Differences in dry matter production, grain production, and photosynthetic rate in barley cultivars under long-term salinity

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ABSTRACT
Soil salinity is a major environmental stress causing significant loss of crop productivity. Barley (Hordeum vulgare L.) is one of the few field crops that can grow in salt-affected fields and varietal differences in productivity under salinity conditions were known. To clarify the trait most responsible for grain production under salt stress, barley cultivars that were salt tolerant (OUE812) or salt sensitive (OUC613) were grown from seedling to harvest stage in vermiculite containing various concentrations of NaCl. Dry weight of aboveground parts and grain weight decreased significantly with increasing NaCl concentration. The dry weight of the aboveground parts and grain weight decreased more significantly in OUC613 than in OUE812 for plants treated with 150 mM and 200 mM NaCl. A marked reduction in ripening percentage caused significantly decreased grain production in OUC613 as compared with OUE812. In plants treated with 200 mM NaCl, the photosynthetic rate decreased three weeks after starting the NaCl treatment, but a significant difference between cultivars in photosynthetic rate did not appear until seven weeks of NaCl treatment. OUE812 kept a higher photosynthetic rate during ripening than did OUC613 and dry matter production during the period from ripening to harvest was significantly larger in OUE812 than in OUC613. Keeping a higher photosynthetic rate might have contributed to higher grain production in OUE812. Higher ripening percentage and higher rate of photosynthesis during ripening might be target traits in breeding to improve the tolerance of barley to long-term salt stress.

Introduction
Soil salinity is a major environmental stress that causes significant losses of crop productivity and adversely affects agriculture around the world. Over 6% of the world’s land is affected by either salinity or sodicity, with saline soils and sodic soils covering approximately 400 and 430 million ha, respectively (FAO Land and Plant Nutrition Management Service, 2008). In addition to salt-affected soils that have developed due to natural soil-forming processes, salinization due to man-made factors, mainly as a consequence of improper methods of irrigation and tree clearing which lead to water table and salt table rise, is an increasingly important issue (Pessarakli & Szabolcs, 2011). Of the current 230 million ha of irrigated land, approximately 20% is salt-affected (FAO Land and Plant Nutrition Management Service, 2008). In saline environments, salt-tolerant crops are more productive (Richards et al., 1987; Ryo & Aragues, 1999). It is important to be able to grow salt-tolerant crops for reliable crop performance in salinized soil, and from this perspective, it is necessary to develop salt-tolerant crops.

Salt tolerance differs significantly between crop species (Rawson et al., 1988; Munns et al., 2006; Munns & Tester, 2008). Among Gramineae crops, for example, salt tolerance of barley is very high and that of rice is low. Salt tolerance for reliable crop performance in salinized soil, and from this perspective, it is necessary to develop salt-tolerant crops.
In Exp. 1, seeds were sown on 4 February 2012 at a rate of 9 plants pot\(^{-1}\) (3 plants hill\(^{-1}\) and 3 hills pot\(^{-1}\)). The plants were grown under a rain-shield glass roof with 1/10 strength Hoagland solution until 70 days after sowing (DAS) and with 1/2 strength Hoagland solution from 70 DAS until harvest. In Exp. 2, seeds were sown on 17 December 2004 and grown with Hoagland solution the same as in Exp. 1.

The nutrient solution at full strength contained 4.0 mM Ca(NO\(_3\))\(_2\), 4.0 mM KNO\(_3\), 1.0 mM MgSO\(_4\), 1.0 mM NH\(_4\)H\(_2\)PO\(_4\), 1.0 mM (NH\(_4\))\(_2\)HPO\(_4\), 1.0 mM NaCl, 36 μM FeNaEDTA, 12.5 μM H\(_3\)BO\(_3\), 0.25 μM CuSO\(_4\), 1.0 μM MnSO\(_4\), 1.0 μM ZnCl\(_2\), and 0.4 μM NaMoO\(_4\).

**NaCl treatment**

In Exp. 1, the NaCl treatment commenced at 30 DAS. Hoagland solution without additional NaCl (with 0 mM NaCl, control) or with 50, 100, 150, or 200 mM NaCl was supplied twice a week until harvest. In Exp. 2, the NaCl treatment commenced at 50 DAS. Hoagland solution without additional NaCl (with 0 mM NaCl, control) or with 200 mM NaCl was supplied twice a week until harvest. Each pot was completely filled with solution of a given NaCl concentration and then the solution was completely drained from the bottom of the pot. This filling-up and draining procedure was done twice each time in order to renew the solution completely and avoid salt accumulation in vermiculite.

**Measurements of leaf area and dry weight**

Plants with the average number of stems or spikes were taken for measurement. After the leaf area was measured with a leaf area meter (AAM-8; Hayashi Denko Co., Tokyo, Japan), leaves, stems and spikes were oven-dried at 80 °C to constant weight and dry weight was determined.

**Measurements of SPAD (chlorophyll), rate of photosynthesis, and leaf water potential**

Leaf color was measured with a chlorophyll meter (SPAD-502; Konica Minolta, Osaka, Japan) and expressed as a SPAD value. SPAD values correlate closely not only to leaf chlorophyll content but also to Rubisco content (Kumagai et al., 2009). Net photosynthetic rate and stomatal conductance were determined between 08:00 and 13:00 using a portable open-flow gas-exchange system (Li-6400; Li-COR, Lincoln, NE, USA). Light intensity was set to 2000 (PPFD) μmol m\(^{-2}\) s\(^{-1}\), leaf temperature to 20 °C (in late February), 23 °C (in late March and early April) or 25 °C (in mid-May), the leaf–air vapor-pressure difference to approximately 1.5 kPa, and the ambient CO\(_2\) concentration...
to 370 μmol mol⁻¹ during measurements. After stomatal conductance (gₛ) and the photosynthetic rate were measured at the ambient CO₂ concentration of 370 μmol mol⁻¹ (A₁₃₇₀), the photosynthetic rate was measured at the intercellular CO₂ concentration of 280 μmol mol⁻¹ (A₂₈₀). The photosynthetic rate without the effect of stomatal conductance, that is, biochemical limitations of photosynthetic rate in the mesophyll can be compared by the measurements of A₂₈₀.

Leaf water potential was measured with a thermocouple psychrometer by the dew point method (Hirasawa & Ishihara, 1978). Leaf discs (6 mm in diameter) were excised and loaded immediately into the sample chamber of a psychrometer (C-52; Wescor Inc., Logan, UT, USA). Leaf osmotic potential was measured by the dew point method, using the same procedure as for the measurement of leaf water potential, after the disks were thawed for approximately 30 min at room temperature. Leaf turgor pressure was calculated as the difference between the water potential and the osmotic potential of a leaf.

**Determination of ion concentration in plant tissues**

Plants were oven-dried at 80 °C and powdered with a ball mill (85,200; Qiagen). Ions in the powdered plant materials were extracted by water at 80 °C for 3 h (for leaves) and 6 h (for other organs) and their concentration was measured by ion chromatography (HIC-6A; Shimadzu Co., Kyoto, Japan) using an electrochemical detector (CDD-6A; Shimadzu, Kyoto, Japan) with a cation exchange column (Shim-pack IC-C4; Shimadzu) or anion exchange column (Shim-pack IC-A3; Shimadzu).

**Statistical analysis**

The varietal differences were tested using Student’s t-test for comparing cultivars under same treatment and Tukey–Kramer’s test for comparing cultivars and treatments. All of the statistics were analyzed using Statcell 3 (OMS Publishing, Tokorozawa, Japan).

**Results**

**Effects of strength of NaCl treatment on dry matter and grain production (Exp. 1)**

The average heading date was 95 DAS and maturity was approximately 150 DAS for both cultivars and all NaCl treatments.

Dry weight of the aboveground parts at harvest decreased as NaCl concentration increased, and in the plants receiving 200 mM NaCl treatment, dry weight of the aboveground parts decreased to between 10 and 16% of the control (Figure 1(A)). No difference was observed between the dry weights of the aboveground parts of OUC613 and OUE812 in the control, and the reduction in dry weight of plants treated with 150 mM and 200 mM NaCl was lower in OUE812 than in OUC613. With the 200 mM NaCl treatment, the dry weight of the aboveground parts was significantly larger in OUE812 than in OUC613.
### Table 1. Yield components of barley plants treated with different concentrations of NaCl.

| Treatment | Cultivar | Spike number (hill⁻¹) | Number of spikelets (panicle⁻¹) | Ripening percentage (%) | 1000-grain weight (g) |
|-----------|----------|-----------------------|---------------------------------|-------------------------|-----------------------|
| 0 mM      | OUC613   | 41.3 ± 3.0            | 27.8 ± 1.0                      | 76.4 ± 6.1              | 45.0 ± 2.1            |
|           | OUE812   | 22.5 ± 2.1            | 47.8 ± 3.5                      | 83.3 ± 1.6              | 53.9 ± 2.6            |
|           |          |                       |                                 |                         |                       |
| 50 mM     | OUC613   | 36.5 ± 1.1            | 28.5 ± 0.9                      | 70.7 ± 3.5              | 47.3 ± 1.5            |
|           | OUE812   | (88)                  | (102)                           | (93)                    | (105)                 |
|           |          |                       |                                 |                         |                       |
| 100 mM    | OUC613   | 19.3 ± 3.8            | 51.8 ± 1.9                      | 77.3 ± 4.6              | 56.1 ± 1.7            |
|           | OUE812   | (86)                  | (108)                           | (93)                    | (104)                 |
|           |          |                       |                                 |                         |                       |
| 150 mM    | OUC613   | 26.5 ± 3.8            | 27.2 ± 1.4                      | 58.2 ± 4.5              | 48.8 ± 1.2            |
|           | OUE812   | 14.5 ± 1.1            | 43.5 ± 1.4                      | 74.1 ± 7.8              | 52.7 ± 3.0            |
|           |          |                       |                                 |                         |                       |
| 200 mM    | OUC613   | 9.5 ± 2.3             | 5.1 ± 2.2                       | 16.3 ± 2.7              | 41.3 ± 1.4            |
|           | OUE812   | 8.8 ± 1.8             | 15.3 ± 0.9                      | 9.9 ± 2.6               | 40.6 ± 3.0            |
|           |          |                       |                                 |                         |                       |

Notes: Mean ± SD (n = 3). Ripening percentage (%) = (No. of ripe grains/No. of spikelets) × 100. Values for grain weight and 1000-grain weight were determined at 12.5% moisture content. Values in parentheses represent percentage values relative to those receiving 0 mM NaCl treatment.

Stem and leaf dry weight in the control was larger in OUC613 than in OUE812. Stem and leaf dry weight decreased and variated difference in the dry weight decreased as NaCl concentration increased. But OUC613 had a larger stem and leaf weight compared with OUE812 even at 200 mM NaCl (Figure 1(B)).

Grain weight decreased as NaCl concentration increased. There was no difference in grain weight between cultivars treated with the 0, 50, and 100 mM NaCl. With the 150 and 200 mM NaCl treatments, grain weight of OUC613 decreased markedly and became smaller than that of OUE812 (Figure 1(C)).

All yield components decreased as NaCl concentration increased (Table 1). In particular, spike number decreased greatly in both cultivars although it was always larger in OUC613. Ripening percentage decreased larger in OUC613 than OUE812 with 100, 150 and 200 mM NaCl treatments. The reduction in ripening percentage contributed to the reduction of grain weight in OUC613 compared with that in OUE812 treated with 150 and 200 mM NaCl.

### Growth and physiological responses to salt stress (Exp. 2)

#### Growth responses

The average heading date was 125 DAS and maturity was approximately 185 DAS in both accessions under all NaCl treatments.

Plant length increased sigmoidally with DAS (Figure S1). The effect of 200 mM NaCl appeared immediately after starting the treatment, but the effect was small. The difference in plant length between plants in the control and those treated with 200 mM NaCl became larger during the period of stem elongation starting approximately three weeks before heading, and plants treated with 200 mM NaCl were significantly shorter than those in the control.

No difference in plant length was observed between OUC613 and OUE812 in the control or between OUC613 and OUE812 treated with 200 mM NaCl, although in both treatments, OUC613 tended to be taller than OUE812 from approximately three to four weeks before heading.

In plants of the control, OUC613 had 11 leaves on the main stem and OUE812 had 13 at full heading; in plants treated with 200 mM NaCl, OUC613 had 11 leaves on the main stem and OUE812 had 12 (Table 2). NaCl treatment started at the beginning of the expansion of the 4th leaf. The effects of the NaCl treatment on leaf area appeared with the sixth leaf, which was fully expanded approximately one month after starting the NaCl treatment. The difference in leaf area between NaCl treatments became clear in the upper leaves from the seventh to the flag leaves from approximately 85 DAS, and the area was significantly smaller with 200 mM NaCl than the control plants. However, no clear difference in the reduction of leaf area was observed between cultivars.

In the early ripening stage (140 DAS), the total leaf area of plants treated with 200 mM NaCl was 33.3% (for OUC613) and 39.2% (for OUE812) of the total leaf area of the respective control plants (Figure 2(A)). Similarly, the dry weight of the aboveground parts of plants treated with 200 mM NaCl was 40.9% (for OUC613) and 44.7% (for OUE812) of the dry weight of the aboveground parts of plants in the control (Figure 2(B)). Total leaf area and the dry weight of the aboveground parts tended to be large in OUE812 compared with OUC613 for the plants with 200 mM NaCl treatment, but the differences were not significant.

At harvest, the dry weight of the aboveground parts of plants treated with 200 mM NaCl was 24.7% (OUC613) and 35.3% (OUE812) of the aboveground dry weight of plants in the control, and in plants treated with 200 mM NaCl the dry weight of OUC613 was smaller than that of OUE812 (Table 3). Grain weight in plants treated with 200 mM NaCl as compared to weights in plants in the control, was 16.9% and 32.3% in OUC613 and OUE812, respectively, and was smaller in OUC613 than in OUE812 (Table 3). The varietal difference in the reduction of grain weight resulted mainly from the marked reduction in the ripening percentage of OUC613. Varietal differences in the reduction of the number of spikes, the number of spikelets per spike, and 1000-grain weight were small compared with the differences in the ripening percentage, although the number of spikes was relatively large and the reduction in the number of spikelets per spike was relatively small in OUE812 compared with OUC613.
Table 2. Areas (cm²) of barley leaves at different positions on the main stem.

| Treatment | Cultivar | 1st   | 2nd   | 3rd   | 4th   | 5th   | 6th   | 7th   | 8th   | 9th   | 10th  | 11th  | 12th  | 13th  |
|-----------|----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0 mM      | OUC613   | 4.2 ± 0.6<sup>b</sup> | 6.8 ± 1.4<sup>a</sup> | 10.7 ± 0.9<sup>a</sup> | 9.6 ± 2.4<sup>b</sup> | 21.8 ± 2.1<sup>ab</sup> | 25.9 ± 0.9<sup>b</sup> | 26.3 ± 1.2<sup>b</sup> | 25.2 ± 1.2<sup>ab</sup> | 29.5 ± 2.0<sup>b</sup> | 31.9 ± 1.4<sup>b</sup> | 14.4 ± 1.3<sup>c</sup> | –     | –     |
|           | OUE812   | 5.5 ± 0.2<sup>a</sup> | 6.5 ± 1.3<sup>a</sup> | 9.4 ± 3.1<sup>a</sup> | 18.1 ± 0.7<sup>a</sup> | 21.9 ± 1.7<sup>ab</sup> | 28.3 ± 2.9<sup>a</sup> | 32.7 ± 2.2<sup>a</sup> | 36.1 ± 2.7<sup>a</sup> | 45.4 ± 3.4<sup>a</sup> | 49.6 ± 2.7<sup>a</sup> | 47.9 ± 2.2<sup>a</sup> | 41.0 ± 2.2<sup>a</sup> | 19.3 ± 1.9<sup>a</sup> |
| 200 mM    | OUC613   | 4.2 ± 0.6<sup>b</sup> | 6.8 ± 1.4<sup>a</sup> | 10.7 ± 0.9<sup>a</sup> | 9.1 ± 1.9<sup>b</sup> | 18.1 ± 0.6<sup>b</sup> | 21.8 ± 0.6<sup>c</sup> | 19.2 ± 1.3<sup>b</sup> | 18.2 ± 2.0<sup>c</sup> | 18.9 ± 0.5<sup>c</sup> | 18.1 ± 1.4<sup>b</sup> | 7.3 ± 0.4<sup>b</sup> | –     | –     |
|           | OUE812   | 5.5 ± 0.2<sup>a</sup> | 6.5 ± 1.3<sup>a</sup> | 9.4 ± 3.1<sup>a</sup> | 14.3<sup>a</sup> | 24.4 ± 0.3<sup>a</sup> | 24.7 ± 1.6<sup>ab</sup> | 24.9 ± 1.2<sup>b</sup> | 26.0 ± 0.8<sup>b</sup> | 25.5 ± 0.4<sup>b</sup> | 24.2 ± 0.4<sup>b</sup> | 20.5 ± 1.7<sup>b</sup> | 10.1 ± 0.6<sup>b</sup> |

Notes: Mean ± SD (n = 3). Same letter represents no significant difference between plants for leaves at each position (p < 0.05, Tukey-Kramer's test). #, measured for only one replication.
Physiological responses

In the control plants, SPAD values were larger in the upper leaves at the early ripening stage (131 DAS) and tended to be larger in OUC613 than in OUE812 (Table 4). On the other hand, in plants treated with 200 mM NaCl, the SPAD value remained large, especially in the lower leaves, compared with the plants in the control. No difference in the SPAD value was observed between OUC613 and OUE812 at any leaf position.

\( A_{\text{ca370}} \) and \( g_s \) decreased significantly at three weeks after starting the 200 mM NaCl treatment; however, no difference between cultivars in \( A_{\text{ca370}} \) or \( g_s \) was observed (Figure S2). At 7 weeks after starting NaCl treatment (Figure 3) and at 14 weeks after starting NaCl treatment (in the early ripening stage, Figure 4), there was no difference between cultivars in \( A_{\text{ca370}} \) or \( g_s \) for upper and lower leaves in the plants in the control. At seven weeks after starting NaCl treatment, plants treated with 200 mM NaCl showed significantly lower \( A_{\text{ca370}} \) and \( g_s \) in the upper and lower leaves compared with the plants in the control; values of \( A_{\text{ca370}} \) and \( g_s \) tended to be smaller but not significant in the upper leaves (Figure 3(A) and (B)) and were significantly smaller in the lower leaves in OUC613 than in OUE812 (Figure 3(D) and (E)). There was no difference in \( A_{\text{ci280}} \) for the upper leaves (Figure 3(C)).

\( A_{\text{ca370}} \) was significantly smaller and \( g_s \) tended to be smaller in OUC613 than in OUE812 for the upper and middle leaves in the early ripening stage (Figure 4(A), (B), (D), (E)). \( A_{\text{ci280}} \) was lower for the upper and middle leaves in OUC613 than in OUE812 (Figure 4(C), (F)). In contrast, for the lower leaves, \( A_{\text{ca370}} \) and \( A_{\text{ci280}} \) but not \( g_s \) remained high in plants treated with 200 mM NaCl compared with the plants in the control.

OUC613 compared with OUE812. Harvest index decreased large in OUC613 at 200 mM NaCl. This was caused mainly by a marked reduction in the ripening percentage.

Table 3. Dry weight of aboveground parts, grain weight, and yield components of barley.

| Treatment | Cultivar | No. of spikes (hill\(^{-1}\)) | No. of spikelets (spike\(^{-1}\)) | Ripening percentage (%) | 1000-grain weight (g) | Grain weight (g) | Above-ground weight (g hill\(^{-1}\)) | Harvest index |
|-----------|----------|-------------------------------|----------------------------------|-------------------------|---------------------|-----------------|-----------------------------|-------------|
| 0 mM      | OUC613   | 53.3\(^{a}\)                   | 28.4\(^{a}\)                     | 85.3\(^{a}\)            | 49.1\(^{a}\)       | 63.4\(^{ab}\)   | 129.4\(^{a}\)               | 0.490\(^{ab}\) |
|           | OUE812   | 27.0\(^{b}\)                   | 53.8\(^{a}\)                     | 85.3\(^{a}\)            | 60.9\(^{a}\)       | 75.6\(^{ab}\)   | 126.5\(^{a}\)               | 0.597\(^{a}\) |
| 200 mM    | OUC613   | 24.3\(^{b}\)                   | 24.5\(^{a}\)                     | 40.3\(^{a}\)            | 45.5\(^{a}\)       | 10.7\(^{a}\)     | 31.9\(^{a}\)                | 0.339\(^{c}\) |
|           | OUE812   | 16.7\(^{b}\)                   | 41.3\(^{a}\)                     | 68.8\(^{a}\)            | 51.8\(^{a}\)       | 24.4\(^{a}\)     | 44.6\(^{a}\)                | 0.551\(^{ab}\) |

Notes: Means ± SD (n = 4). Ripe grains were selected with a sieve (2.2 mm mesh). Grain weight was measured at 12.5% moisture. Items labeled with the same letter within a column do not differ significantly (p < 0.05, Tukey-Kramer’s test).

Table 4. SPAD value of barley leaves at different positions on a stem at the early ripening stage (131 DAS; 80 days after starting NaCl treatment).

| Treatment | Cultivar | 5th | 6th | 7th | 8th | 9th | 10th | 11th | 12th | 13th |
|-----------|----------|-----|-----|-----|-----|-----|------|------|------|------|
| 0 mM      | OUC613   | 33.1 ± 1.9\(^{a}\) | 38.6 ± 3.4\(^{ab}\) | 43.6 ± 0.5\(^{b}\) | 48.6 ± 1.2\(^{b}\) | 52.7 ± 1.5\(^{ab}\) | 54.1 ± 1.4\(^{ab}\) | – | – |
|           | OUE812   | 30.2 ± 6.5\(^{b}\) | 35.3 ± 2.4\(^{b}\) | 39.7 ± 1.2\(^{b}\) | 42.4 ± 1.3\(^{a}\) | 45.8 ± 1.8\(^{a}\) | 49.9 ± 1.6\(^{ab}\) | 51.1 ± 1.2\(^{ab}\) | 51.1 ± 0.5\(^{a}\) | 51.2 ± 1.1 |
| 200 mM    | OUC613   | 33.4 ± 3.5\(^{b}\) | 48.3 ± 3.3\(^{ab}\) | 52.4 ± 4.0\(^{b}\) | 52.0 ± 2.6\(^{a}\) | 58.8 ± 1.7\(^{a}\) | 58.3 ± 1.1\(^{a}\) | 54.9 ± 0.8\(^{a}\) | – | – |
|           | OUE812   | 40.0 ± 4.2\(^{a}\) | 46.4 ± 3.1\(^{ab}\) | 49.3 ± 2.5\(^{b}\) | 54.2 ± 1.7\(^{a}\) | 58.9 ± 0.9\(^{a}\) | 60.7 ± 1.9\(^{a}\) | 56.3 ± 1.8\(^{a}\) | 55.3 ± 1.9\(^{a}\) | – | – |

Notes: Mean ± SD (n = 3). Same letter represents no significant difference between plants for leaves at each position (p < 0.05, Tukey-Kramer’s test).
The same results were obtained for the middle leaves (data not shown).

Discussion

Degree of salt stress and growth stage at which a critical difference between cultivars appears

In barley, dry weight of aboveground part and grain weight at harvest decreased to half of the control before the concentration of NaCl treatment increased to 150 mM (equivalent to approximately 15 dS m⁻¹) and differences of cultivars in dry matter and grain production became

(Figure 4(G)–(I)). In the lower leaves of plants treated with 200 mM NaCl, $A_{\text{ca370}}$ was significantly higher in OUE812 than OUC613 and, however, differences were not found in $g_s$ and $A_{\text{ci280}}$ between cultivars.

Leaf water and osmotic potential in the upper leaves decreased significantly in plants treated with 200 mM NaCl compared with plants in the control (Figure 5(A), (B)). Since the reduction in osmotic potential was substantial compared with the change in water potential, leaf turgor pressure increased somewhat in plants treated with 200 mM NaCl (Figure 5(C)). No significant difference in leaf water potential, osmotic potential, or turgor pressure was observed between OUC613 and OUE812 in either the control or the 200 mM NaCl treatment. The same results were obtained for the middle leaves (data not shown).

Figure 3. The rate of photosynthesis at the ambient CO₂ concentration of 370 μmol mol⁻¹ ($A_{\text{ca370}}$; A, D), stomatal conductance ($g_s$; B, E), and the rate of photosynthesis at the intercellular CO₂ concentration of 280 μmol mol⁻¹ ($A_{\text{ci280}}$; C) of barley leaves at 93–97DAS (approximately 7 weeks after starting NaCl treatment).

Notes: A, B, and C show results for leaves approximately 10 days after full expansion (upper leaves), and D and E show results for leaves approximately 35 days after full expansion (lower leaves). Error bars represent SD ($n = 6$). C-0 and E-0 represent cvs. OUC613 and OUE812 treated with 0 mM NaCl, respectively, and C-200 and E-200 represent OUC613 and OUE812 treated with 200 mM NaCl, respectively. Leaf temperature was controlled at 23 °C. Bars labeled with the same letter in each figure do not significantly differ ($p < 0.05$, Tukey–Kramer’s test).
under long-term salt stress as target traits for breeding. In Figure 6, potential causes of the cultivar difference in grain weight are shown for plants under salt stress in the case of this study. Leaf expansion and the photosynthetic rate of a leaf under salt stress are expected to be important for dry matter production, and dry matter production and photoassimilate translocation to the grains are expected to be important for yield (Hay & Porter, 2006). Spikelet number, ripening percentage, and one grain weight determine grain weight (yield) and, therefore, affect harvest index significantly.

Leaf growth and leaf photosynthesis were affected significantly from approximately 1 month and 3 weeks after starting the 200 mM NaCl treatment, respectively (Figure S2 and Table 2). However, no significant difference in the reduction of leaf growth was observed between OUC613 and OUE812 (Figure S2 and Table 2). Significant cultivar difference was observed in leaf photosynthetic rate of the lower leaves at seven weeks after starting the 200 mM NaCl treatment (93-97 DAS) (Figure 3). However, no significant difference in the SPAD value under salt stress was observed between OUC613 and OUE812 even at 131 DAS.

Richards et al. (1987) also observed that differences of cultivars in grain yield and biomass in wheat and barley increased as the soil electrical conductivity increased. In rice, which is one of the most salt-sensitive crops, differences in dry matter production appeared in cultivars treated with as little as approximately 80 mM NaCl (Yamanouchi, 1989). The hydroponic NaCl concentration at which dry matter production decreases to half is approximately 70 mM for rice and 125 mM for barley (Munns & Tester, 2008). These results indicate that for barley, differences in response to salt stress and a need to identify tolerant cultivars appear under relatively severe salt stress conditions. In this point, OUE812 has important traits for growing in highly salinized soil.

A number of characteristics have already been examined with respect to tolerance of short- and long-term salt stress (Rawson et al., 1988; Tsuchiya et al., 1994; Dubey & Pessarakli, 1995; Dubey, 1996; Munns et al., 2003; Munns & Tester, 2008; Pessarakli & Szabolcs, 2011). From an agronomic perspective, the most important traits affecting dry matter and grain production should be selected under long-term salt stress as target traits for breeding. In Figure 6, potential causes of the cultivar difference in grain weight are shown for plants under salt stress in the case of this study. Leaf expansion and the photosynthetic rate of a leaf under salt stress are expected to be important for dry matter production, and dry matter production and photoassimilate translocation to the grains are expected to be important for yield (Hay & Porter, 2006). Spikelet number, ripening percentage, and one grain weight determine grain weight (yield) and, therefore, affect harvest index significantly.

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weeks of starting the 200 mM NaCl treatment, but it took several weeks thereafter to observe any significant cultivar differences.

However, differences in leaf water potential appear within a few days in barley seedlings grown in hydroponic solution containing 100 mM NaCl (Watanabe et al., 2008). OUE812 maintained a higher leaf water potential and a higher rate of photosynthesis than OUC613. These results indicate that although a significant difference appears immediately after starting the NaCl treatment (short-term salt stress), this significant difference quickly disappears and under long-term salt stress a significant difference takes several weeks to reappear and become larger with time after NaCl treatment (long-term salt stress). It is said short-term salt stress is induced by water stress and that long-term stress is induced by salt accumulation in a plant (Munns et al., 2003). The appearance and disappearance of the difference between cultivars might therefore reflect different mechanisms underlying short- and long-term salt stress. This remains a topic for future research.

**Cultivar difference in photosynthesis**

Stomatal conductance ($g_s$) and $C_i$ decreased large in OUC613 in the lower leaves at seven weeks after starting the NaCl treatment (Figure 3(E)). This suggests that the cause of the cultivar difference in $A_{ca370}$ was mainly the reduction in $g_s$. At 14 weeks after starting the NaCl treatment, $g_s$ decreased without significant cultivar difference and $A_{ci280}$ decreased with significant cultivar difference in the upper and middle leaves of plants treated with 200 mM NaCl (Figure 4(B)–(F)). This suggests that biochemical limitations in the mesophyll contributed to the cultivar difference in $A_{ca370}$ in the upper and middle leaves after long-term salt stress (Dubey, 1996). In contrast, $A_{ci280}$ in the lower leaves of plants treated with 200 mM NaCl increased significantly at 14 weeks after starting the NaCl treatment, suggesting that mesophyll photosynthetic activity could have contributed to the increase in $A_{ca370}$ probably due to keeping higher leaf nitrogen content and slow leaf senescence as suggested by the SPAD results (Table 4). Long-term water stress significantly increases leaf nitrogen content and chlorophyll content of growing leaves, because growth suppression is larger than the suppression of mineral uptake (Pessarakli et al., 1989; Dubey & Pessarakli, 1995; Kramer & Boyer, 1995). Various kinds of nitrogen compound accumulate in the plants under salt stress as well as under water stress (Dubey & Pessarakli, 1995). A number of nitrogen containing compounds accumulate in plants under salt stress (Mansour, 2000; Sharma & Dubey, 2011). Some compatible solute accumulated under salt stress might retard senescence of lower leaves under salt stress (Demiral & Türken, 2006).
matter production appeared during the period from early ripening to harvest (Table 3). Keeping a high photosynthetic rate during ripening might have contributed to the higher grain weight via increasing dry matter production in OUE812.

The reduction in the ripening percentage was severe in both cultivars, but much less so in OUE812, and this is another cause of the remarkable reduction in grain weight in OUC613 as compared with OUE812 (Figure 1, Table 1). It is well known that drought during the meiosis and flowering stages induces severe infertility in many crops (Kramer & Boyer, 1995; Tajima, 1995). Salt stress induces severe infertility in rice (Khatun & Flowers, 1995; Rao et al., 2008). Infertility might be a cause of the significant reduction in the ripening percentage. However, there are no reports of salinity-induced infertility in barley to our knowledge. Transport of Na$^+$ and Cl$^-$ in the phloem to the apex is sufficiently well-controlled to prevent the concentration of these ions reaching toxic levels in the cells of the reproductive apex in barley under salt stress (Munns & Rawson, 1999). We also observed that Na$^+$ and Cl$^-$ concentrations were far lower in the tissues in the spike than in the whole shoot of the plants treated with 100 mM NaCl (Figures S5 and S6), and that Na$^+$ and Cl$^-$ concentrations in spike organs were rather low in OUC613 as compared with OUE812 (Figure S6). However, ripening percentage even in 100 mM NaCl treatment was severely affected in the salt-sensitive variety, OUC613 compared with OUE812 (Table 1). This demonstrates that some mechanism other than the direct effects of salt is involved in the reproductive organs in OUC613. This remains a topic for a future research.

**Conclusion**

We can summarize cultivar differences in effects of long-term salinity as in Figure 6. Long-term salinity decreased

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**Figure 6.** A scheme showing potential causes of the varietal difference in the reduction of grain weight of plants under salt stress in this study. Notes: Responses observed with and without significant varietal difference are shown in open and shaded boxes, respectively. Responses in parentheses have not been examined.

Leaf water status affects the rate of photosynthesis (Hsiao, 1973). However, there was no difference in leaf water potential or turgor pressure between cultivars treated with 200 mM NaCl (Figure 5). Very small difference in $g_s$ was observed between OUC613 and OUE812 except for the lower leaves at 93-97 DAS (Figures 3 and 4). Very small cultivar difference in transpiration might cause no difference in leaf water potential (Figure 5). Accumulation of Na$^+$, Cl$^-$, and K$^+$ affects the rate of photosynthesis of plants under salt stress (Yeo et al., 1985; Maegawa et al., 1987; Dionisio-Sese & Tobita, 2000). Some difference between the cultivars in the rate of photosynthesis was observed in plants treated with 100 mM NaCl at the early ripening stage (Figure S3). The leaf concentration of Na$^+$ and Cl$^-$ was larger in OUC613 than in OUE812 and the leaf concentration of K$^+$ was much smaller in the leaves of OUC613 than in OUE812 (Figure S4). As a result, the concentration ratio of K$^+$ to Na$^+$ became larger in OUE812 than in OUC613. The maintenance of photosynthesis in OUE812 under conditions of salt stress might be a result of keeping low concentrations of Na$^+$ and Cl$^-$ and a low Na$^+$/K$^+$ ratio (Munns & Tester, 2008).

**Cultivar differences in grain weight and ripening percentage**

A remarkable difference between cultivars in grain weight was observed with the 150 and 200 mM NaCl treatments (Figure 1). Almost all photosynthate produced after heading is translocated to the grains in barley same as other cereal crops (Hay & Porter, 2006). Although the varietal difference in the reduction of $A_{\text{net}}$, was observed in the lower leaves before heading (Figure 3), the aboveground weight did not differ significantly between cultivars even at early ripening (Figure 2). OUE812 kept a higher photosynthetic rate from the upper to lower leaves during ripening than OUC613 (Figure 4), and a difference in dry matter production appeared during the period from early ripening to harvest (Table 3). Keeping a high photosynthetic rate during ripening might have contributed to the higher grain weight via increasing dry matter production in OUE812.

The reduction in the ripening percentage was severe in both cultivars, but much less so in OUE812, and this is another cause of the remarkable reduction in grain weight in OUC613 as compared with OUE812 (Figure 1, Table 1). It is well known that drought during the meiosis and flowering stages induces severe infertility in many crops (Kramer & Boyer, 1995; Tajima, 1995). Salt stress induces severe infertility in rice (Khatun & Flowers, 1995; Rao et al., 2008). Infertility might be a cause of the significant reduction in the ripening percentage. However, there are no reports of salinity-induced infertility in barley to our knowledge. Transport of Na$^+$ and Cl$^-$ in the phloem to the apex is sufficiently well-controlled to prevent the concentration of these ions reaching toxic levels in the cells of the reproductive apex in barley under salt stress (Munns & Rawson, 1999). We also observed that Na$^+$ and Cl$^-$ concentrations were far lower in the tissues in the spike than in the whole shoot of the plants treated with 100 mM NaCl (Figures S5 and S6), and that Na$^+$ and Cl$^-$ concentrations in spike organs were rather low in OUC613 as compared with OUE812 (Figure S6). However, ripening percentage even in 100 mM NaCl treatment was severely affected in the salt-sensitive variety, OUC613 compared with OUE812 (Table 1). This demonstrates that some mechanism other than the direct effects of salt is involved in the reproductive organs in OUC613. This remains a topic for a future research.
dry matter production largely by decreasing photosynthetic rate probably due to the changes in the concentration of Na⁺, Cl⁻ and K⁺ and the ratio of K⁺ to Na⁺ in leaves, and decreased grain weight largely by decreasing ripening percentage in OUC613 compared with OUE812. Higher ripening percentage and higher rate of photosynthesis during ripening might be target traits in breeding to improve the tolerance of barley to long-term salt stress.

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