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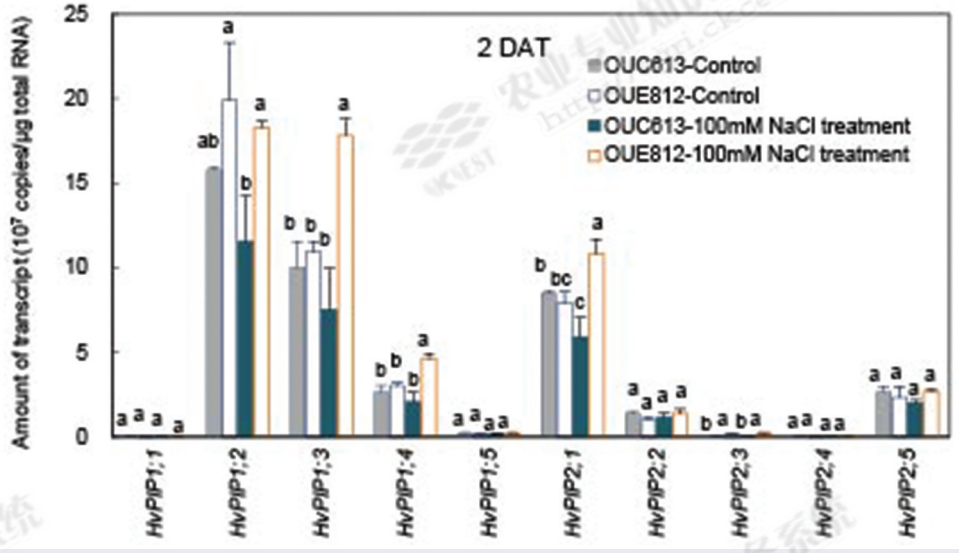
Accession difference in leaf photosynthesis, root hydraulic conductance and gene expression of root aquaporins under salt stress in barley seedlings

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ABSTRACT

Soil salinity causes considerable losses of crop productivity. Barley (*Hordeum vulgare*) is one of the most salt-tolerant Gramineae crops. Previously, we found that net photosynthetic rate (A_n) was kept remarkably higher in the salt-tolerant barley accession OUE812 than in the salt-sensitive accession OUC613 after heading under salt stress due to the low level of salt accumulation in leaves. Here we grew seedlings in Hoagland solution with 100 mM NaCl (salt treatment) or without added NaCl (control), and compared A_n, stomatal conductance (g_s), salt accumulation in leaves, root hydraulic conductance and gene expression of root aquaporins between the accessions under salt stress for a few days. A_n, g_s and root hydraulic conductance of the plants with salt treatment decreased significantly in OUC613 compared to OUE812 with no accession difference in salt accumulation in leaves at 2 days after the onset of treatment (DAT). The reduction in root hydraulic conductance in OUC613 was caused by the reduction of the root hydraulic conductivity (Lp_r). Salt treatment also decreased the transcript levels of some plasma membrane intrinsic aquaporin genes (*HvPIP*s) in OUC613 and, on the contrary, increased those of some *HvPIP*s in OUE812, resulting in a large difference between OUC613 and OUE812 in the transcript levels at 2 DAT. The accession difference in *HvPIP*s expression and thus Lp_r was closely associated with the accession difference in A_n and g_s under the short-term salt stress.



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1. Introduction

Soil salinity causes considerable losses of crop productivity. Salt tolerance differs significantly among crop species. Among Gramineae crops, barley (*Hordeum vulgare*) is the most salt tolerant, whereas rice (*Oryza sativa*) is the most susceptible (Munns et al., 2006; Munns & Tester, 2008; Rawson et al., 1988). In barley, wheat (*Triticum aestivum*), and rice, salt tolerance differs among cultivars (Mano, 2007; Rawson et al., 1988;

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Supplemental data for this article can be accessed here.

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Richards et al., 1987; Royo & Aragues, 1999; Slavich et al., 1990; Yamanouchi, 1989). To adopt gene mining and molecular breeding for the improvement of crop salt-stress tolerance, it would be important to clarify natural variations in salt-stress tolerance and its characteristics in each crop species.

In most cases, crops grow under salinity conditions from seed germination to harvest, and thus investigation of the response to salt stress over the whole life of the plants is needed for characterization of their salt tolerance. The harmful effects of soil salinity on plant growth and yield are caused by two main factors: osmotic stress and ion toxicity (Horie et al., 2012; Munns & Tester, 2008). Plant salt tolerance can be characterized from these viewpoints.

Long-term salinity increases Na^+ and Cl^- concentrations in plants, which causes ion toxicities (Munns & Tester, 2008). Differences in ion exclusion capacity underlie cultivar differences in salt-stress tolerance in rice (Tsuchiya et al., 1994), wheat (Colmer et al., 2006), and barley (Shabala et al., 2010; Zhu et al., 2016). *HvNax3* and *HvNax4* were identified in barley as QTLs related to Na^+ exclusion (Rivandi et al., 2011; Shavrukov et al., 2010). A wheat cultivar carrying a Na^+ transporter gene, *TmHKT1;5-A*, has decreased leaf Na^+ concentration and increased grain yield under salinity (Munns et al., 2012).

Soil salinity leads to a decrease in water uptake and, consequently, water potential in plants. The osmotic and water-deficit-inducing effects of salinity lead to reduced leaf photosynthesis and growth (Munns & Tester, 2008). Salinity also affect hydraulic conductivity of roots (Lp_r; root hydraulic conductance per unit root surface area) (Aroca, Porcel & Ruiz-Lozano, 2012; Afzal et al., 2016; Chaumont & Tyerman, 2014; Javot & Maurel, 2002). Changes in Lp_r, in turn, affect water potential in plants through changing plant hydraulic conductance (Hirasawa, 2018). Lp_r is highly affected by root aquaporins, by which water transport across cell membranes is facilitated (Chaumont & Tyerman, 2014; Maurel et al., 2015) and the transcriptional regulation of root aquaporins and accession difference in aquaporins expression under salt stress were reported (Boursiac et al., 2005; Horie et al., 2011; Katsuhara et al., 2011; Kirch et al., 2000; Martins et al., 2015). However, the studies on the association of the accession difference in aquaporin expression with salt tolerance are very limited (Katsuhara et al., 2011; Kreszies et al., 2020).

Among approximately 6000 accessions of barley from the collection at Okayama University, Mano (2007) identified several accessions as salt tolerant and several accessions as salt sensitive. We selected OUE812 as a particularly salt tolerant and OUC613 as a particularly salt sensitive accession and grew them under long-term salt stress from 30 days after sawing to maturity for approximately 5 months (Hirasawa et al., 2017). OUE812 produced higher dry matter yield at maturity than did OUC613. OUE812 maintained significantly higher net photosynthetic rate (A_n) than did OUC613 during ripening, with no difference in leaf water potential (LWP) between the accessions. OUE812 maintained lower concentrations of Na^+ and Cl^- and a higher concentration of K^+ in leaves than did OUC613, suggesting that the difference in A_n between the accessions was caused by the differences in ion accumulation in leaves (Hirasawa et al., 2017).

Plants grow under the conditions of salt stress from the seedling stage. However, we have not examined the accession difference in physiological responses to salt stress at the seedling stage. In the current study, to investigate the accession difference in salt-stress tolerance at the seedling stage and the causal mechanisms, we compared A_n, stomatal conductance (g_s), LWP, ion accumulation in leaves, root hydraulic conductance, Lp_r, and gene expression of root aquaporins between OUE812 and OUC613 seedlings under salt stress for a few days.

2. Materials and methods

2.1. Plant materials

Seeds of cultivated barley (*Hordeum vulgare* L.) accessions OUC613 (a two-rowed landrace collected in China) and OUE812 (a six-rowed landrace collected in Ethiopia) were sterilized with 10% H_2O_2 for 10 min and immersed in distilled water with aeration for 1 day. Germinated seeds were transplanted on a seeding plate with 61 holes for planting in 3.5-L pots filled with 0.25 mM $CaSO_4$ and were hydroponically cultured with aeration for 2 days at 25°C in the dark. Plants were then grown in half-strength Hoagland solution in a growth chamber (KG-50HLA, Koito Manufacturing, Yokohama, Japan) at 14 h day/10 h night, 25°C, 60% relative humidity, and approximately 600 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) at the leaf surface. Plants were thinned periodically for preventing mutual shading.

The full-strength Hoagland solution contained 4.0 mM $Ca(NO_3)_2$, 4.0 mM KNO_3, 1.0 mM $MgSO_4$, 1.0 mM $NH_4H_2PO_4$, 1.0 mM $(NH_4)_2HPO_4$, 1.0 mM NaCl, 36 µM FeNaEDTA, 12.5 µM H_3BO_3, 0.25 µM $CuSO_4$, 1.0 µM $MnSO_4$, 1.0 µM $ZnCl_2$, and 0.4 µM $NaMoO_4$.

2.2. Salt treatment

The treatment started 8 days (for OUC613) or 10 days (for OUE812) after sowing, when the first leaf had fully expanded. Plants were grown in half-strength Hoagland solution without additional NaCl (control) or with 100 mM

NaCl (salt treatment). The solution was renewed every 2 days. Each treatment consisted of three or five replications. For the measurements of A_n, g_s, LWP, leaf ion concentration, and root aquaporin expression, plants grown in the same pot were grouped as one replication. For the measurements of root hydraulic conductance, plants sown on the same day were grouped as one replication.

2.3. Measurements of A_n, g_s and LWP

Measurements of A_n, g_s, and LWP were started 4 to 5 h after the onset of the light period using fully expanded leaves. A_n and g_s were determined with a portable open-flow gas-exchange system (LI-6400; LI-COR, Lincoln, NE, USA) at the center of a leaf. During measurements, leaf temperature, the leaf–air vapor-pressure difference and the ambient CO_2 concentration were controlled at 25°C, approximately 1.5 kPa, and 370 µmol mol^{-1}, respectively. PPFD was controlled at 2000 µmol m^{-2} s^{-1} to obtain light-saturated A_n as no detectable photoinhibition was observed during the measurements.

LWP was measured with a thermocouple psychrometer by the dew-point method. Leaf discs (6 mm in diameter) were excised at the center of a leaf so that they did not include mid-rib tissues, and were loaded immediately into the sample chamber of a psychrometer (C-52; Wescor Inc., Logan, UT, USA). After equilibration for 3 h at 25°C, the dew point was measured with a microvoltmeter (HR-33 T; Wescor Inc.).

Two or three independent experiments were conducted for the comparison of A_n, g_s and LWP between the accessions.

2.4. Determination of ion concentrations

Fully expanded leaves were collected, oven-dried at 80°C, and powdered with a ball mill (85,200; Qiagen, Haan, Germany). Ions were extracted from the powdered material with water at 80°C for 3 h, and their concentration was measured by high-performance liquid chromatography on an HIC-6A instrument (Shimadzu Co., Kyoto, Japan) equipped with an electrochemical detector (CDD-6A; Shimadzu) on a cation exchange column (Shim-pack IC-C4; Shimadzu) or anion exchange column (Shim-pack IC-A3; Shimadzu).

2.5. Measurements of root hydraulic conductance, root surface area and Lp_r

Root hydraulic conductance was estimated according to Fiscus (1975). Plants were cut at the base of the stem. Roots were placed in a pressure chamber (3005, Soil Moisture Equipment, Santa Barbara, CA, USA) containing Hoagland solution without added NaCl. Pressure of 0.2 MPa was applied to the solution containing the roots for 10 min. Then the pressure was changed stepwise to 0.2, 0.35, 0.25, and 0.3 MPa. The exuded xylem sap was collected for 10 min at each pressure by attaching sanitary cotton at the cut end. The cotton weighed before and after exudate collection to determine the mass of the exudate. Root hydraulic conductance (m^3 s^{-1} MP^{-1}) was calculated as the slope of the line fitted by linear regression of the exudation rate against applied pressure (Figure S1) (Miyamoto et al., 2001). At the end of the measurements, pressure of 0.2 MPa was applied again for 10 min to confirm the exudation rate at 0.2 MPa doesn't change from that of the first measurement (Figure S1).

Upon completion of the exudation measurements, root systems were removed from the chamber and stored in 70% ethanol. Root surface area was measured with an image analysis system (Win-RHIZO; Regent Instruments, Quebec, Canada). The ratio of root hydraulic conductance to root surface area was calculated for Lp_r (m s^{-1} MPa^{-1}) (Miyamoto et al., 2001). Root hydraulic conductance and Lp_r values of three to five plants per replicate were measured and averaged.

The root exudation rate was almost constant for at least 100 min after a given pressure was applied to the solution containing the root system (Figure S2). In the plants with salt treatment, the exudation rate was small, and showed no increase or decrease for approximately 100 min after air pressure was applied to the solution without additional NaCl (Figure S2). Lp_r was kept constant during the time from 10:00 to 20:00 in a day (Figure S3). Based on these results, each measurement was taken between 10:00 and 20:00 within 100 min.

2.6. Extraction of RNA and quantitative PCR

Whole root tissues were collected and immediately frozen in liquid nitrogen. Total RNA was extracted from 100 mg root tissue using an RNeasy Plant Mini Kit (Qiagen). First-strand cDNA was synthesized using a High Capacity cDNA Reverse Transcript Kit (Applied Biosystems, Waltham, MA, USA). PCR mixtures were prepared using *Power* SYBR Green PCR Master Mix (Applied Biosystems), and PCR was performed in a 7300 Real Time PCR System (Applied Biosystems) under the following conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. As PIPs are the main aquaporins mediating water transport in root cells (Horie et al., 2011; Javot & Maurel, 2002), the transcript levels of *HvPIP*s were observed. PCR primer sets are listed in Table S1. Serial dilution of a known copy number of each cRNA was reverse transcribed, and produced cDNA was amplified as a standard for absolute quantification (Katsuhara et al.,

2011). Two and four independent experiments were conducted for the comparison between the accessions at 1 DAT and 2 DAT, respectively.

2.7. Statistical analysis

Accession differences and effects of salt treatment were tested using Tukey's HSD test. Differences between treatments (T) and accessions (G) and T × G interactions were tested by two-way analysis of variance (ANOVA). All statistical analyses were performed using JMP v.13 software (SAS Institute, Cary, NC, USA).

3. Results

3.1. A_n, g_s and LWP under salt stress

At all days after the onset of treatment (DAT) analyzed, significant difference in A_n, g_s and intercellular CO_2 concentration (C_i) was not observed between accessions in the control. Salt treatment decreased A_n, g_s, and C_i in all measurements except for A_n and C_i of OUC613 at 1 DAT (Figure 1). In salt-treated plants, there was not significant accession difference in A_n and g_s at 1 DAT although they tended to be lower in OUE812 than in OUC613. A_n and g_s became significantly higher in OUE812 than in OUC613 at 2 DAT (Figure 1). Under salt stress, C_i was significantly lower in OUE812 than in OUC613 at 1 DAT but became significantly higher at 2 DAT (Figure 1). There was no significant difference in A_n/C_i between OUC613 and OUE812 at 1 DAT (0.068 ± 0.003 and 0.066 ± 0.009 mol m^{-2} s^{-1}, respectively) and at 2 DAT (0.067 ± 0.01 and 0.072 ± 0.005 mol m^{-2} s^{-1}, respectively). The higher A_n, g_s and C_i in OUE812 tended to be kept under salt stress at 7 DAT and 14 DAT (Figure S4).

LWP was approximately −0.3 to −0.4 MPa in the control in both accessions (Figure 1). At 1 DAT, salt treatment decreased LWP to approximately −0.6 MPa in both accessions. At 2 DAT, the salt treatment also decreased LWP in both accessions and LWP tended to be higher in OUE812 than in OUC613 in the plants with and without treatment. The reduction of LWP by the salt treatment (T) was significant at 1 and 2 DAT and the accession (G) difference was not significant at 1 DAT but significant at 2 DAT (Table 1). T × G interactions was not significant at 1 and 2 DAT.

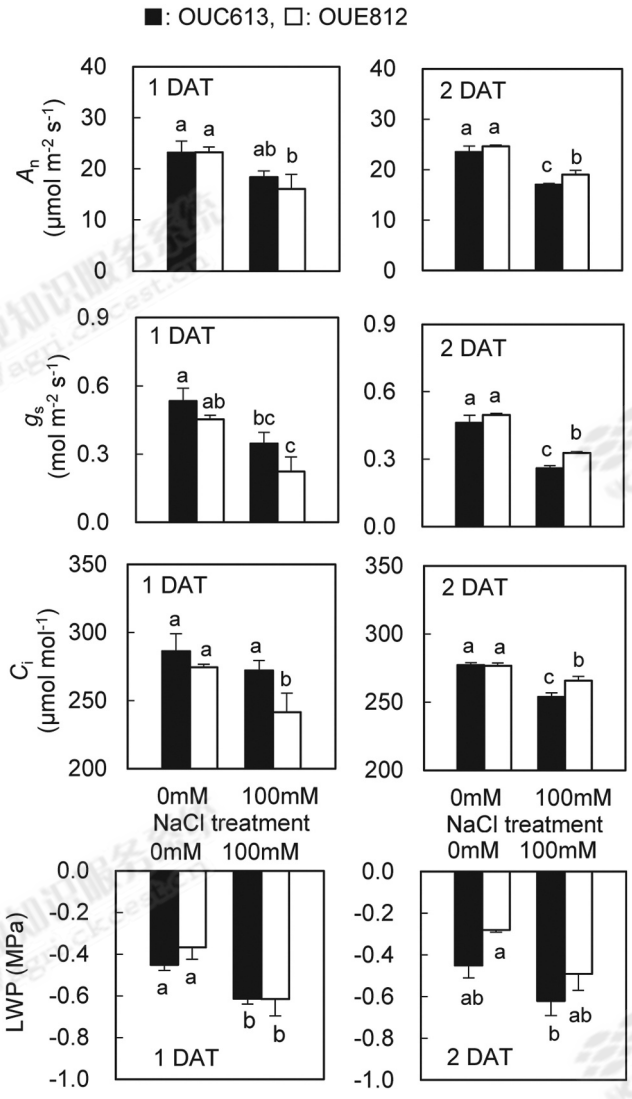


Figure 1. Photosynthetic rate (A_n), stomatal conductance (g_s), intercellular CO_2 concentration (C_i) and water potential (LWP) of the first leaf in OUC613 and OUE812 at 1 and 2 days after the onset of salt treatment (DAT). Error bars represent SD (n = 3). The same letters indicate no significant difference at the 5% level by Tukey's HSD test.

Table 1. Analysis of variance (ANOVA) of leaf water potential between salt treatments (T) and accessions (G), and T × G interactions.

	T	G	T × G
1 DAT	***	ns	ns
2 DAT	**	*	ns

*P<0.05; **P<0.01; ***P<0.001; ns, not significant (two-way ANOVA).

3.2. Ion concentrations in leaves under salt stress

Salt treatment markedly increased the leaf concentration of Na^+, with no significant differences between the accessions either in the control or salt treatment at any DAT (Figure 2). The leaf concentration of Cl^- was significantly higher in OUC613 than in OUE812 in the control and salt treatment at all DAT (Figure 2). The concentration slightly but significantly increased in salt-treated plants, but no difference in the increase was observed between the two accessions. Salt treatment gradually decreased the leaf concentration of K^+ and markedly decreased the ratio of K^+ concentration to Na^+

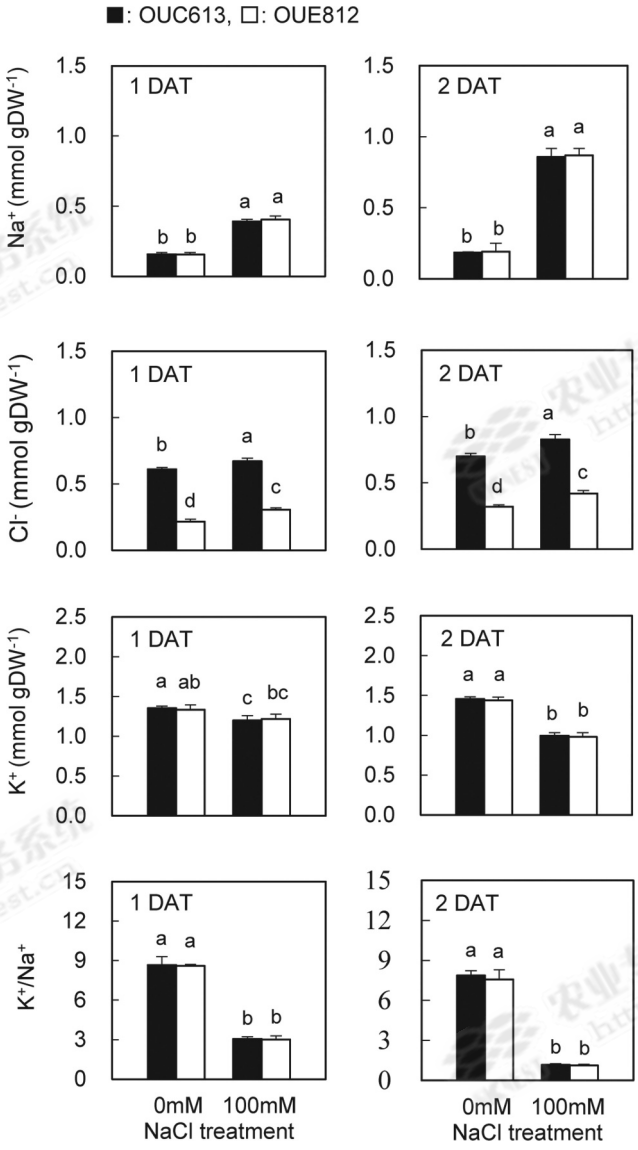


Figure 2. Concentrations of Na^+, Cl^-, and K^+, and the K^+/Na^+ ratio in the first leaf of OUC613 and OUE812 at different days after the onset of treatment (DAT). Error bars represent SD (n = 3). The same letters represent no significant difference at the 5% level by Tukey's HSD test.

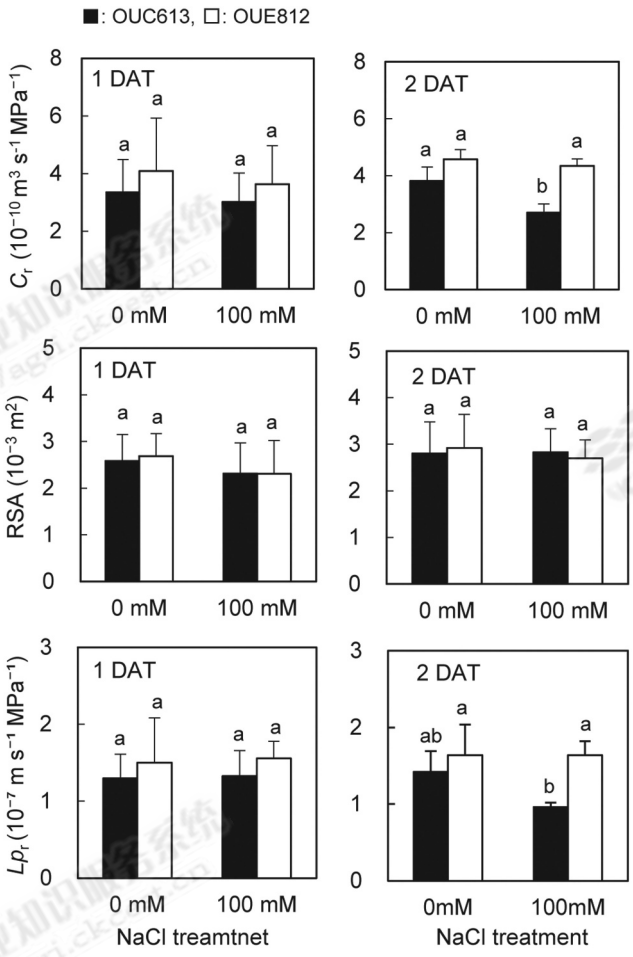


Figure 3. Root hydraulic conductance (C_r), root surface area (RSA), and root hydraulic conductivity (Lp_r) at 1 and 2 days after the onset of treatment (DAT). Error bars represent SD (n = 3 to 5). The same letters represent no significant difference at the 5% level by Tukey's HSD test.

concentration, with no difference between the accessions (Figure 2).

3.3. Root hydraulic conductance and Lp_r under salt stress

At 1 DAT, salt treatment did not affect the root hydraulic conductance of both accessions; there was no difference between the accessions either in the control or salt treatment (Figure 3). At 2 DAT, no significant difference in root hydraulic conductance was observed between OUC613 and OUE812 in the control. Salt treatment did not affect root hydraulic conductance in OUE812 but decreased it significantly in OUC613 (Figure 3). At 1 and 2 DAT, difference in root surface area was not significant between the accessions in either the control or salt treatment (Figure 3). At 1 DAT, no significant difference in Lp_r was observed between the accessions in either the control or salt treatment (Figure 3). At 2 DAT, salt treatment did not decrease Lp_r in OUE812 but decreased it in OUC613, although the reduction was not significant, resulting in a significant difference in Lp_r between the accessions with salt treatment (Figure 3). These results indicate that the significant reduction of root hydraulic conductance in OUC613 under salt stress at 2 DAT was resulted from the reduction in Lp_r.

3.4. Expression of aquaporins under salt stress

The transcript levels of *HvPIP1;2, HvPIP1;3, HvPIP1;4*, and *HvPIP2;1* were higher than those of other *HvPIP*s at 1 DAT

and 2 DAT, and those of *HvPIP1;2, HvPIP2;1*, and *HvPIP2;5* increased at 2 DAT in both accessions regardless of salt treatment (Figure 4). At 1 and 2 DAT, there was no difference in the transcript levels between the two accessions for all *HvPIP*s in the control except for *HvPIP2;3* (Figure 4), and no accession difference was observed in the total transcript level (Figure S5). Salt treatment had no significant effects on the *HvPIP* transcript levels in either accession at 1 DAT. Salt treatment affected the transcript levels of *HvPIP*s differently between OUC613 and OUE812 at 2 DAT (Figure 4). The transcript levels of *HvPIP1;2, HvPIP1;3*, and *HvPIP2;1* decreased significantly or tended to decrease in salt-treated OUC613. In contrast, a large and significant increase in the transcript levels of *HvPIP1;3, HvPIP1;4*, and *HvPIP2;1* was observed in salt-treated OUE812 in comparison with the control. In the salt treatment, the transcript levels of *HvPIP1;2, HvPIP1;3, HvPIP1;4*, and *HvPIP2;1* were higher in OUE812 than in OUC613 (Figure 4) and the total transcript level was significantly higher in OUE812 than that in OUC613 at 2 DAT (Figure S5).

4. Discussion

Under salt stress A_n and g_s decreased in both of the salt-tolerant accession OUE812 and the salt-sensitive accession OUC613 (Figure 1). A_n and g_s did not differ between the accessions at 1 DAT, but became higher in OUE812 than OUC613 at 2 DAT. Physiological mechanisms underlying the accession difference in A_n and g_s under salt stress for 2 days were discussed.

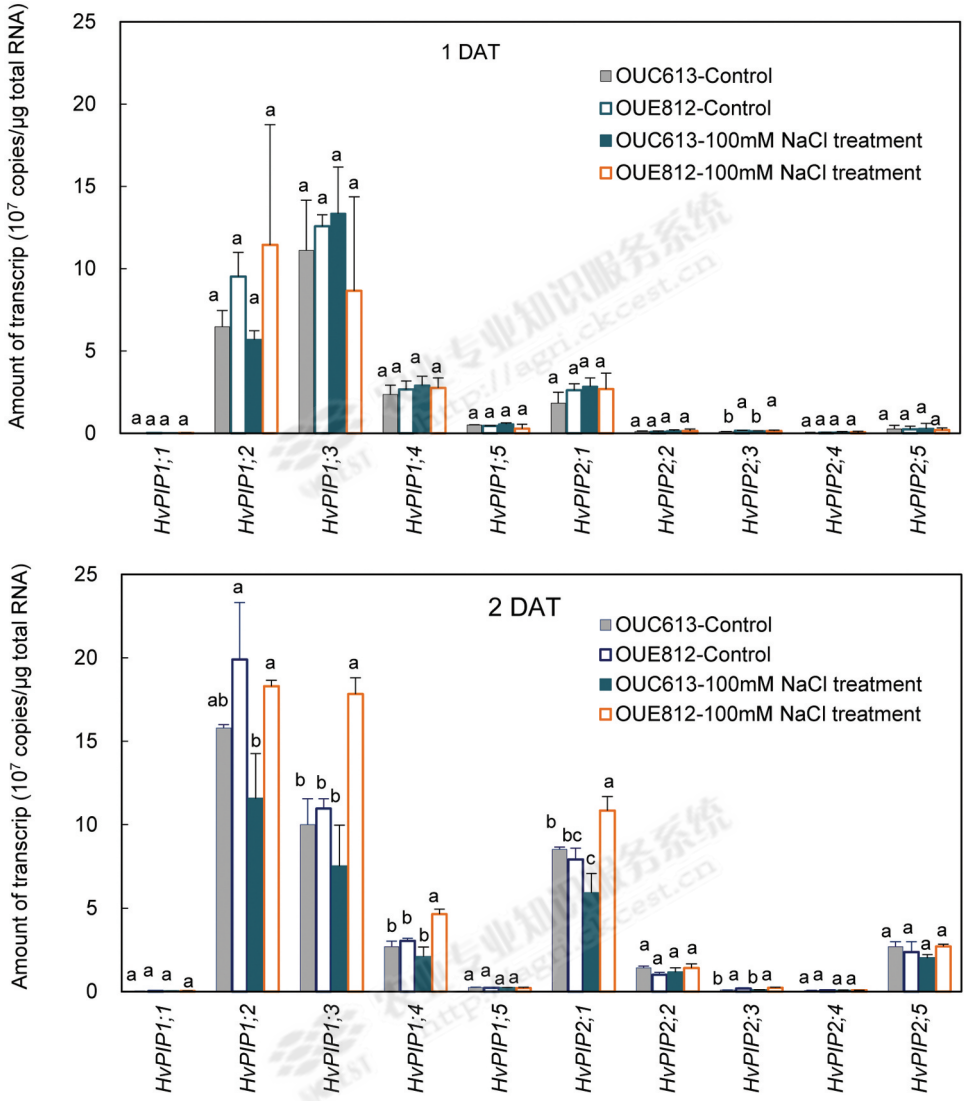


Figure 4. Representative transcript levels of aquaporin genes in OUC613 and OUE812 at 1 and 2 days after the onset of treatment (DAT). Error bars represent SD (n = 3). The same letters represent no significant difference at the 5% level by Tukey's HSD test.

4.1. Ion accumulation in leaves was not a cause of the accession difference in A_n under salt stress at 2 DAT

Most plants let in only 2% of Na^+ or Cl^- in the soil solution owing to their salt exclusion mechanisms (Munns, 2005). In the current study, leaf Na^+ and Cl^- concentration increased in plants with salt treatment, but the rate of the increase per day was a few % or less of the estimated value for the plants without salt exclusion mechanisms. Previously we have shown that OUE812 maintained high A_n in comparison with OUC613 under long-term salt stress, and OUE812 kept low leaf Na^+ and high leaf K^+/Na^+ ratio (Hirasawa et al., 2017) probably due to a high ion exclusion capacity compared with OUC613 (Rivandi et al., 2011; Shavrukov et al., 2010). Compared with OUC613, OUE812 showed high g_s, i.e., high transpiration rate under salt treatment (Figure 1), but showed no large increase in the concentration of Na^+ or Cl^- in leaves (Figure 2). This suggests that OUE812 has a high ion excluding capacity even at the seedling stage. A_n was kept higher in OUE812 than in OUC613 at 2 DAT under salt stress (Figure 1). However, there was no difference in the increase of leaf Na^+ and Cl^- concentrations and in the decrease of leaf K^+/Na^+ ratio between the accessions (Figure 2). These results suggest that the accession difference in A_n under salt stress at 2 DAT was not caused by the difference in ion accumulation in leaves.

4.2. Stomatal limitation of photosynthesis was a major cause of the accession difference in A_n under salt stress at 2 DAT

C_i is determined by the diffusion rate of CO_2 into the substomatal cavity from the atmosphere through stomata and the rate of CO_2 fixation in mesophyll cells. We can estimate that the major limitation of photosynthesis is the decreased CO_2 diffusion into the substomatal cavity by the stomatal closure (stomatal limitation of photosynthesis) for the leaf where C_i decreases along with the reduction in A_n, and is the inhibited photosynthesis metabolism in mesophyll cells (biochemical limitation of photosynthesis) for the leaf where C_i increases along with the reduction in A_n (Hirasawa et al., 1989; Kramer & Boyer, 1995). In the salt treatment, C_i decreased along with the reduction in A_n, and the reduction in both A_n and C_i was larger in OUC613 than in OUE812 at 2 DAT with no accession difference in A_n/C_i (Figure 1). These results indicate that the reduction in A_n under salt stress in both accessions and the larger reduction of A_n in OUC613 were caused mainly by the reduction in g_s (stomatal limitation of photosynthesis), not by biochemical limitation of photosynthesis in the mesophyll. The mechanism involved in the accession difference in A_n reduction differs in the current study from that of the previous study under long-term salt stress where the main cause of the accession difference in A_n was biochemical limitation in the mesophyll due to salt accumulation (Hirasawa et al., 2017).

4.3. Difference in root hydraulic conductance is a probable cause of the accession difference in A_n and g_s under salt stress at 2 DAT

A_n and g_s decreased both in OUE812 and OUC613 under salt stress. OUE812 kept high root hydraulic conductance even under salt stress at 2 DAT and maintained high A_n and g_s compared with OUC613 in which root hydraulic conductance decreased (Figures 1 and Figures 3). It is well known that A_n and g_s decrease in the plants under water stress and the reduction is remarkable in the plants with low hydraulic conductance compared to those with high hydraulic conductance (Christmann et al., 2013; Comstock, 2002; Hirasawa, 2018). The results of the current study were consistent with the results of many previous studies, and it was suggested that the difference in root hydraulic conductance was a probable cause of the accession difference in A_n and g_s under salt stress at 2 DAT, and keeping high root hydraulic conductance under salt stress might be a key trait for salt tolerance.

Physiological mechanisms underlying the relations between g_s (or A_n) and root hydraulic conductance have not been uncovered sufficiently yet. Plants with high hydraulic conductance could keep high leaf water potential compared to plants with low hydraulic conductance when transpiration rate is same in both plants (Hirasawa, 2018). Many studies showed the decrease of A_n and g_s with no clear reduction of LWP under soil moisture stress and close correlations of A_n or g_s with the plant hydraulic conductance (Christmann et al., 2013; Comstock, 2002; Hirasawa, 2018; Hubbard et al., 2001; Meinzer, 2002; Saliendra et al., 1995). It is suggested that A_n and g_s are primarily modulated by hydraulic signals (Christmann et al., 2013, 2007; Tombesi et al., 2015). In the current study, the accession difference in A_n and g_s at 2 DAT in salt treatment was consistent with the accession difference in root hydraulic conductance, but not with the difference in LWP (Figures 1 and Figures 3). Hydraulic signals or other signals might underlie the relations between g_s (A_n) and root hydraulic conductance in the current study (Christmann et al., 2013, 2007; McAdam & Brodribb, 2014). The reduction in g_s suppresses the reduction in LWP and this might make it difficult to find a close correlation of A_n and g_s with LWP in the plants especially

under mild osmotic stress like in the current study. LWP tended to be lower in OUC613 than in OUE812 (Figure 1, Table 1). LWP might decrease beyond the critical value of stomatal closure in the plants with salt treatment and cause the larger reduction in g_s and A_n in OUC613 (Figure 1).

4.4. Regulation of aquaporin genes expression is a probable cause of the accession differences in Lp_r, root hydraulic conductance and A_n under salt stress at 2 DAT

The accession difference in root hydraulic conductance was caused by the accession difference of Lp_r in response to salt stress (Figure 3). Changes in aquaporin gene expression corresponded to the accession difference in Lp_r in the salt treatment at 2 DAT (Figures 3 and Figures 4, S5). Among the *PIP*s of barley, *HvPIP1;2, HvPIP1;3, HvPIP2;1, HvPIP2;2, HvPIP2;4*, and *HvPIP2;5* are relatively highly expressed (Horie et al., 2011; Katsuhara et al., 2011). In line with these reports, we found relatively high transcript levels of *HvPIP1;2, HvPIP1;3, HvPIP1;4*, and *HvPIP2;1* in both accessions regardless of salt treatment (Figure 4). In *Arabidopsis*, the reduction in expression of aquaporin genes under salt treatment is larger for highly expressed genes than for genes expressed at low levels (Boursiac et al., 2005). In the current study, changes in transcript levels in response to salt treatment also tended to be large for highly expressed genes.

Salt stress decreases the expression of root aquaporins in *Arabidopsis* and barley (Afzal et al., 2016; Boursiac et al., 2005; Horie et al., 2011; Katsuhara et al., 2011), but increases it in ice plant (Kirch et al., 2000) and sweet orange (Martins et al., 2015). The response of aquaporin expression to salinity depends on the duration of salt stress (Aroka et al., 2012). Transcription of *PIP*s was down-regulated within 1 day after the onset of salinity (Boursiac et al., 2005; Horie et al., 2011; Katsuhara et al., 2011); it was up-regulated under salinity for 14 days (Kirch et al., 2000) or 20 days (Martins et al., 2015). In our study, the expression of aquaporin genes was not affected by salt treatment at 1 DAT in either accession and the expression of some *HvPIP*s increased in OUE812 at 2 DAT, but decreased in OUC613 (Figure 4). Water flow into roots at a given water potential difference between soil (culture solution) and roots would be increased by the up-regulation of root aquaporin genes. The effects of salt stress are reportedly mitigated by the overexpression of aquaporin genes (Hu et al., 2012; Sreedharan et al., 2013). The increase in the expression of *HvPIP*s might keep high water uptake by maintaining high Lp_r and high root hydraulic conductance in OUE812 under salt treatment at 2 DAT, and, on the contrary, water uptake might decrease markedly in OUC613 under salt treatment by the suppression of *HvPIP*s expression and the decreased Lp_r at 2 DAT (Figures 3 and Figures 4, S5). Some accessions of *Arabidopsis* do not decrease hydraulic conductivity of a root cell (Lp_{cell}) (and probably Lp_r) in salt treatment (Sutka et al., 2011). However, how the difference in Lp_{cell} (or Lp_r) is associated with the accession difference in salt tolerance was unclear. Recently, the higher expression of root aquaporin genes and larger Lp_r have been observed in salt stress tolerant wild barley compared with cultivated barley (Kreszies et al., 2020). Our results suggest that changes in the expression of *HvPIP*s in response to salt stress cause the difference in A_n and g_s between the cultivated barley accessions under salt stress through changes in Lp_r and root hydraulic conductance.

Both the amount and activity of root aquaporins affect Lp_r (Horie et al., 2011; Johansson et al., 1998; Kaneko et al., 2015; Maurel et al., 1995; Van Wilder et al., 2008). Cultivar difference in aquaporin activity was assumed in barley within 24 h after the onset of salt stress treatment (Kaneko et al., 2015). It was reported recently that osmotic stress decreases Lp_r by suberization in barley (Kreszies et al., 2020, 2019). Although we did not examine the connection of aquaporin activity and suberization with the accession difference in Lp_r, our results reveal that changes in the expression of aquaporin genes induced by salt treatment cause the accession difference in Lp_r, which is associated with the accession difference in the maintenance of A_n and g_s. Mapping populations derived from OUE812 and OUC613 developed by Kodama et al. (2018) can be a useful material to identify quantitative trait locus (loci) regulating the *HvPIP*s expression level under salt stress. It would provide the beneficial knowledge for the future breeding to improve the salt tolerance in barley.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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