

Salt acclimation induced salt tolerance in wild-type and abscisic acid-deficient mutant barley

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Abstract: Salt acclimation is a process to enhance salt tolerance in plants. The salt acclimation induced salt tolerance was investigated in a spring barley (*Hordeum vulgare* L.) cv. Steptoe (wild type, WT) and its abscisic acid (ABA)-deficient mutant *Az34*. Endogenous ABA concentration in leaf was significantly increased by salt stress in WT, while it was not affected in *Az34*. Under salt stress, the salt acclimated *Az34* plants had 14.8% lower total soluble sugar concentration and 93.7% higher sodium (Na) concentration in leaf, compared with salt acclimated WT plants. The acclimated plants had significantly higher leaf water potential and osmotic potential than non-acclimated plants in both WT and *Az34* under salt stress. The salt acclimation enhanced the net photosynthetic rate (by 22.9% and 12.3%) and the maximum quantum yield of PS II (22.7% and 22.0%) in WT and *Az34* under salt stress. However, the stomatal conductance in salt acclimated *Az34* plants was 28.9% lower than WT under salt stress. Besides, the guard cell pair width was significantly higher in salt acclimated *Az34* plants than that in WT plants. The results indicated that the salt acclimated WT plants showed a higher salt tolerance than *Az34* plants, suggesting that ABA deficiency has a negative effect on the salt acclimation induced salt tolerance in barley.

Keywords: water relation; phytohormone; ion toxicity; salinization; chlorophyll *a* fluorescence

Soil salinization is a growing problem in agriculture, which limits crop growth and productivity. Multiple physiological processes in plants are modified by salt stress, such as the morphology of guard cell and ion concentrations in leaf (Liang et al. 2018). As one of the most important agricultural food crops worldwide, barley is often considered to possess moderate salt tolerance (El-Esawi et al. 2018). However,

high salt stress leads to a great reduction in grain yield in a large area (Tabassum et al. 2018). Salt stress depresses photosynthetic efficiency through decreasing chlorophyll concentration and stomatal closure in barley (Mahlooji et al. 2017). In addition, salt stress-induced over accumulations of Na⁺ and Cl⁻ in plant cells cause ionic toxicity, resulting in oxidative stress (Wu et al. 2014).

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Higher plants have various strategies to cope with salt stress, including regulating the ion uptake in roots and translocations in the shoot, activation of antioxidative enzyme systems, and modification of membrane structure (Liang et al. 2018). Salt acclimation has been documented to improve salt tolerance in various plant species (Janda et al. 2016, Pandolfi et al. 2016). For instance, salt acclimation by irrigating wheat plants with 25 mmol NaCl solution resulted in a decrease in chlorotic symptoms and impairment of the photosynthetic processes of the plants exposed to subsequent high salt stress (Janda et al. 2016). Osmotic adjustment is important for the induction of salt tolerance in various plant species (Tang et al. 2015). In saline soils, plants accumulate sufficient solutes to match, in osmolarity, the higher ion levels in the soil solution (Munns and Tester 2008). The osmotic adjustment helps to maintain cell turgor in cells, to benefit for salt tolerance in plants.

Phytohormones play crucial roles in response to salt stress in plants, especially abscisic acid (ABA), which regulates multiple physiological processes and the adaptation to abiotic stresses, such as salt (Zuo et al. 2017). The positive effects of ABA on salt tolerance have been well illuminated by molecular and biochemical studies. Under salt stress, plants could enhance the endogenous ABA level by activating the synthetic pathway of ABA and/or inhibiting its degradation pathway (Merilo et al. 2018). However, the roles of ABA in the process of salt acclimation induced salt tolerance and the physiological alterations involved are still unclear.

To explore the physiological mechanisms of salt acclimation induced salt tolerance and the involvement of ABA in this process, a spring barley cv. Steptoe and its ABA-deficient mutant *Az34* firstly experience a salt acclimation process, and subsequently exposed to high salt stress. The objective was to examine whether ABA deficiency could affect the process of salt acclimation induced salt tolerance.

MATERIAL AND METHODS

Genetic materials and experimental design. A spring barley cv. Steptoe (WT, wild type) and its ABA-deficient mutant *Az34* (= *nar2a* in Steptoe genetic background) were grown in plastic pots (15 cm high and 25 cm in diameter) filled with 4.8 kg clay soil in Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun, China. The soil contains 8.06 g/kg organic carbon, 1.3 g/kg

total N, 83.1 mg/kg available N, 0.05% P, 1.75% Na, and 0.65% K, and the soil pH is 6.1. The soil was sieved by passing through a 1 cm mesh. Eight seeds were sown in each pot, and four seedlings remained after thinning at the 2-leaf stage. At 5-leaf stage, barley plants were exposed to four treatments: C – non-stress control; AC – salt acclimation (25 mmol NaCl for 10 days); ST – salt stress (500 mmol NaCl for 4 days); AC + ST – salt acclimation + salt stress. The plants were grown in a greenhouse, where the photosynthetic active radiation (PAR) was > 500 $\mu\text{mol}/\text{m}^2/\text{s}$, and the photoperiod was 12 h. The relative humidity was $70 \pm 5\%$. The experiment was a randomized block design. Each treatment consisted of three replicates (each replicate had 4 pots).

Physiological trait determination. Just after the salt stress treatment, the net photosynthetic rate (P_n) and stomatal conductance (g_s) was determined on the latest fully expanded leaf with a portable photosynthesis system (LI-6400XT, Li-Cor, Lincoln, USA) at 400 $\mu\text{mol}/\text{mol}$ of CO_2 concentration and 1200 $\mu\text{mol}/\text{m}^2/\text{s}$ of PAR. The gas exchange measurements were carried out from 9:30 to 11:30. The maximum quantum yield of PS II (F_v/F_m) and performance index on the absorption basis (PI_{ABS}) were measured on the same leaf as for the gas exchange with the plant efficiency analyzer (Pocket-PEA, Hansatech, Norfolk, UK) after a 30 min of dark adaptation. The relative chlorophyll content of the same leaf was measured with a chlorophyll meter (SPAD 502, soil plant analysis development, Minolta, Tokyo, Japan). The stomatal morphology was analyzed based on the images of stomata in the latest fully expanded leaf, processed with a Dino-Lite digital microscope (AM411, Vidy Precision Equipment, Wuxi, China). After these non-destructive measurements, the latest fully expanded leaf was used to measure the midday leaf water potential (Ψ_l) and osmotic potential (Ψ_o) with a pressure chamber (soil moisture equipment, Santa Barbara, USA) and a psychrometer (C-52 sample chambers, Wescor, Logan, USA) connected with a microvolt meter (HR-33T, Wescor, Logan, USA), respectively.

The leaf samples from each treatment were collected just after salt stress for analysis of concentrations of ABA, total soluble sugar (TSS), and Na^+ and K^+ . The leaf ABA concentration was measured with ELISA following our previous protocol (Li et al. 2015). The TSS concentration was determined with dry leaf samples (Li et al. 2013). Concentrations of Na^+ and K^+ were analyzed after high-pressure digestion with

nitric acid (UltraClave III, MLS, Leutkirch, Germany) using inductively coupled plasma optical emission spectrometry (ICP-OES 720, Varian, Palo Alto, USA).

Statistical analysis. The data was firstly tested for homogeneity of variance with boxplot and subjected to the one-way ANOVA using SigmaSTAT (V3.5, Systat Software Inc., Chicago, USA).

RESULTS AND DISCUSSION

As expected, the ABA concentration in leaf was significantly increased by salt stress and the combination of salt acclimation and salt stress in WT, while it was not affected by both treatments in ABA-deficient mutant *Az34* (Figure 1). The remarkable increases in endogenous ABA concentration have been found in various plant species when exposed to salt stress (Jakab et al. 2005). ABA has been demonstrated to function as crucial cellular signaling of salt acclimation, probably regulating carbohydrate metabolism (Garcia de la Garma et al. 2015).

The chlorophyll content (SPAD) and TSS concentration were both significantly reduced by salt stress (Figure 1). Under salt stress, the SPAD value was significantly higher in salt acclimated plants than non-acclimated plants in WT and *Az34* by 24.6% and 11.7%, respectively. This indicated that salt acclimation increased the chlorophyll content and TSS concentration in leaf, which benefited the salt tolerance of barley plants. However, it should be noted that the salt-induced reduction in chlorophyll content was larger in the *Az34* compared with WT. This might be related to the lower level of endogenous ABA in *Az34*. Soluble sugar contributes to the osmotic adjustment in plants when exposed to salt stress (Janda et al. 2016). Here, salt acclimation increased leaf TSS concentration in WT barley exposed to subsequent salt stress, while the TSS concentration was not affected by salt acclimation in *Az34*. Thus, it was suggested that salt acclimation induced salt tolerance by regulating the osmotic adjustment process, which may be ABA-dependent.

The Ψ_l were significantly decreased by 57.1% and 72.0% in WT and *Az34* under salt stress, compared with the control (Figure 2). Under salt stress, the salt acclimated plants had 15.9% and 13.4% higher Ψ_l than the non-acclimated plants in both WT and *Az34*. A similar trend was found in Ψ_o . It has been well documented that salt stress induces dehydration in plant tissues (Parida and Das 2005). Nonetheless, salt acclimated plants had less dehydration in leaf than non-acclimated plants,

suggesting that salt acclimation helps to maintain a better water status in barley under salt stress.

The salt acclimation, salt stress, and their combination all increased significantly the leaf Na^+ concentration in WT and *Az34* (Figure 3). Soil salinity leads to ion toxicity, which is one of the major negative effects on plant growth (Janda et al. 2016). Modifications of Na^+ uptake and translocation are among the mechanisms which may lead to higher salt tolerance in cereals (Janda et al. 2016). In the present study, salt acclimation reduced the Na^+ ac-

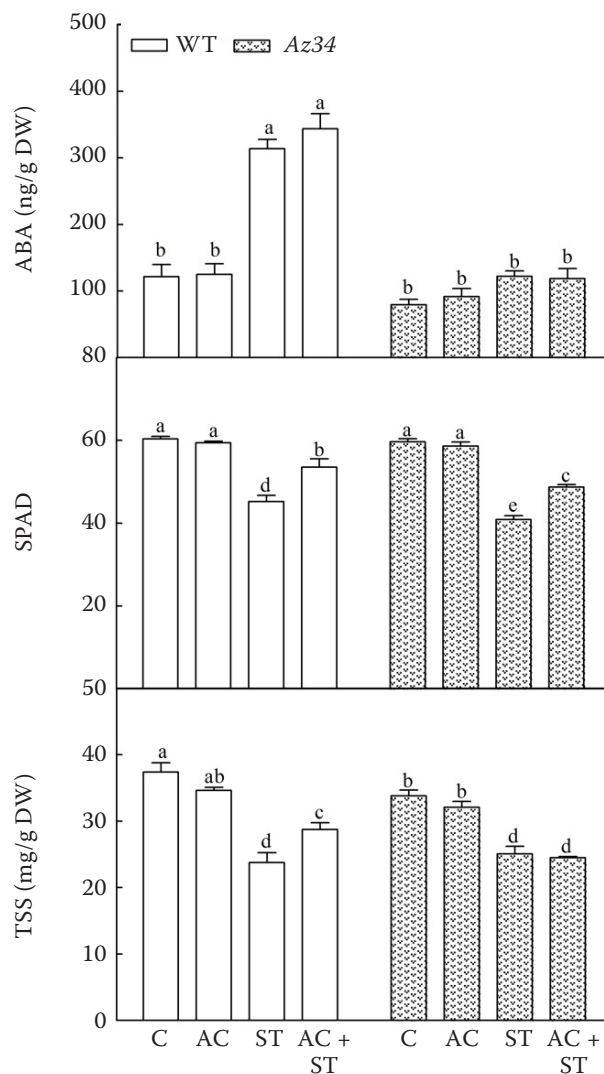


Figure 1. The concentration of abscisic acid (ABA), chlorophyll content (SPAD), and concentration of total soluble sugars (TSS) in the latest fully expanded leaf of wild type (WT) barley and its ABA-deficient mutant (*Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level; DW – dry weight

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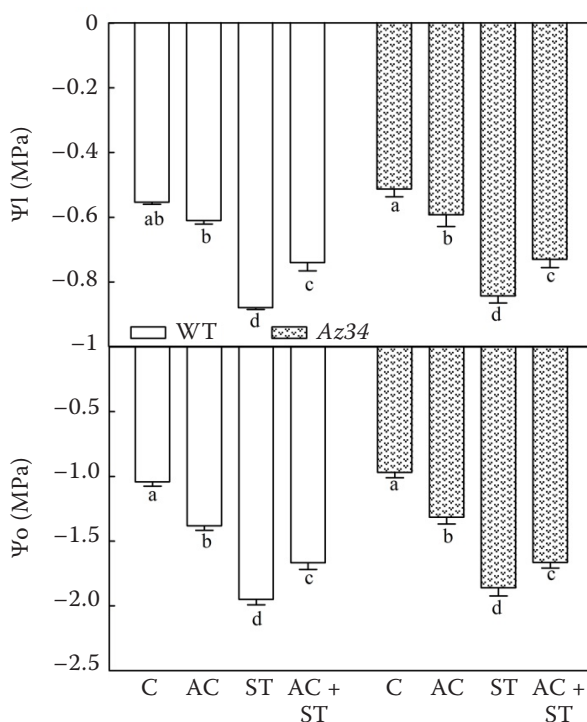


Figure 2. Leaf water potential (Ψ_l) and osmotic potential (Ψ_o) of the latest fully expanded leaf in barley (wild type (WT) and *Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level

cumulation by 24.9% in leaf under subsequent salt stress in WT, indicating that salt acclimation modified the Na^+ translocation in leaf. However, it was not the same case in *Az34*, indicating that ABA might be involved in the regulation of Na^+ translocation in leaf. In *Phaseolus vulgaris*, ABA inhibited the Na^+ transport from root to shoot, alleviating the toxicity caused by Na^+ overaccumulation (Karmoker and Van Steveninck 1979). Normally, plants increase Na^+ uptake and reduce K^+ uptake under salt stress, hence disrupting the balance between Na^+ and K^+ (Tavakkoli et al. 2011). In the present study, the leaf K^+ concentration was significantly decreased by 32.2% and 40.4% by salt stress in WT and *Az34*, compared with the non-stress control (Figure 3). However, salt acclimated plants had significantly higher K^+ concentration in leaf than non-acclimated plants under salt stress. Accumulated evidence indicates that salt acclimation could enhance K^+ stability in plant tissues (Pandolfi et al. 2016). Here, the salt acclimation induced tolerance to the subsequent salt stress could be linked to the higher K^+ level in

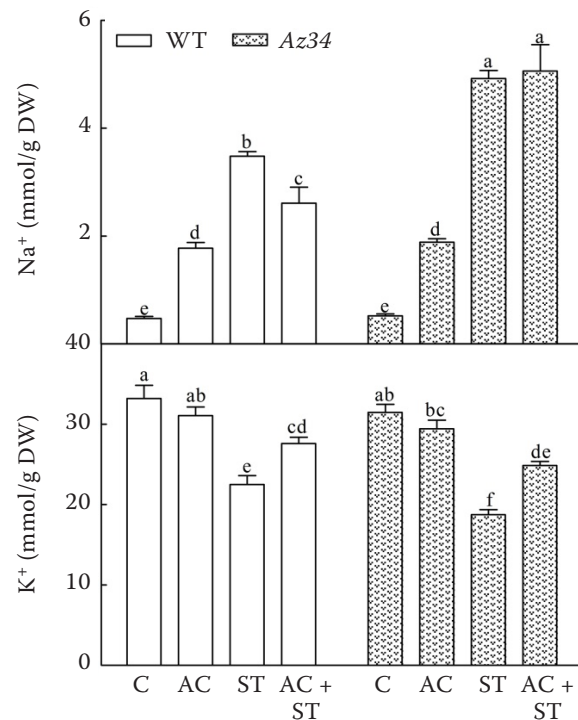


Figure 3. Concentrations of Na^+ and K^+ in barley leaves (wild type (WT) and *Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level

tissues. In addition, WT and *Az34* barley showed a similar trend, indicating that ABA deficiency had no effect on the transport and accumulation of K^+ under salt stress.

Salt stress has direct effects on photosynthesis by modulating photosynthetic proteins in chloroplasts; besides, it also affects photosynthesis indirectly by limiting stomatal functioning (Munns and Tester 2008). In the present study, the P_n and g_s were both significantly decreased by salt stress in WT and *Az34* (Figure 4). Interestingly, the salt acclimated plants had significantly higher P_n and g_s than the non-acclimated plants under salt stress, except for the g_s in *Az34*. This indicated that the greater P_n of salt acclimated plants could be partially ascribed to their higher g_s in relation to the non-acclimated plants under salt stress (Wang et al. 2017).

F_v/F_m and PI_{ABS} were widely used to test the response of photosynthetic electron transport to salt stress (Živčák et al. 2017). Here, the significant reductions in F_v/F_m and PI_{ABS} were observed in WT and *Az34* barley under salt stress; however, the salt

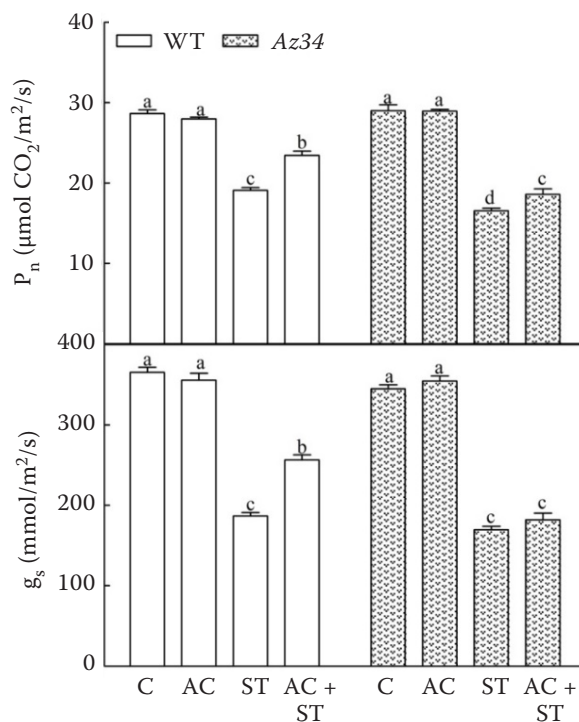


Figure 4. Net photosynthetic rate (P_n) and stomatal conductance (g_s) of the latest fully expanded leaf in barley (wild type (WT) and *Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level

acclimated plants showed less reduction in F_v/F_m and PI_{ABS} , in relation to the non-acclimated plants (Figure 5). F_v/F_m and PI_{ABS} reflect the maximum quantum yield of PS II and the light absorption process, respectively (Živčák et al. 2017). Greater F_v/F_m and PI_{ABS} contributed to the higher P_n in salt acclimated barley under salt stress, which is in line with the early study (Wang et al. 2017).

The plants could regulate the g_s to control the photosynthetic rate through modulating the aperture of the stomatal pore in the short term (Sun et al. 2016). In this process, ABA plays an important role in inducing stomatal closure (Dodd and Davies 1994). Here, both guard cell length and guard cell pair width were reduced by salt stress, compared with the non-salt control (Figure 6). For either guard cell length or guard cell pair width, no significant difference was found between ST and AC + ST plants. This demonstrates that salt acclimation had no remarkable effect on the stomatal response to salt stress in barley. Notably, for a given treatment, the *Az34* barley had significantly higher guard cell pair

width than WT plants under salt stress, indicating that the lowered endogenous ABA concentration resulted in less sensibility of stomata to salt stress. It might depress the salt tolerance of plants, due to higher water loss of plants under salt stress (Liang et al. 2018).

In conclusion, leaf ABA was significantly increased by salt acclimation in WT, while it was not affected in *Az34*. The salt acclimation enhanced the P_n in WT and *Az34* under salt stress. However, the g_s in salt acclimated *Az34* plants were significantly lower than WT under salt stress. In addition, the guard cell pair width was remarkably higher in salt acclimated *Az34* plants than that in WT plants under salt stress. The salt acclimation enhanced the tolerance to the subsequent high salt stress in both WT and *Az34* barley. The salt acclimated WT plants had higher salt tolerance than ABA-deficient *Az34* plants. It was suggested that ABA had a key role in the process of salt acclimation induced salt tolerance in barley.

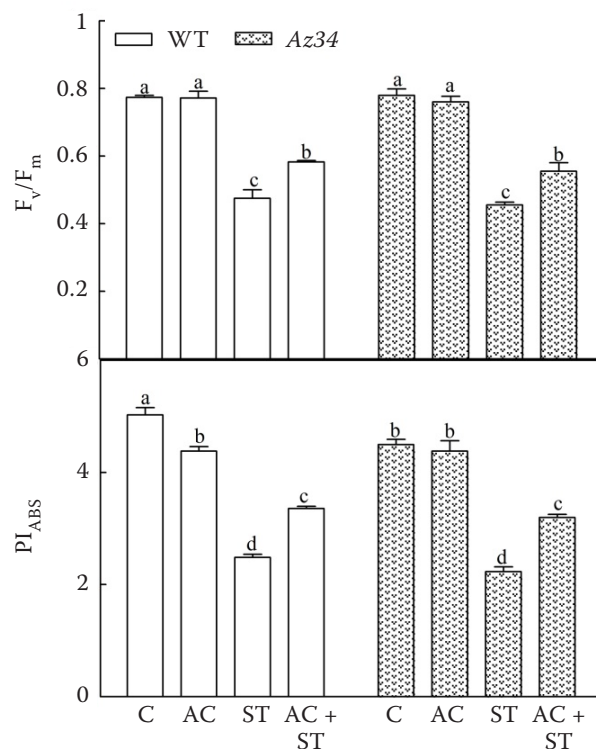


Figure 5. The maximum quantum efficiency of photosystem II (F_v/F_m) and performance index on absorption basis (PI_{ABS}) of the latest fully expanded leaf in barley (wild type (WT) and *Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level

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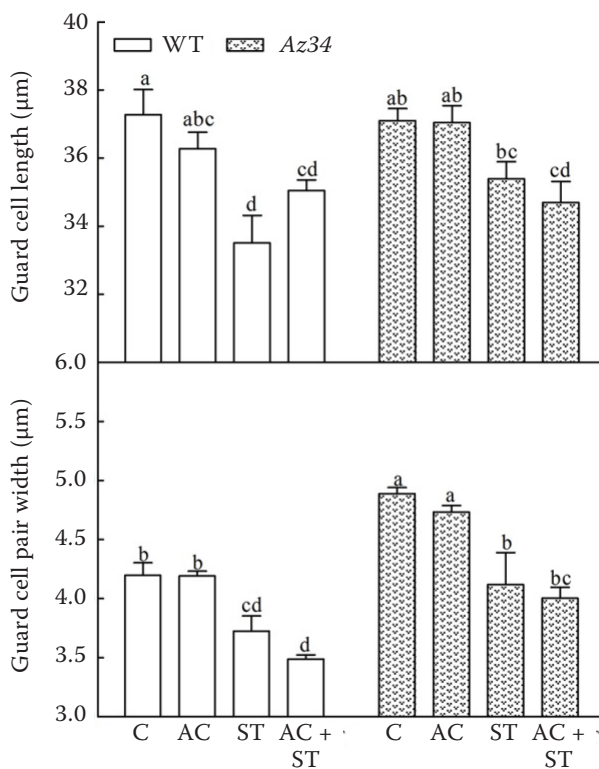


Figure 6. Guard cell length and guard cell pair width of the latest fully expanded leaf in barley (wild type (WT) and *Az34*) as affected by salt acclimation and salt stress. C – non-stress control; AC – salt acclimation; ST – salt stress; AC + ST – salt acclimation + salt stress. Different small letters mean significant difference at $P < 0.05$ level

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