute to maintaining water content through a decreased transpiration rate, which could be favourable for minimizing Na^+ transport towards the shoots (Tester and Davenport 2003). At the same time, closed stomata causes a diffusion barrier resulting in a decrease in CO_2 carboxylation (Flexas *et al.* 2004). On the other hand, the higher g_s may lead to considerable carbon assimilation providing better growth rate and/or improved grain yield (James *et al.* 2008, Rahnama *et al.* 2010). Plants responding to osmotic or ionic stress with relative high g_s are able to maintain their CO_2 assimilation rate (P_N) more successfully compared to plants reacting with low g_s (James *et al.* 2008, Dulai *et al.* 2010, 2011, 2014).

Salt stress has many consequences for nonstoma-dependent processes as well. Salt-induced nonstomatal inhibition (L_{ns}) can be observed when CO_2 assimilation is disturbed by the presence of toxic ions in the mesophyll cells. This limitation may be associated with limited Rubisco activity a reduced amount of Rubisco protein or poor efficiency of PSII during the second stage of salt stress (Muranaka *et al*. 2002, Kalaji *et al.* 2011), when a high concentration of toxic Na^+ and Cl^- ions occurs in the leaves (Munns and Tester 2008). During salt stress, photosynthesis is often hindered by the secondary effect of disturbed ion homeostasis. This often leads to the plant absorbing more light energy than that which can be used by CO_2 fixation, which causes over-reduction of the linear electron transport chain leading to oxidative damage (Asada 2006). This may also contribute to suppressing the repair of PSII, resulting in photoinhibition (Allakhverdiev *et al.* 2002). Under these circumstances the downregulation of PSII by nonradiative energy dissipation is an essential defence mechanism (Qiu *et al.* 2003). It has also been reported that PSII is usually more sensitive to stress conditions than PSI (Apostolova *et al*. 2006). In fact, PSI activity may even be enhanced by salt as observed in cyanobacterium *Spirulina platensis* (Sudhir *et al.* 2005). Moreover, the higher quantum yield of PSI (ϕ_{PSI}) compared to that of PSII (ϕ_{PSII}) may favour the cyclic electron flow (CEF) around PSI. CEF may have a role in maintaining an adequate ΔpH for nonphotochemical quenching (NPQ), which could act as a protective mechanism in the case of both osmotic (Golding and Johnson 2003) and Na^+ ionic stress (Lu *et al.* 2008). The ability to maintain better photosynthesis and consequently achieve higher growth and production/yield is based on these intensive protecting/regulating mechanisms during salt stress.

Recently, several new wheat barley addition lines have been developed using wheat cv. Asakaze and barley cv. Manas in order to increase the allelic variation of wheat (Molnár-Láng *et al.* 2012). It has recently been reported by Darkó *et al.* (2015), that among the added barley chromosomes tested (2H, 3H, 4H, 6H, 7H), the 7H addition line has elevated salt tolerance as compared to the wheat parent, and that the salt tolerance of the 7H addition line is associated with elevated osmotic adjustment capacity, similar to that found in Manas. However, except for a short preliminary study showing that increasing salt concentrations caused a less pronounced decline in net photosynthesis in the wheat (Asakaze)–barley (Manas) 7H addition line (7H add) than in the parental variety Asakaze (Dulai *et al*. 2010), the photosynthetic responses of this line, focusing to the role of photoprotective mechanisms and electron transport processes connected to PSII and PSI under salt treatment have not yet been studied in detail.

The aim of the present study was to clarify the effects of the added 7H barley chromosome on the photosynthetic processes under salt-stress conditions using the 7H add line. For this purpose the salt stress responses of several parameters (gas exchange, chlorophyll *a* fluorescence induction, and P700) were examined and compared to those of the parental genotypes to obtain deeper knowledge on the mechanisms responsible for the salt tolerance of photosynthesis in this line.

Materials and methods

Plant materials and treatments: The seeds of wheat (*Triticum aestivum* L. cv. Asakaze, Japanese facultative), barley (*Hordeum vulgare* L.) cv. Manas (Ukrainian six-row, winter) and the 7H wheat (Asakaze)–barley (Manas) addition line (7H add) required for the experiments were provided by Márta Molnár-Láng, Agricultural Institute of

the Hungarian Academy of Science (Martonvásár). The effects of salt stress were investigated on the 7H add developed from the Asakaze × Manas hybrid (Molnár-Láng *et al*. 2000, 2007, 2012), together with the parental lines.

The seeds were germinated on filter paper moistened with distilled water in Petri dishes for two days. The germinated seeds were grown in half-strength modified Hoagland nutrient solution (Nagy and Galiba 1995) in 1,500-ml pots in growth chambers under normal CO_2 concentration, 75% relative humidity, a light intensity of 200 μmol(photon) m^{-2} s^{-1}, a temperature of 20–25°C, and 12/12 h of light/dark cycle. Salt stress was induced in five-week-old plants by applying increasing (100, 200, and 300 mM) concentration of NaCl (*Sigma*, St. Louis, USA) in seven-day cycles. Measurements were made before the treatment (control), after each seven-day treatment, and after two and seven days of regeneration without NaCl. All the experiments were performed on intact leaves or leaf segments.

Fluorescence *in situ* hybridization: The presence of the added barley chromosome was checked using genomic *in situ* hybridization (GISH) on individual plants of the wheat–barley 7H disomic addition lines used for the physiological experiments (Fig. 1). Root tips collected from germinated seeds were fixed for chromosome preparations as described earlier (Molnár-Láng *et al.* 2000). Total barley genomic DNA was used as a probe and unlabelled wheat genomic DNA was used as blocking DNA. Labelling, *in situ* hybridization and detection were carried out as reported by Molnár-Láng *et al.* (2012). The slides were screened using a *Zeiss Axio Imager M2* fluorescence microscope with the appropriate filter sets. Images were captured with a *Zeiss AxioCam MRm CCD* camera and processed with *Zeiss Axiovision 4.8.2*. software.

Chlorophyll (Chl) fluorescence: The *in vivo* Chl *a* fluorescence was measured in dark-adapted intact leaves using a dual channel P700 and Chl fluorescence measuring system (*Dual PAM-100*, *Walz*, Effeltrich, Germany) with *DUAL-E* and *DUAL-DB* measuring heads containing a PIN photodiode for detection. The initial level of fluorescence (F_0) was detected after 15-min dark adaptation. The maximal fluorescence level of the dark- (F_m) and light-adapted (F_m') leaves were determined by applying saturating flashes [15,000 μmol(photon) m^{-2} s^{-1}] lasting for 0.8 s. Photosynthesis was induced by continuous illumination of the leaf at 221 μmol(photon) m^{-2} s^{-1} for 15 min. The fluorescence parameters were calculated as described by van Kooten and Snel (1990) and Klughammer and Schreiber (2008a) on the basis of the following equations: maximal quantum yield of PSII, $F_v/F_m = (F_m - F_0)/F_m$; effective quantum yield of PSII, $\phi_{PSII} = (F_m' - F)/F_m' = \Delta F/F_m'$; quantum yield of regulated energy dissipation, $\phi_{NPQ} = (F/F_m') - (F/F_m)$; quantum yield of nonregulated energy dissipation, $\phi_{NO} = F/F_m$.

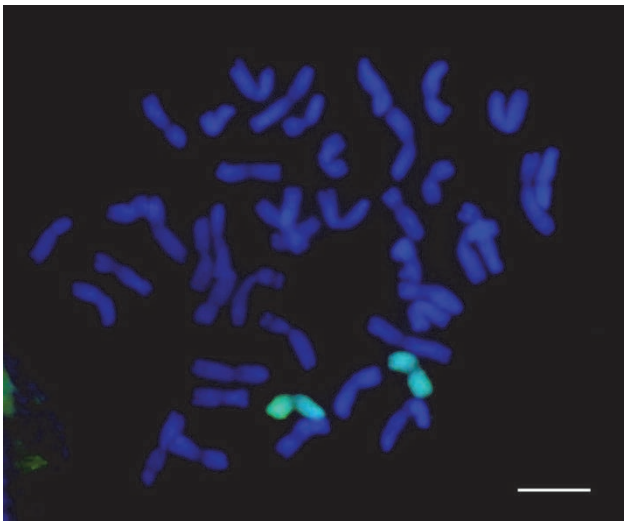

Fig. 1. Detection of the added 7H barley chromosomes in mitotic meristematic cells of an Asakaze–Manas disomic addition line (7H add) using GISH. Barley chromosomes were detected using total barley genomic DNA as a probe (labelled with biotin-16-dUTP and detected with streptavidin-FITC, light gray), wheat chromosomes are dark gray as a result of counterstaining with DAPI. Bar = 10 μm.

P700 measurements: P700 was measured simultaneously with Chl fluorescence *via* changes in absorbance in the near infrared spectrum (difference signal measured at 875–830 nm) as described by Klughammer and Schreiber (1994, 2008b) using the *DUAL-E* and *DUAL-DB* measuring heads equipped with a PIN photodiode and a special pulse preamplifier with maximal time resolution of 30 μs for measuring P700. The complementary PSI quantum yields were calculated on the basis of the following equations: photochemical quantum yield of PSI, $\phi_{PSI} = 1 - (\phi_{ND}) - (\phi_{NA})$; nonphotochemical quantum yield of PSI, related to limitation on the donor side, $\phi_{ND} = 1 - P700_{red.}$; nonphotochemical quantum yield of PSI, related to limitation on the acceptor side, $\phi_{NA} = (P_m - P_m')/P_m$. The yield of the cyclic electron flow around PSI was estimated from the difference between ϕ_{PSI} and ϕ_{PSII}, $\phi_{CEF} = \phi_{PSI} - \phi_{PSII}$ (Huang *et al.* 2010).

Gas exchange: The CO_2 assimilation of intact leaves was measured with an infrared gas analyser (*GFS-3000FL*, *Walz, Effeltrich*, Germany). The net assimilation rate (P_N), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were calculated in the light-saturated state of photosynthesis [1,000 μmol(photon) m^{-2} s^{-1}] using the equations reported by von Caemmerer and Farquhar (1981). The gas-exchange chamber parameters were set at 25°C, 20% of relative humidity. The CO_2 concentration of the reference air was 360 μL L^{-1}. The maximal assimilation rate (P_{Nmax}) was determined at saturating light intensity [1,000 μmol(photon) m^{-2} s^{-1}] and CO_2 concentration (1,200 μL L^{-1}). The response of P_N to changes in

ambient CO_2 concentration was measured between 0–1,200 μL(CO_2) L^{-1} at the above mentioned conditions. P_{Nmax} was determined at light intensity of 1,000 μmol(photon) m^{-2} s^{-1} and 1,200 μL(CO_2)L^{-1}. The stomatal (L_s) and nonstomatal (L_{ns}) limitation were determined on the basis of C_i *vs*. P_N curves, as described by Lawlor (2002). The carboxylation efficiency [ε, mol(CO_2) m^{-2} s^{-1}] was calculated as the initial slope of C_i *vs*. P_N curves according to Pfanz *et al.* (2007).

Determination of relative water content and dry matter production: The water status of the plants was traced by determining the relative water content (RWC) according to the following equation: RWC [%] = [(FM – DM)/(SM – DM)] × 100, where FM is the fresh mass, SM is the water-saturated mass, and DM is the oven dry mass. FM of the leaves was measured, after which they were dried at 105°C for 12 h. To determine the SM, the leaves were incubated in distilled water in a Petri dish for 24 h at room temperature. The shoot and root DM [g per plant] was determined in nine-week-old plants at the end of the whole experimental period and the data were compared with the values for control plants of same age, grown in Hoagland solution without NaCl.

Statistical analysis: All the experiments were repeated three times. Four measurements were performed on each genotypes and treatment for Chl fluorescence and P700 measurements, while five measurements were performed for CO_2 gas-exchange analyses. The RWC content was determined in five replicates of each genotypes and treatments. Biomass production was determined on 16 measurements per treatments.

The results are presented as the means ± standard deviations (SD) of three independent experiments. Differences between treatments or genotypes within each treatment were determined by means of *Tukey*'s post hoc test ($p \leq 0.05$) using the *SPSS 16.0* software (Table 1S, *supplement available online*).

Results

Genomic stability of the 7H Asakaze–Manas disomic addition line: As the wheat-barley addition lines have a certain level of genetic instability leading to the loss of barley chromosomes, it is needed to prove the presence of barley chromosome 7H in the experimental plants. By the use of total barley genomic DNA as a probe for genomic *in situ* hybridization to the mitotic cells of 7H add, a pair of barley chromosome 7H were unambiguously detected. The GISH on the experimental population of the 7H add showed that 100% of the seeds contained the added barley chromosome pair (Fig. 1), so the photosynthetic response to the salt stress was not affected by the lack of barley chromosome in these plants.

Relative water content and gas-exchange parameters: The RWC of the leaves decreased in parallel with increasing salt concentration in all the genotypes (Fig. 2*A*). When 100 mM NaCl was applied, a decrease in RWC was observed in Asakaze and the 7H add line, but further increases in salt concentration only resulted in a mild water loss. In the case of Manas, the decrease in RWC was not statistically significant up to 200 mM NaCl compared with the untreated control, but a great decline was observed at 300 mM. At this stage, the difference between Manas and the other lines was statistically significant. During the regeneration period, the genotypes recovered their water contents completely by the 7th day.

The stomatal conductance (g_s) decreased in all the genotypes (Fig. 2*B*). The highest initial g_s and the strongest stomatal closure were detected in Asakaze. In this genotype, the g_s value was only 46% of the control at 100 mM NaCl, and this reduction in g_s continued as the salt concentration intensified. In contrast to Asakaze, the decrease in g_s was moderate in barley and the 7H add up to 200 mM NaCl, and the value of this parameter was still higher than that in Asakaze even at 300 mM NaCl. Although, the absolute difference in the g_s values in the genotypes was not substantial at severe stress, more than 70% of the original activity was lost in the case of Asakaze, but less than 40% in the 7H add.

Like g_s, the intercellular CO_2 concentration (C_i) decreased continuously in Asakaze, as the salt stress became more severe, but swiftly returned to the control level in parallel with the opening of the stomata during the recovery period (Fig. 2*B*,*C*). C_i was practically unaltered in Manas and decreased in the 7H add up to 200 mM NaCl and then increased significantly in both genotypes when the highest salt concentration was applied.

In respect to net assimilation rate (P_N), there was no significant difference between the untreated genotypes (Fig. 2*D*). Like g_s, P_N decreased in Asakaze in parallel with the salt treatment being significantly lower even at 100 mM NaCl, while in the other lines, the values remained close to the control level up to 200 mM NaCl. In this NaCl range, the CO_2 fixation values were significantly higher in Manas and the 7H add than that in Asakaze. At a salt concentration of 200 mM, P_N was strongly inhibited in the latter genotype leading to the loss of more than 43% of the original activity. At the 300 mM NaCl, P_N decreased more intensively in all the genotypes, and the differences between the genotypes were less pronounced. During the regeneration period, P_N was almost fully restored by the 7th day in Asakaze and Manas, while the value was somewhat lower for 7H add line.

The inhibition of P_N in Asakaze even at a moderate stress suggests that the limitation of CO_2 fixation in wheat might partly occur due to other factors than in Manas and the 7H add. During salt stress, the maximal assimilation

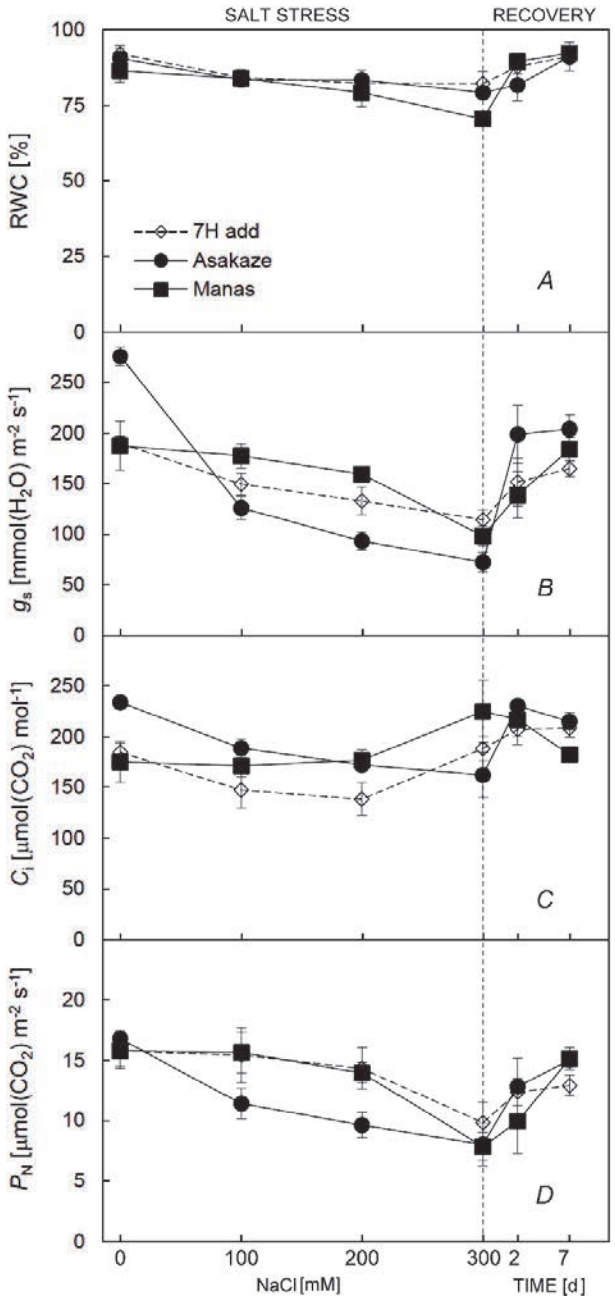

Fig. 2. Effects of increasing NaCl concentration followed by seven days of regeneration on relative water content (RWC) (*A*), stomatal conductance (g_s) (*B*), intercellular CO_2 concentration (C_i) (*C*), net assimilation rate (P_N) (*D*) in 7H wheat-barley addition line (7H add), wheat (Asakaze), and barley (Manas). Each value (± SD) is the mean of the data of five plants per treatment.

rate (P_{Nmax}) determined at saturating-light intensity [1,000 µmol(photon) m^{-2} s^{-1}] and CO_2 concentration (1,200 µL L^{-1}) decreased continuously in Asakaze, while it was fully sustained up to 200 mM NaCl in Manas and the 7H add (Fig. 3*A*). More severe salt treatment (300 mM) resulted in

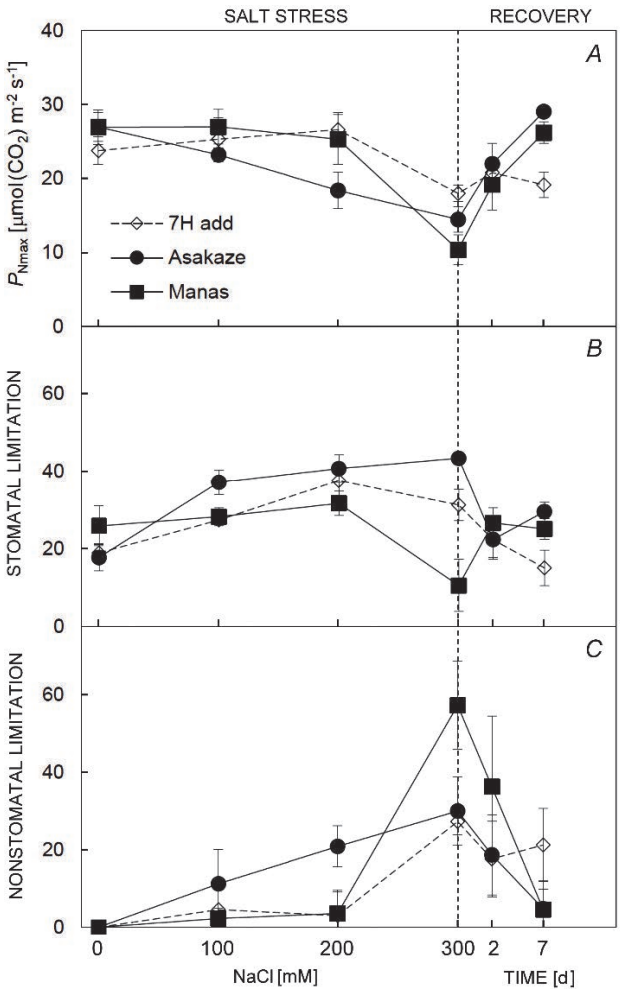

Fig. 3. Effects of increasing NaCl concentrations followed by seven days of regeneration on maximal assimilation rate measured at saturating CO_2 concentration (P_{Nmax}) (*A*), stomatal limitation (*B*), nonstomatal limitation (*C*) in wheat-barley 7H addition line (7H add), wheat (Asakaze), and barley (Manas). Each value (± SD) is the mean of the data of five plants per treatment.

an inhibition of P_{Nmax} in all the genotypes. Parallelly with the decrease in P_{Nmax} and the intensification of salt stress, nonstomatal limitation (L_{ns}) calculated on the basis of C_i *vs*. P_N curves increased continuously in Asakaze, while it was negligible in Manas and the 7H add up to 200 mM NaCl. At severe stress, however, L_{ns} was the highest in Manas, which contrasted strikingly with the other lines. When salt was removed from the medium, L_{ns} dropped to almost zero by the 7th day with the exception of the 7H add. Stomatal limitation (L_s) increased significantly in Asakaze and the 7H add at lower salt concentrations, and remaining almost unchanged in Manas (Fig. 3*B*,*C*). When the stress became more intensive (300 mM NaCl), however, L_s dropped considerably for Manas in parallel with the dramatic rise in the nonstomatal limitation in this genotype.

Table 1. Effects of increasing NaCl concentrations followed by 7 days of regeneration on the carboxylation efficiency [ε, mol(CO_2) $m^{-2}s^{-1}$] in the leaves of Asakaze, Manas, and the 7H addition line (Asakaze – wheat, Manas – barley, 7H add – wheat-barley 7H addition line). ε was calculated as the initial slope of C_i *vs*. P_N curves according to Pfanz *et al.* (2007). Each value (± SD) is the mean of the data of five plants per treatment. The asterisks indicate significant differences between untreated control and treatments within a genotype at $p \leq 0.05$ level. Insignificant differences between the untreated control and treatments are marked by ns. *Different letters* show significant differences from the other lines (a), between Manas and 7H add (b) and between Asakaze and 7H add (c) at $p \leq 0.05$ level within the same treatment.

Genotypes	Control	NaCl [mM]			Recovery [d]	
		100	200	300	2	7
Asakaze	0.091 ± 0.006	0.079 ± 0.007^{ns}	0.068 ± 0.003$^{*, a}$	0.062 ± 0.002*	0.072 ± 0.008*	0.087 ± 0.004$^{ns, c}$
Manas	0.090 ± 0.010	0.092 ± 0.007^{ns}	0.088 ± 0.0025^{ns}	0.045 ± 0.009*	0.056 ± 0.013$^{*, b}$	0.085 ± 0.005^{ns}
7H add	0.084 ± 0.007	0.097 ± 0.008^{ns}	0.097 ± 0.015^{ns}	0.059 ± 0.007*	0.070 ± 0.006$^{ns, b}$	0.068 ± 0.006$^{ns, c}$

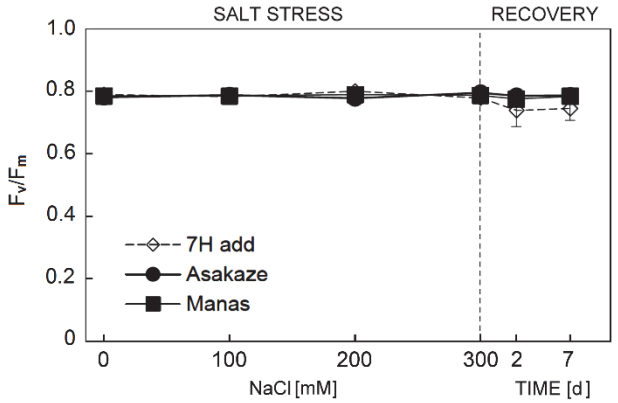

Fig. 4. Effects of increasing NaCl concentrations followed by seven days of regeneration on maximal quantum yield of PSII (F_v/F_m) measured at 221 μmol(photon) m^{-2} s^{-1} of actinic light intensity in wheat-barley 7H addition line (7H add), wheat (Asakaze), and barley (Manas). Each value (± SD) is the mean of the data of four plants per treatment.

The application of 100 and 200 mM NaCl caused no substantial change in the initial slope of the C_i *vs*. P_N curves, representing the maximal carboxylation efficiency (ε), in Manas or the 7H add (Table 1). In Asakaze, on the other hand, there was a substantial reduction in ε by the 200 mM NaCl compared to the initial level and with the other two genotypes. A considerable decrease in ε was observed when the strongest salt treatment was applied both in Manas and the 7H add. At this salt concentration, Asakaze and the 7H add showed almost the same value, while 300 mM NaCl reduced ε to half in Manas. The value of ε recovered in wheat and barley, but the control level was not fully regained by the 7H add even on the 7th day after salt removal.

Chl *a* fluorescence induction and P700 parameters: Chl fluorescence and P700 measurements provide a relatively fast and sensitive method for analysing the functional state of the photosynthetic apparatus. Salt stress resulted practically in no decrease of the values for optimal quantum yield (F_v/F_m) in any of the genotypes, where the values varied between 0.74 and 0.8, irrespective of the treatment (Fig. 4). In untreated plants and under mild (100 mM NaCl) stress conditions, ϕ_{PSII} was lower and ϕ_{NPQ} higher in Asakaze than that in barley and the 7H add, and no further changes in these parameters could be observed in wheat cv. Asakaze at severe stress (Fig. 5*A*,*B*). At the same time, there was a pronounced decrease in ϕ_{PSII} in Manas and the 7H add at severe stress (300 mM NaCl), which differed significantly from both the untreated control and the values recorded for Asakaze. In parallel with the reduction in ϕ_{PSII}, ϕ_{NPQ} increased in Manas and the 7H add, and these parameters only partially recovered during the regeneration phase. By contrast, Asakaze showed a significant increase in ϕ_{PSII} and decrease in ϕ_{NPQ} during a recovery phase (Fig. 5*A*,*B*). However, there were moderate differences only in the nonregulated energy dissipation (ϕ_{NO}) between either the genotypes or the treatments (Fig. 5*C*).

The photochemical quantum yield of PSI (ϕ_{PSI}) did not change significantly during salt stress and recovery in Asakaze, while there was a decrease at 300 mM NaCl in Manas and the 7H add. However, this returned almost to the initial level during the recovery period (Fig. 5*D*). As indicated by the values of ϕ_{ND} and ϕ_{NA}, the nonphotochemical quantum yields of PSI, the donor side limitation of PSI increased considerably in Manas, while the acceptor side limitation decreased at severe stress (Fig. 5*E*,*F*). These changes were slight for Asakaze, while the 7H add line showed moderate changes, representing a level intermediate between the wheat and barley genotypes. The differences between Asakaze and the other genotypes were statistically significant at 300 mM NaCl.

The quantum yield of cyclic electron transport around PS I (ϕ_{CEF}) was significantly higher in Asakaze than that in Manas and the 7H add both in the control and salt treatments up to 200 mM. Salt stress was found to have little effect on ϕ_{CEF} in Asakaze (Fig. 6), while in Manas and the 7H add, ϕ_{CEF} only reached values similar to that of Asakaze at severe stress (300 mM NaCl).

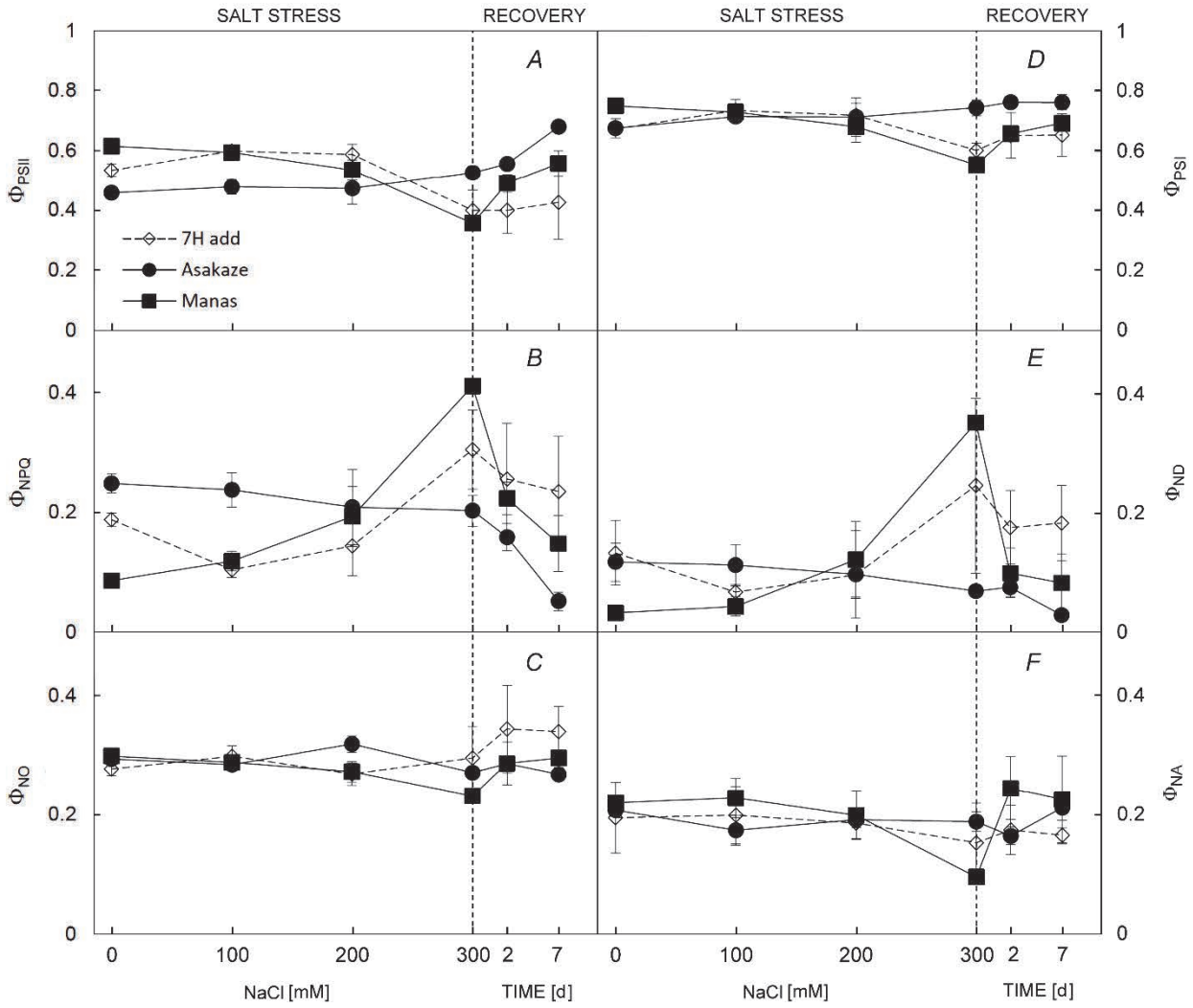

Fig. 5. Effects of increasing NaCl concentrations followed by seven days of regeneration on effective quantum yield of PSII photochemistry (ϕ_{PSII}) (*A*), quantum yield of regulated energy dissipation (ϕ_{NPQ}) (*B*), quantum yield of nonregulated energy dissipation (ϕ_{NO}) (*C*), photochemical quantum yield of PSI photochemistry (ϕ_{PSI}) (*D*), quantum yield of the donor side limitation of PSI (ϕ_{ND}) (*E*), quantum yield of the acceptor side limitation of PSI (ϕ_{NA}) (*F*) measured at 221 μmol(photon) m^{-2} s^{-1} of actinic light intensity in wheat-barley 7H addition line (7H add), wheat (Asakaze) and barley (Manas). Each value (± SD) is the mean of the data of four plants per treatment.

Biomass production: The root DM production in all the genotypes was negatively affected by 300 mM NaCl treatment, especially in the case of Asakaze, where it decreased by 69% (Table 2).

By contrast, the barley and 7H add lost only 42 and 56% of their root DM production in the presence of salinity, respectively. The decrease of shoot DM was also more pronounced in Asakaze than those in Manas and 7H add (Table 2). The salt-induced suppression of root biomass production was reflected in the increment of the shoot/root ratio in all the genotypes, but the most significant change was observed in the Asakaze (data not shown).

Discussion

Both wheat and barley are glycophytic plants; however, barley responds better to salinity, suggesting it could be a good candidate to improve the salt tolerance of wheat (Colmer *et al*. 2005, 2006). It is also known that plant growth and productivity during salt stress correlate well with photosynthetic ability (James *et al.* 2002), which partly depends on the capacity of regulating/protecting mechanisms as reviewed by Chaves *et al.* (2009). In the present study, the photosynthetic performance of an earlier developed 7H add (Molnár-Láng *et al.* 2007, 2014) was investigated under salt-stress conditions in order to reveal how the presumed tolerance of photosynthesis to moderate

salt stress in barley cv. Manas (Dulai *et al.* 2010) is manifested in the genetic background of wheat. In these experiments, deeper knowledge was obtained about the mechanisms responsible for the tolerance of photosynthesis to various levels of salt stress and of the regeneration capacity after salt elimination. Fluorescence *in situ* hybridization demonstrated that besides the whole wheat genome the 7H barley chromosome was present in the 7H add (Fig. 1), the manifestation of salt-tolerance traits was not limited by the lack of the 7H barley chromosome.

Several photosynthetic processes are modified during salt stress. Prior to the accumulation of toxic ions, moderate salt stress has also osmotic effects, influencing the water balance, stomatal behaviour, and net carbon fixation processes of plants (Munns 2002, Munns and Tester 2008). In most cases, stomatal closure can be observed, as indicated by a decrease in g_s (Centritto *et al.* 2003). While RWC decreased moderately in Manas and the 7H add, wheat cv. Asakaze exhibited a dramatic drop

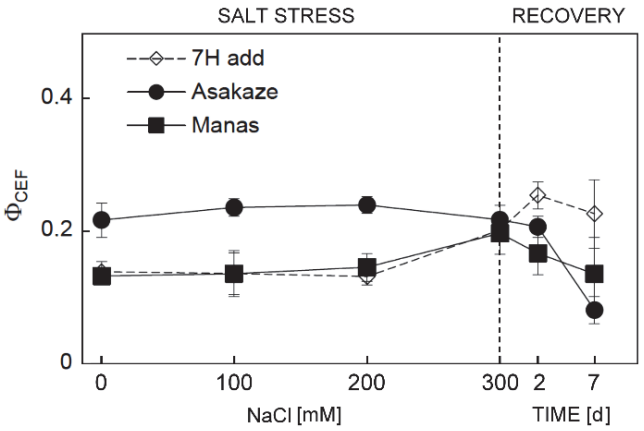

Fig. 6. Effects of increasing NaCl concentrations followed by seven days of regeneration on quantum yield of cyclic electron flow around PSI (Φ_{CEF}) measured at 221 μmol(photon) m^{-2} s^{-1} of actinic light intensity in wheat-barley 7H addition line (7H add), wheat (Asakaze), and barley (Manas). Each value (± SD) is the mean of the data of four plants per treatment.

Table 2. The biomass production of roots and shoots expressed in terms of dry matter for 300 mM NaCl-treated (stress) and control plants of similar age grown in nutrient solution without NaCl (control). (Asakaze – wheat, Manas – barley, 7H add – wheat-barley 7H addition line). *Different letters* indicate statistically significant differences at $p \leq 0.05$, using *Tukey*'s post hoc test.

Genotypes	Root dry mass [g per plant]		Shoot dry mass [g per plant]	
	Control	Stress	Control	Stress
Asakaze	0.899[a]	0.281[d] (31.2%)	1.897[b]	1.085[e] (57.2%)
Manas	0.823[a]	0.479[b] (58.2%)	1.881[b]	1.263[d] (67.1%)
7H add	0.844[a]	0.37[c] (43.8%)	2.191[a]	1.413[c] (64.5%)

in g_s even during mild stress (Fig. 2*A*,*B*). Although stomatal closure is the most efficient way of reducing water loss, allowing water saving and improving water-use efficiency (Chaves *et al*. 2009), Manas and the 7H add were able to avoid drastic water losses, as well as exhibiting only a moderate decrease in g_s. These results showed that Manas and the 7H add were able to maintain their water status at a level similar to that of Asakaze without intense stomatal closure. This suggests that an efficient osmoregulation mechanism exists in these genotypes, as also demonstrated by Darkó *et al.* (2015). Teulat *et al.* (1998) also suggested that the 7H homologous chromosome played a role in osmotic adjustment in barley. These results indicated that barley and the 7H add are better able to adjust osmotic pressure, contributing to efficient water uptake and tolerance of moderate salt stress.

As the stomatal closure not only affects the regulation of water loss, but also restricts CO_2 diffusion into the leaves (Chaves *et al.* 2009), thus influencing mesophyll conductance and photosynthetic CO_2 fixation (Centritto *et al.* 2003), the maintenance of adequate photosynthesis may require relatively high g_s (James *et al.* 2008). In Asakaze, P_N decreased substantially as g_s fell even at a moderate stress level, while it hardly differed from the control in Manas and the 7H add, which responded with less intense stomatal closure. These results showed that barley and the 7H add were able to maintain photosynthesis in parallel with moderate stomatal closure as indicated by the relatively high g_s. These genotypes were able to prevent significant water loss due to their better capacity for osmotic adjustment. It seems that plants responding to mild or moderate salt stress with relatively low stomatal closure, in parallel with improved osmotic adjustment, follow an efficient strategy for sustaining photosynthetic activity. These results are in accordance with earlier results where the photosynthetic rate was reported to be a good tool for discriminating between salt-tolerant and susceptible plants (Belkhodja *et al.* 1999).

Many authors suggest that stomatal and nonstomatal factors may contribute to the inhibition of P_N under salt stress (Centritto *et al.* 2003, Hu *et al.* 2013). In nonstressed but light-saturated C_3 plant, P_N does not reach the maximum level which would otherwise be measurable at saturating CO_2 concentration (P_{Nmax}, Lawlor and Cornic 2002). As long as stomatal limitation is exclusive in the inhibition of P_N, exposure of leaves to saturating CO_2 should be effective in restoring P_{Nmax} in salt-stressed plants. As demonstrated in Fig. 3*A*, P_{Nmax} was fully restored at moderate salt stress in Manas and the 7H add, indicating that the regulation of P_N was definitely affected

by stomatal limitation. Although P_{Nmax} was not fully recovered in Asakaze, the strong stomatal closure and decrease in C_i indicated that stomatal limitation was also responsible to a considerable extent for the inhibition of P_N in Asakaze. This was also manifested in the L_s values calculated on the basis of C_i *vs*. P_N curves (Fig. 3*B*) as described by Lawlor (2002). Since P_{Nmax} is not fully restored by saturating CO_2, P_N must also be influenced by the processes responsible for nonstomatal limitation (L_{ns}). P_{Nmax} decreased and L_{ns} increased in Asakaze in parallel with the severity of salt stress (Fig. 3) even under the moderate treatment, indicating the increased importance of mesophyllic or metabolic factors in the restriction of photosynthesis, which were negligible for Manas and the 7H add. Consequently, the susceptibility of photosynthesis to salt was more pronounced in this wheat genotype at moderate stress level than that in barley or the 7H add, where the dominant role of stomatal limitation was observed. Very similar results were obtained when the sensitivity of photosynthetic CO_2 fixation to stress was estimated as the ability to restore the maximal assimilation rate or by calculating limitations on the basis of C_i *vs*. P_N curves (Lawlor 2002).

There are thought to be several biochemical (metabolic) and diffusional factors in the background of nonstomatal limitation (Lawlor and Cornic 2002, Chaves *et al.* 2009, Flexas *et al.* 2004). In the present case, the rapid decline in g_s caused in Asakaze by the initial salinity resulted in a decrease in CO_2 availability for carboxylation. Thus, it may be that the rapid, substantial increase in stomatal resistance in response to the initial osmotic shock caused by NaCl led indirectly to the nonstomatal limitation observed at 200 mM NaCl *via* a gradual reduction in ε (Table 1). As reported by Downton *et al.* (1988) conclusions drawn on the basis of C_i may be uncertain due to the patchy stomatal closure (Buckley *et al.* 1997) and the estimation of C_i can be biased also by the cuticular transpiration (Boyer *et al.* 1997). However, since the decrease in C_i was not proportional to the strong drop in g_s (Fig. 2*B*,*C*), possibly a mesophyllic diffusion barrier or an alternative electron sink might operate in Asakaze in the early stages of stress, influencing the CO_2 concentration in the intercellular spaces. It was suggested by Kozaki and Takeba (1996) and Chaves *et al.* (2009), that photorespiration might be also involved in the protection of the photosynthetic apparatus when intense stomatal closure restricts CO_2 diffusion into the leaves. In this case, protective mechanisms may be important even at normal light intensity.

When salt stress becomes severe and CO_2 assimilation is significantly disturbed, the role of nonstomatal factors in the limitation of photosynthesis usually becomes more pronounced (Brugnoli and Lauteri 1991; Qin *et al.* 2010). As it can be seen in Fig. 2*D*, P_N was significantly inhibited at 300 mM NaCl in all the genotypes. The significant increase in C_i also indicated the importance of nonstomatal factors (Qin *et al.* 2010) in Manas and to some extent in the 7H add. L_{ns} was substantially higher in Manas than those in Asakaze or the 7H add. It is interesting to note, that the salt-induced changes in nonstomatal limitation in the 7H add were similar to those recorded for Manas at moderate salinity levels (up to 200 mM NaCl), but resembled those in Asakaze in the case of severe salt stress (at 300 mM NaCl). Consequently, the overall photosynthetic performance of 7H add appeared to exhibit the better traits of the parental genotypes under both moderate and severe stress, although this is not true of all the processes involved in photosynthesis.

Photochemical and electron transport processes may also affect photosynthetic CO_2 fixation during salt stress both in wheat and barley, but the contribution of these processes to the limitation of CO_2 assimilation usually depends on the duration/intensity of the salt treatment (Kalaji *et al.* 2011). In the present work, the level of salt stress increased slowly and gradually. The optimal quantum yield (F_v/F_m) and the quantum yield of nonregulated energy dissipation (ϕ_{NO}) were practically unaffected by salt stress even at 300 mM NaCl (Figs. 4, 5*C*). These results suggest that salinity had no noticeable effect on the capacity of primary charge separation, and no PSII damage was observed in the range of treatment applied as also reported by Hanachi *et al*. (2014). As shown by the slight changes observed in ϕ_{PSII} values, electron transport processes were also unlimited in all the genotypes up to 200 mM NaCl. It is unlikely that the salt sensitivity of PSII or the salt-induced downregulation of electron transport processes was the main reason for the decrease in the assimilation rate or for the nonstomatal limitation observed in Asakaze. This is supported by the fact that ϕ_{PSII} did not decrease further under severe stress in this genotype. Since ϕ_{PSII} was lower in Asakaze than in Manas or the 7H add in untreated plants and at mild salt stress, parallelly with higher ϕ_{NPQ}, it is likely that the PSII activity was originally slightly downregulated by unknown mechanisms in Asakaze as compared to Manas and the 7H add. Although, further investigations are required to reveal the reason for this phenomenon, it is evident that the difference in fluorescence parameters observed between Asakaze and Manas was not a consequence of the salt treatment. In the case of barley and the 7H add, severe salt stress caused a significant decrease in ϕ_{PSII} (more than 40 and 25% of the original activity was lost, respectively), indicating that electron transport processes were downregulated in these genotypes. Parallelly with this, photoprotective mechanisms were more intensely accelerated in these lines than in Asakaze, as indicated by the ϕ_{NPQ} and ϕ_{ND} values (Fig. 5*B*,*E*). These processes compete with primary photochemistry for the absorbed excitation energy, leading to a decrease in ϕ_{PSII} (Genty *et al.* 1989) and an increase in nonradiative energy dissipation in the LHC (Horton and Ruban 2005, Chaves *et al.* 2009). Considering that the acceptor-side limitation did not increase, as reflected in ϕ_{NA}, while L_{ns} increased substantially (Figs. 3*C*, 5*F*), the downregulation of PSII-driven electron transport might be

partly responsible for the nonstomatal limitation of photosynthesis in Manas and the 7H add at severe salt stress.

In parallel with linear electron transport, electrons may also follow a cyclic route, driven solely by PSI and known as cyclic electron flow (CEF), which generate ΔpH across the thylakoid membranes leading to the formation of ATP but not NADPH, thus preventing the over-reduction of the acceptor side of PSI. CEF is considered to be essential for efficient photosynthesis even if plants are grown in under stress-free conditions (Munekage *et al*. 2004). Several studies have shown that it may be stimulated by water deficit (Golding and Johnson 2003, Dulai *et al.* 2014) or salt stress (Lu *et al*. 2008). CEF is also thought to support the regulation of light-harvesting processes *via* the enhancement of NPQ, thereby contributing to the protection of PSII (Golding and Johnson 2003). The higher values of ϕ_{NPQ} and ϕ_{CEF} (Figs. 5*B*, 6) in the leaves of Manas and the 7H add compared with the control at severe stress suggest that CEF may help to prevent the over-reduction of the electron transport chain and subsequent oxidative damage. This was confirmed by the fact that the acceptor-side limitation of PSI, represented by ϕ_{NA}, did not increase at this stage of stress. Interestingly, the values of ϕ_{NPQ} and the activity of CEF were originally higher in Asakaze than those in Manas and the 7H add, and were hardly influenced by salt treatment.

When the genotypes were compared, both CO_2 assimilation and photosynthetic electron transport processes showed a similar tendency in barley and the 7H add, but differed in wheat. In the former genotypes, the slight decrease of CO_2 assimilation and stomata closure was accompanied with slight changes of NPQ under mild and moderate salt stress induced by 100 or 200 mM NaCl. In the case of Asakaze, the fluorescence induction parameters were practically unaffected by salt stress, but the assimilation rate decreased significantly even at mild (100 mM) salinity. At severe (300 mM) salt stress, CO_2 fixation and NPQ changed significantly in Manas and the 7H add, so the CO_2 assimilation rate and g_s parameters became similar to that of Asakaze.

The ability to recover from stress-induced down-regulation or injury may depend both on the level of stress-induced damage (Chaves *et al.* 2009) and on the sensitivity of the plants. When photosynthesis is mainly limited by stomatal factors, CO_2 fixation may recover to the normal level relatively rapidly after the elimination of the stress *via* the restoration of g_s, as observed in the case of Asakaze in the present experiments, where 76% of the original activity was restored by the second day. By contrast, when L_{ns} is the dominant factor and key photosynthetic processes are affected, the regeneration capacity of CO_2 fixation may slow down, as found in Manas, where the greatest extent of L_{ns} was recorded. However, complete regeneration was recorded in Manas by the 7th day. In the case of 7H add, slow regeneration was observed throughout the regeneration period. In agreement with these results, Kirschbaum (1988) also demonstrated biphasic recovery from severe water stress. It has been suggested that the maintenance of photoprotective mechanisms is responsible for the slow or incomplete recovery of CO_2 assimilation (Chaves *et al.* 2009). It should also be mentioned that the 7H add started to head during the third week of the salt treatment and showed moderate leaf senescence. This earliness, possibly induced by salt stress, might also have retarded the recovery process and may be related to the relatively moderate stomatal closure and osmotic adjustment, as indicated by González *et al*. (1999).

The sensitivity of photosynthetic capacity to moderate salt stress was manifested as a considerable reduction in dry matter production in Asakaze, where the root biomass production in particular was strongly inhibited (Table 2). Although, the relationship between the net photosynthesis of leaves and growth or biomass production is not simple and often could be indirect (Flood *et al*. 2011), the more promising dry matter production for Manas and 7H add suggested better tolerance to moderate salinity of the latter genotypes.

In conclusion, the results proved that the 7H add was able to maintain its photosynthetic rate under moderate salt stress. This line seemed to respond to moderate salt stress with low stomatal closure which may result in an efficient strategy for sustaining photosynthetic activity based on osmotic adjustment, as found by Darkó *et al.* (2015). As the better tolerance of photosynthesis to moderate salt stress exhibited by the barley parent cv. Manas appeared to be manifested in the 7H add, it is a good candidate for improving the tolerance of bread wheat to salt stress.

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