

Salt response of photosynthetic electron transport system in wheat cultivars with contrasting tolerance

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ABSTRACT

Soil salinity significantly decreases the photosynthetic efficiency and plant growth in wheat (*Triticum aestivum* L.). However, sensitivity of the photosynthetic electron transport system of wheat in relation with salt stress is unclear. Two wheat cultivars with contrasting salt tolerance were exposed to soil salinity, and the physiological responses and performance of photosynthetic electron system were investigated. The depressed photosynthetic carbon assimilation was mainly caused by stomatal closure and lower photosynthetic electron transport efficiency. Under salt stress, the salt-resistant cv. YN19 had higher efficiency in photosynthetic electron transport, hence maintaining higher photosynthetic rate under salt stress, compared with the salt-sensitive cv. JM22. In addition, the parameters derived from fast chlorophyll *a* fluorescence induction curve, i.e. the quantum yield for electron transport (Φ_{E_0}) and the probability that an electron moves further than QA (Ψ_{E_0}), can be used as indicators for rapid screening of wheat cultivars tolerant to soil salinity.

Keywords: soil salinization; water uptake; gas exchange; photosynthesis; damage to photosynthetic apparatus

Soil salinization is a growing problem in agriculture causing a decrease of land area for cultivation and decline in crop yield (Deinlein et al. 2014, Janda et al. 2016). Soil salinity reduces the ability of water uptake of crop plants, due to increased amounts of Na⁺ and Cl⁻ in roots (Deinlein et al. 2014). The Na⁺ and Cl⁻ impair metabolic processes and cause damage to photosynthetic apparatus, thus declining the photosynthetic efficiency and plant growth (Flowers and Yeo 1995).

Photosynthesis converts light energy to ATP and redox equivalents, which is the primary metabolic sink for plant growth (Brestič et al. 2012, 2014,

2015). Excess salts rapidly affect the photosynthesis by direct and indirect effects (Munns and Tester 2008). For instance, excess salts cause the modulation of photosynthetic proteins in chloroplasts, which directly affects functioning of photosystem (PS) (Zhao et al. 2013). Salt stress also induces a rapid stomatal closure, which results in an imbalance between light capture and energy utilization, leading to disturbances in all photosynthetic processes (Silveira and Carvalho 2016). Light energy is trapped by the antenna of PS I and PS II to drive the charge separation in the reaction centres (RCs) (Li et al. 2014, 2016). This

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process is disturbed by salt stress, as the chlorophyll antenna complexes trap more energy than the capacity of biochemical procession in RCs, resulting to over-energized status in the thylakoid membranes (Ensminger et al. 2006).

Higher plants, such as wheat (*Triticum aestivum* L.), have developed several survival mechanisms to improve their tolerance to salt stress (Janda et al. 2016). High salt-resistant cultivars can maintain a better water status and reduce the effects of salt-induced osmotic stress by control of ion uptake by roots and their transport to photosynthetic tissues in leaves (Parida and Das 2005). The adjustment of photosynthetic processes is also a protective mechanism enabling the plants to tolerate salt stress (Parida and Das 2005). However, the adjustment of photosynthetic processes in wheat to salt stress is rarely known. It has documented that the damage caused by salt stress is more prominent at the donor side rather than the acceptor side of PS II (Mehta et al. 2010). However, it is still unclear whether the sensitivity of photosynthetic electron transport system of wheat plants to salt stress is related to their salt tolerance.

In the present study, two contrasting wheat cultivars, i.e. JM22 (salt-sensitive) and YN19 (salt-resistant) were exposed to salt stress, and the physiological responses and performance of photosynthetic electron system were investigated. The hypotheses were that: (1) the sensitivity of photosynthetic electron transport system in contrasting wheat cultivars is different under salt stress; and (2) the sensitivity of photosynthetic electron transport system of wheat plants to salt stress is related to their salt tolerance.

MATERIAL AND METHODS

Experimental setup. A pot experiment was conducted in a climatic chamber at the Lianyungang Experimental Station April–July 2012. Two wheat cultivars were used in this study, i.e. a salt susceptible cv. JM22 and a salt tolerant cv. YN19. Previous stress trials conducted at the station indicated that these cultivars varied in salt tolerance. Selected seeds of wheat were grown in plastic pots (22 cm in high and 25 cm in diameter), which were filled with 7.5 kg of clay soil. The soil contained 1.1 g/kg total N, 32.4 mg/kg P and 132.4 mg/kg K. Before filling the pots, the soil was pre-mixed

with 1.2 g N, 0.16 g P and 0.75 g K per pot. Eight seeds were sown in each pot, and four seedlings were retained after thinning at the 3-leaf stage. At 5-leaf stage, half of the pots were irrigated with a solution containing 200 mmol NaCl for two weeks and used as a salt treatment. The remaining pots were irrigated daily with water as control. The experiment was a randomized block design, with three replicates for each treatment. Each replication consisted of 4 pots.

Physiological index determination. After the salt treatment, plant height and dry weight were recorded. The RWC was determined according to the protocol of Jensen et al. (2000). Just after the salt treatment, photosynthetic rate (P_n) and stomatal conductance (g_s) of the last fully expanded leaves were taken for gas exchange measurement with a portable photosynthesis system (LI-6400, LI-Cor, Lincoln, USA) at a CO_2 concentration of 380 $\mu\text{mol/mol}$ and photosynthetically active radiation of 1200 $\mu\text{mol/m}^2/\text{s}$. Using the same leaves as for gas exchange measurement, relative chlorophyll content was measured non-destructively with a SPAD 502 chlorophyll meter (Soil Plant Analysis Development; Minolta, Japan). Total leaf area was measured with a leaf area meter (LI-3100, Li-Cor Inc., Lincoln, USA). Images of stomatal morphology in the same leaf as for gas exchange analysis were processed with a Dino-Lite digital microscope (AM411 series with Version 1.4.1, Vidy Precision Equipment Co. Ltd, Wuxi, China) without damage of the leaf. The guard cell length and guard cell pair width were measured. The image of calibration sample was also saved to calculate the actual value using AxioVision SE64 software (Rel. 4.4.3, Carl Zeiss Microscopy, Köln, Germany).

Chlorophyll a fluorescence. The fast chlorophyll a fluorescence induction curve was measured using a Plant Efficiency Analyzer (Pocket-PEA; Hansatech, Norfolk, UK). Before measuring, plants were dark adapted for 0.5 h. The collected data were processed by the programme PEA Plus 1.04, and Biolyzer 3.0 software (Bioenergetics Lab., Geneva, Switzerland, http://www.fluoromatics.com/biolyzer_software-1.php) was used to calculate the fast chlorophyll a fluorescence induction (OJIP) test parameters. These measurements were carried out just after the end of the salt treatment.

Statistical analysis. All data were firstly tested for homogeneity of variance with boxplot and then subjected to two-way ANOVA to determine the

significant differences between treatments and cultivars using the software of SigmaSATA, V3.5, Systat Software, San Jose, USA).

RESULTS AND DISCUSSION

The plant height and dry weight were significantly decreased in saline (200 mmol NaCl) as compared to non-saline (0 mmol NaCl) treatment (Figure 1). However, the salt-induced decrease in plant growth was larger in the susceptible cultivar (JM22) compared with the tolerant cultivar (YN19). Salt stress induced various physiological and metabolic modifications and eventually decreased plant growth in wheat, which results in decreased grain yield (Akram et al. 2002, Mathur

et al. 2013). Initially, plant growth is depressed by salt-induced osmotic stress and followed by ion toxicity (Rahnama et al. 2010). In addition, osmotic stress reduces water absorption capacity of roots and affects leaf water status in wheat (Rahnama et al. 2010). In accordance with this, significantly lower relative water content (RWC) was found in both cultivars exposed to salt stress (Figure 1). However, cv. YN19 had relatively higher RWC than cv. JM22 when exposed to salt stress. This indicated that cv. YN19 can maintain a better water status compared with cv. JM22 under soil salinity. It was noted that chlorophyll content (SPAD) decreased in cvs. JM22 and YN19 under saline stress. No significant difference in SPAD was found between cvs. YN19 and JM22 under saline stress. It was suggested that the chlorophyll content is not a sensitive indicator of salinity tolerance (Ashraf 1989).

Photosynthesis is a process highly sensitive to any changes in environmental conditions; it is essential to keep the balance between light energy absorbed by photosystems and energy consumed by metabolic sinks (Ensminger et al. 2006, Brestič et al. 2015). In the present study, P_n and g_s were significantly reduced by salt stress in both cultivars (Figure 2). However, higher P_n and g_s were found in cv. YN19 as compared to cv. JM22 under salt stress. It has been well documented that stress-induced inhibition of photosynthesis is attributed to stomatal and non-stomatal limitations (Farquhar and

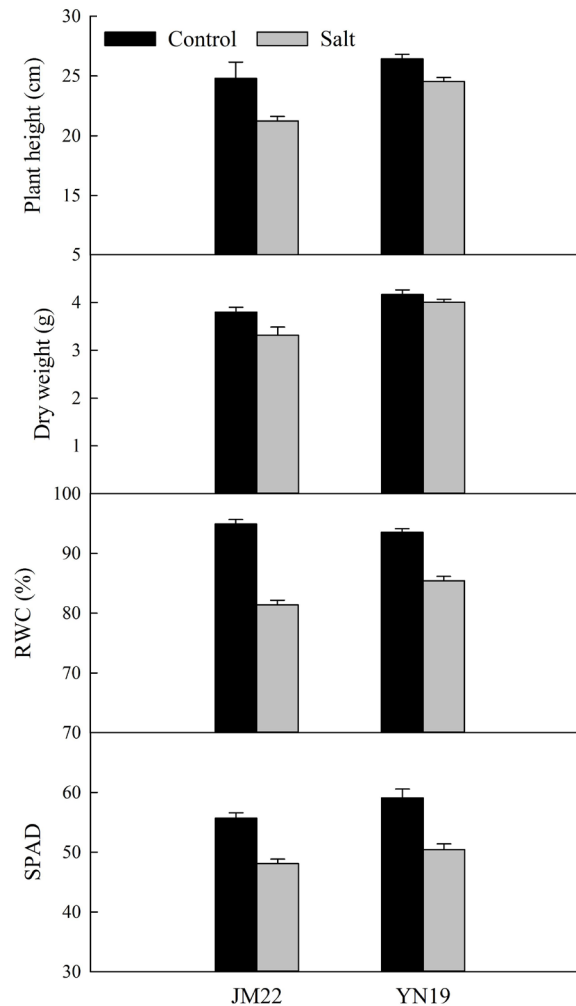


Figure 1. Effect of salt stress on plant height, dry weight, leaf relative water content (RWC) and chlorophyll content (SPAD) in wheat. Mean values \pm standard error are shown ($n = 3$)

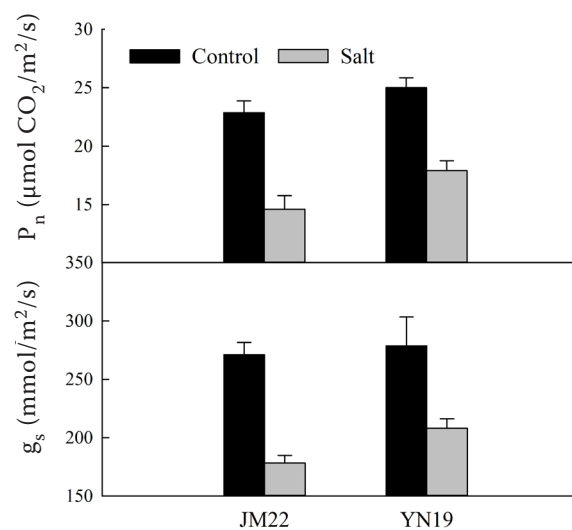


Figure 2. Effect of salt stress on net photosynthetic rate (P_n) and stomatal conductance (g_s) in wheat. Mean values \pm standard error are shown ($n = 3$)

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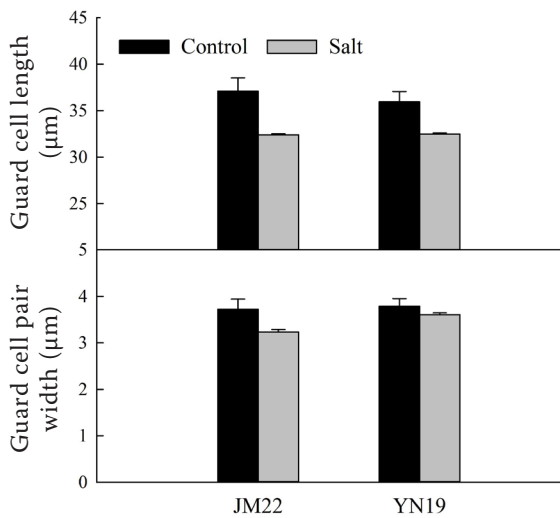


Figure 3. Effect of salt stress on guard cell length and guard cell pair width in wheat. Mean values \pm standard error are shown ($n = 3$)

Sharkey 1982). Here, relatively higher P_n , together with relatively higher g_s , in salt-resistant cultivar (YN19) suggested that salt-induced reduction in photosynthesis was at least partly due to stomatal limitation. In agreement with our results, Diao et al. (2014) documented that the reduction of photosynthesis in salt-stressed tomato plants was partly caused by stomatal closure. In addition, higher guard cell pair width was found in cv. YN19 compared with cv. JM22 under salt stress, while there was no significant difference in guard cell length between cv. YN19 and cv. JM22 (Figure 3). The plasticity in stomatal size in tomato leaves is reported to be sensitive to high temperature and drought stress (MacDonald et al. 2010). In the present study, the relatively lower reduction in guard cell pair width in cv. YN19 in relation to cv. JM22 under salt stress indicated that the width of guard cell might be considered as an indicator for salt tolerance in wheat breeding.

The stomatal closure and changes in plant water status caused by salt stress also affect the water use efficiency (WUE) (Barbieri et al. 2012). The WUE describes the relationship between a unit of biomass produced per unit of water used (Richardson 2013). Different WUE definitions exist, however, they depend on scale at which the relation is represented. Depending on gas exchange in leaf, intrinsic water use efficiency (iWUE) can be calculated as the ratio of P_n to g_s (Topbjerg et al. 2015). In the present study, iWUE was slightly

reduced by salt stress in both cultivars (Figure 4). No significant difference was found in iWUE between cv. JM22 and cv. YN19 after exposure to soil salinity. This can be explained by the similar reduction in P_n and g_s of wheat leaves under salt stress. iWUE is a complex indicator which is affected by a range of mechanisms related to plant water uptake and use (Topbjerg et al. 2015). When plants encounter salt or osmotic stress, one of the first responses is a decreasing g_s (Yordanov et al. 2000). The changes in g_s significantly affect the water use in plants; besides, the leaf area also has a significant effect on plant water use. Under salt stress, leaf area index and leaf area duration were reduced in wheat (Zheng et al. 2008). The smaller leaf area decreases the transpiration, which is related to WUE. Here, the leaf area was slightly reduced by soil salinity, and no significant difference in leaf area was found between cv. JM22 and cv. YN19.

The fast chlorophyll *a* fluorescence induction curve was widely used to investigate the photosynthetic electron transport as influenced by abiotic stress, including salt and temperature stress (Mehta et al. 2010, Li et al. 2014). In the present study, all parameters derived from the chlorophyll *a* fluorescence induction curves were decreased by soil salinity (Figure 5). However, cv. YN19 possessed higher F_v/F_m compared with cv. JM22 when exposed to soil salinity, indicating that cv. YN19 plants had higher quantum yield of PSII and increased electron

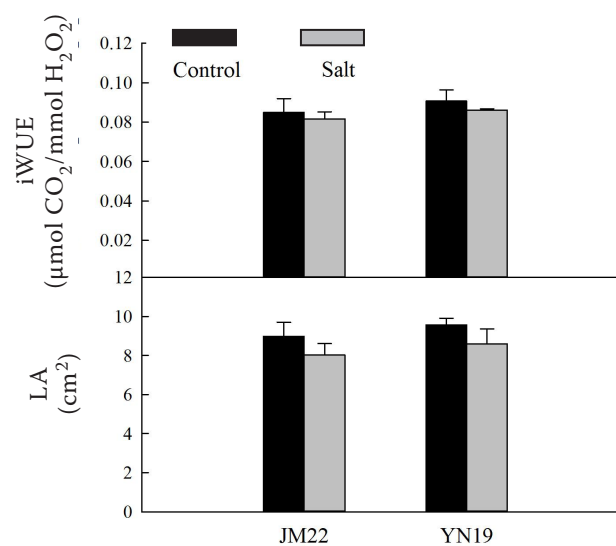


Figure 4. Effect of salt stress on intrinsic water use efficiency (iWUE) and leaf area (LA) in wheat. Mean values \pm standard error are shown ($n = 3$)

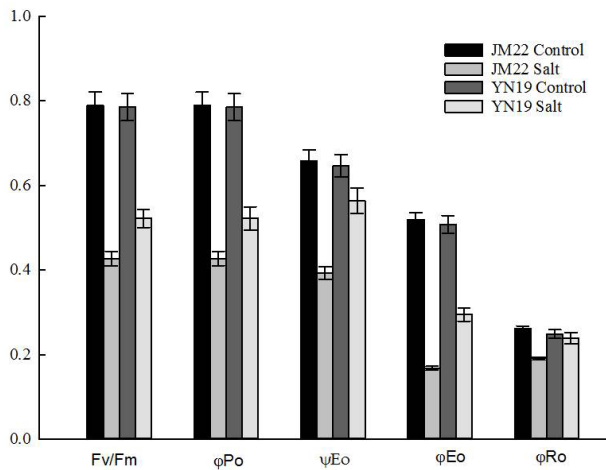


Figure 5. Fluorescence transient chlorophyll a parameters deduced from analysis of the JIP- test of wheat leaves as affected by salt stress. Mean values ± standard error are shown ($n = 3$). Subscript o indicates that the parameter refers to illumination onset, when all RCs are assumed to be open. F_o – minimal fluorescence, when all PSII RCs (reaction centres) open; $F_v = F_t - F_o$, variable fluorescence at time t ; F_m – maximal recorded fluorescence intensity; ϕP_o – maximum quantum yield for primary photochemistry; ϕE_o – quantum yield for electron transport (ET); ϕR_o – quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE); ψE_o – probability that an electron moves further than Q_A

donation to PSII under salt stress (Spoustová et al. 2013). The ϕP_o represents the efficiency of the light photochemical reactions (Chen et al. 2011), which was higher in cv. YN19 in relation to cv. JM22 under salt stress. This indicated that the

salt-resistant cultivar possessed higher efficiency in the light reaction process in photosystem. It was reported that the decrease in efficiency of light reaction results in increased energy dissipation as heat, which depresses the efficiency of reaction centres (Chen et al. 2011). Furthermore, the efficiency balance of the dark reactions after Q_A , which is expressed as ψE_o (Strasser et al. 2004), was also higher in cv. YN19 compared with cv. JM22. This indicated that salt-resistant cultivar had higher efficiency than salt-sensitive cultivar in the process that trapped exciton transfers electron into the electron transport chain beyond Q_A (Chen et al. 2011). In addition, higher maximum quantum yield of primary photochemistry (ϕE_o) and quantum yield for the reduction of the end electron acceptors at the PSI acceptor side (ϕR_o) was seen in cv. YN19 when exposed to soil salinity. The ϕE_o also represents the maximum quantum yield for electron transport beyond Q_A (Li et al. 2014). Thus, higher ϕE_o is helpful for photosynthetic electron transport efficiency of wheat plants under salt stress.

The derived parameters can be further visualized by the dynamic energy pipeline leaf model of the photosynthetic apparatus (Li et al. 2014). The leaf model of phenomenological energy fluxes (per cross-section) was used in the present study (Figure 6). In the present study, electron transport in PS II cross-section (ETo/CSm) was reduced significantly in wheat leaves under salt stress, indicated by significantly smaller size of blue arrow in salt treatment. ETo/CSm presented the reoxidation of reduced Q_A via electron transport over a cross-section of active and inactive RCs, which

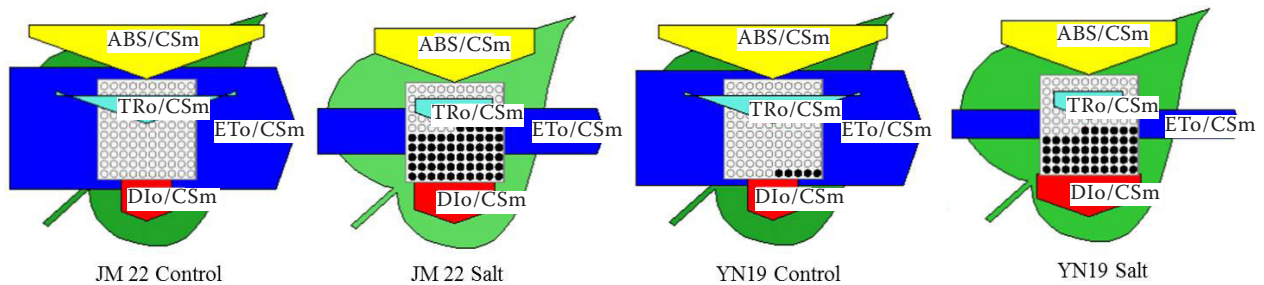


Figure 6. Energy pipeline leaf model of phenomenological fluxes (per cross-section, CS) in last fully expanded leaves as affected by salt stress. Data are means ± standard error ($n = 3$). Each relative value is drawn by the width of the corresponding arrow, standing for a parameter. Empty and full black circles indicate, respectively, the percentage of active (Q_A reducing) and non-active (non Q_A reducing) reaction centres of PS II. TRO/CSm – trapped energy flux per CS; ETO/CSm – electron transport flux per CS; ABS/CS_m – absorption flux per CS; DIO/CSm – non-photochemical quenching per CS

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was higher in cv. JM22 as compared to cv. YN19 under salt stress. However, it was noted that the density of the active RCs in PS II cross-section, which is indicated as open circles, was lower in cv. JM22 than that in cv. YN19. This suggests that the electron transport was not enhanced under salt stress, through less active RCs were converted into inactive RCs in the salt-resistant cultivar. The size of red arrows showed the difference in non-photochemical quenching (DI_O/CSm). The decreased ETo/CSm and relatively higher DI_O/CSm contributed to the balance between light energy absorbed by photosystems and energy consumed by metabolic sinks in the plant under salt stress. Therefore, the parameters related to the density of active RCs in PS II can be used as indicators for rapid screening wheat cultivars tolerant to soil salinity. In addition, there was no significant difference in energy absorbed per excited cross-section (ABS/CSm) and trapped energy flux per CS (TR_O/CSm) between cvs. JM22 and YN19 under salt stress.

In conclusion, soil salinity significantly affected the plant water status and reduced the photosynthetic rate and plant growth in wheat. The depressed photosynthetic carbon assimilation was mainly caused by stomatal closure and lower photosynthetic electron transport. The salt-resistant cv. YN19 had higher efficiency in photosynthetic electron transport, hence maintaining higher photosynthetic rate under salt stress, compared with the salt-resistant cv. JM22. In addition, the parameters derived from fast chlorophyll *a* fluorescence induction curve, i.e. ϕ_{Eo} and ψ_{Eo} , can be used as indicators for salt tolerance in wheat.

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