Accurate evaluation and verification of varietal ranking for flooding tolerance at the seedling stage in barley (*Hordeum vulgare* L.)

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Soil flooding or waterlogging is a major abiotic stress in upland crops. In barley, there have been several reported studies of selection for flooding-tolerant genotypes, but it is difficult to obtain varietal rankings that are consistent among researchers. Our objectives were to establish experimental conditions that could be applied by other research groups and to verify the varietal ranking conducted in an earlier study. We conducted greenhouse experiments on 14 barley varieties. At the 2.5-leaf stage, they were flooded with 0% or 0.1% soluble starch solution (mimicking reducing conditions). At 13 to 15 days after the start of treatment, the degree of leaf injury and the shoot dry weight ratio (treatment:control) were recorded. Reliable and highly repeatable results were obtained for the criterion of leaf injury under reducing conditions, whereas shoot dry weight ratio was unstable. The varieties OUJ820 and OUA301 were highly tolerant, whereas OUA002 and OUJ247 were sensitive; these results matched those of the earlier study. The experimental conditions that we developed here may be useful for selection testing and genetic analysis of flooding tolerance in other laboratories.

**Key Words:** barley, flooding, genetic resources, soil reduction, variation, waterlogging.

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**Introduction**

Soil flooding or waterlogging is a major abiotic stress that results in substantial yield losses in both winter and summer crops. In Japan, which is located in the Asian monsoon region, upland crops are sometimes grown in upland paddy fields in order to maximize land productivity. In such fields, however, temporary flooding often occurs because of inadequate drainage of the soils. Of the winter crops, barley shows sensitivity to soil flooding (Ikeda *et al.* 1955, Kato *et al.* 2007, Wang *et al.* 1996) and barley seedlings are damaged under oxygen-deficient flooded or waterlogged soils. Also, in spring, the soil temperature gradually increases, resulting in a reducing soil environment. Therefore, in addition to low oxygen stress, production of toxic chemical species (e.g., Fe$^{2+}$, H$_2$S) is induced by soil flooding, in particular under higher temperatures (Yamasaki 1952).

As with a number of other abiotic stress tolerances, flooding tolerance is a complex trait involving many morphological, anatomical and physiological characteristics. Flooding tolerance is an inherited quantitative trait with relatively low heritability in terms of its ability to prevent a reduction of grain yield in wheat (Collaku and Harrison 2005) and barley (Hamachi *et al.* 1990); the exception was a study by Okubo and Takeda (1994), who obtained moderate to high heritabilities in a barley diallel analysis. With such a complex trait, varietal ranking could change according to the stage of growth, and the results of screening for flooding tolerance in the early growth stages are not always representative of final yield (Dickin *et al.* 2009). Therefore, the evaluation of flooding tolerance at each stage and the pyramiding of multiple factors (genes) associated with flooding tolerance are essential for establishing varieties that are tolerant of flooding throughout all stages of growth. Such an attempt has been made by several researchers, but it is still difficult to obtain varietal rankings for flooding tolerance that are consistent among researchers (Mano and Oyanagi 2009, Oyanagi 2011). One reason may be the difficulty in conducting evaluations of the same trait in different laboratories; another may be a lack of diversity in the breeding lines that are usually evaluated for flooding tolerance for practical breeding purposes.

The University of Tasmania, Australia, has recently been studying waterlogging tolerance in barley (Pang *et al.* 2004, Zhou *et al.* 2007), and successful quantitative trait loci (QTL) detection has been achieved by using tolerant (TX9425 and Yerong) and sensitive varieties (Franklin; Li *et al.* 2008, Xue *et al.* 2010). These studies have emphasized the importance of the screening methods used (Zhou 2011). They have used mainly soil from one frequently waterlogged site (at the Cressy Research Station in Tasmania) in their greenhouse experiments; it would therefore be difficult
to establish the same experimental conditions (using the same soil) in other countries or laboratories.

In general, stress tolerance is evaluated by measuring the reduction in biomass of plants grown under stress conditions (e.g., Kubo et al. 2007, Malik et al. 2011). In addition, leaf injury is reliable for evaluating flooding tolerance and is used widely for QTL analyses in barley (Li et al. 2008, Zhou 2011), maize (Mano et al. 2006) and soybean (Cornelious et al. 2005, Reyna et al. 2003). However, the degree of leaf injury under flooded conditions can differ widely according to soil type.

In a study in rice, application of soluble starch to soils reliably induced reducing soil conditions (low soil redox potential, Eh) under flooded conditions (Ishihara et al. 1981). Also, in a study in maize, mimicking reducing soil conditions by using commercial potting compost with soluble starch was effective in treating stable and severe flooding stress and detecting clear varietal differences (Mano et al. 2006). In this study, we applied soluble starch treatments to barley to standardize the soil conditions.

In earlier studies of large numbers of varieties, superior genotypes for flooding tolerance and pre-germination flooding tolerance have been selected in barley (Qi and Ke 1991, Stanca et al. 2003, Takeda 1989, Takeda and Fukuyama 1986) and wheat (Takeda et al. 1987). By using unique barley germplasm resources that were selected by Takeda (1989) because they exhibit high flooding tolerance at the seedling stage, we attempted to: (1) establish experimental conditions for flooding tolerance that could be applied by other research groups; and (2) verify the varietal ranking devised in an earlier study (Takeda 1989). The experimental conditions that we developed may be useful in selection tests and genetic analysis of flooding tolerance by other laboratories.

### Materials and Methods

#### Plant materials

A total of 14 barley varieties were used (Table 1). The 13 varieties starting with the code “OU” were provided by the Institute of Plant Science and Resources, Okayama University with the support in part by the National Bio-Resource Project of the MEXT, Japan. The remaining variety, Norin-37 (N-37), was provided by the NARO Institute of Crop Science. In a previous seedling test using 4096 varieties (Takeda 1989), 7 showed flooding tolerance, 4 showed sensitivity and 3 were of unknown status (Table 1). Of the 3 with unknown status, OUJ251 and OUJ247 have been classified in field experiments into a moderate to sensitive group (Hamachi et al. 1985, Kato et al. 2007) and N-37 has been classified into a moderate group (Yoshioka et al. 2005).

#### Comparison of soil types (Experiment 1)

To determine the soil conditions suitable for reliable evaluation of flooding tolerance, greenhouse experiments with natural daylight (daylength approximately 11 h) were conducted in two periods in autumn (October–November and November 2009) at the NARO Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan. The seedlings of three of the varieties (OUA301, which is tolerant at the seedling stage; OUE265, which is tolerant; and OUJ251, whose tolerance is unknown) were grown in Wagner pots (11-cm diameter, 15-cm depth) filled with either of the following two types of commercial potting compost: (1) Granular soil (designated Gr-soil): “Kureha Engei Baido” (Kureha Chemical Industry, Tokyo, Japan; fertilized with 0.4 g/kg N, 1.0 g/kg P and 0.6 g/kg K, volume-weight of 0.85 kg/L, size of granular soil ranged from 1.5-3 mm in diameter).

| Code       | Name                  | Flooding tolerance | Origin | Hull type | Row type | Uzu semi-brachytic |
|------------|-----------------------|--------------------|--------|-----------|----------|-------------------|
| 1          | OUJ820                | Tolerant           | Japan  | Naked     | Six-rowed | Uzu               |
| 2          | N-37c                 | Sayakaze           | Unknown| Covered   | Six-rowed | Non-uzu           |
| 3          | OUA301                | Tolerant           | Canada | Covered   | Six-rowed | Non-uzu           |
| 4          | OUC034                | Tolerant           | China  | Covered   | Six-rowed | Non-uzu           |
| 5          | OUI003                | Tolerant           | India  | Covered   | Six-rowed | Non-uzu           |
| 6          | OUE265                | Tolerant           | Ethiopia| Covered | Six-rowed | Non-uzu           |
| 7          | OUK121                | Tolerant           | North Korea| Covered | Six-rowed | Non-uzu           |
| 8          | OUS623                | Sensitive          | Japan  | Naked     | Six-rowed | Non-uzu           |
| 9          | OUB057                | Tolerant           | Egypt  | Covered   | Six-rowed | Non-uzu           |
| 10         | OUA604                | Sensitive          | Canada | Covered   | Six-rowed | Non-uzu           |
| 11         | OUA610                | Sensitive          | Canada | Covered   | Six-rowed | Non-uzu           |
| 12         | OUJ251                | Unknown            | Japan  | Covered   | Two-rounded| Non-uzu          |
| 13         | OUJ247                | Unknown            | Japan  | Covered   | Two-rounded| Non-uzu          |
| 14         | OUA002                | Sensitive          | Canada | Covered   | Two-rounded| Non-uzu          |

a Accession numbers beginning with “OU” are from the Barley Germplasm Center, Institute of Plant Science and Resources, Okayama University.

b Flooding tolerance at the seedling stage, as evaluated by Takeda (1989).

c Norin-37.
(2) Mixture of granular soil and organic soil (M-soil), consisting of a mixture (at a 4:3 weight-based ratio) of Gr-soil and the organic soil “Frontier-2” (Ings Corporation, Tochigi, Japan; 0.4 g/kg N, 2.0 g/kg P and 0.4 g/kg K, volume-weight of 0.5 kg/L).

The porosity of these potting composts was much larger compared to paddy soil.

We had previously used Gr-soil in a flooding study of maize in summer (Mano et al. 2006). In the present study we tested an additional type of soil, “M-soil”, to increase soil reduction by adding organic matter. A single seedling was grown per pot and a total of 16 pots per variety (8 pots per soil type) were tested.

After the seedlings had reached the 2.5-leaf stage, they were divided into two groups, one flooded with 0% soluble starch solution (FL group) and the other with 0.1% soluble starch solution (Wako, Osaka, Japan) (RD [i.e., reduced] group) \( n = 4 \) for each) to a depth of 1 cm above the soil surface. During RD treatment, ventilation in the greenhouse was performed to prevent the release of noxious gases from the soil. At 14 days after the start of the treatment, the degree of leaf injury (LI) was visually recorded according to the criteria shown in Table 2.

Variations in flooding tolerance (Experiment 2)

Using the 14 barley varieties listed in Table 1, we conducted flooding experiments in the same greenhouse as used in Experiment 1, but over 3 periods between February and May 2011 with 2 replications (Table 3). Daylengths during the experiments ranged from 10 h (January) to 14 h (May). The minimum temperature in the greenhouse was above 10°C throughout all experiments. Soil temperature was measured at 11 am during the flooding treatment (Table 3).

The seedlings were grown at four plants per pot in Wagner pots (16-cm diameter, 19-cm depth) filled with M-soil and a total of three pots per variety were tested. This soil type was chosen on the basis of the results of Experiment 1. After reaching the 2.5-leaf stage, they were divided into three groups. Two groups were flooded with 0% (FL) or 0.1% soluble starch solution (RD) to a depth of 1 cm above the soil surface, and the remaining group was not flooded (C [i.e., control]). At 13 to 15 days after the start of the flooded treatment, the degree of leaf injury was recorded (see Table 2). In addition, the shoots of each plant were harvested, dried at 70°C for 3 days and then weighed.

### Table 2. Key used to score flooding tolerance under reducing soil conditions at the seedling stage in barley

| Score | Class          | Degree of leaf injury                                      |
|-------|----------------|------------------------------------------------------------|
| 1     | Sensitive      | Chlorosis in the first, second and over 50% of the third, leaves from the bottom |
| 2     | Moderate       | Chlorosis in the first and second leaves                    |
| 3     | Tolerant       | No, or only slight, chlorosis in the first leaf and clear in the upper leaves |

Intermediate scores (e.g., 1.5, 2.5, 3.5 and 4.5) were given when appropriate.

### Table 3. Experimental conditions of soil temperature, Eh and pH at 11 am during flooding treatments (Experiment 2)

| Period | Replication | Date        | Soil temperature \(^\circ\mathrm{C}\) | Eh (mV) | pH   |
|--------|-------------|-------------|-------------------------------------|---------|------|
|        |             | Sowing      | FL\(^a\) | RD\(^a\) | FL\(^b\) | RD\(^b\) | FL\(^c\) | RD\(^c\) | FL\(^d\) | RD\(^d\) |
|        |             | Treatment   | C\(^e\) | FL\(^f\) | RD\(^g\) | C\(^h\) | FL\(^i\) | RD\(^j\) | C\(^k\) | FL\(^l\) | RD\(^m\) |
| 1      | 1           | 1 Jan 2011  | 16.1 (2.8) | 15.1 (2.2) | 14.4 (1.7) | 450 (31) | 231 (15) | 397 (27) | 131 (4) | 6.67 (0.03) | 6.47 (0.08) | 6.59 (0.04) | 6.31 (0.03) |
|        | 2           | 14 Jan 2011 | 17.0 (4.2) | 15.6 (2.6) | 16.4 (3.0) | 477 (26) | 213 (33) | 347 (23) | 118 (28) | 6.60 (0.08) | 6.88 (0.10) | 6.62 (0.07) | 6.73 (0.04) |
| 2      | 1           | 15 Mar 2011 | 17.9 (1.7) | 17.6 (1.7) | 17.4 (1.5) | 523 (25) | 99 (32)  | 308 (23) | 132 (19) | 6.62 (0.14) | 6.31 (0.06) | 6.71 (0.08) | 6.09 (0.05) |
|        | 2           | 25 Feb 2011 | 21.3 (2.3) | 19.0 (1.6) | 19.1 (1.9) | 520 (10) | 178 (135) | 349 (54) | 115 (23) | – (0.06) | – (0.03) | – (0.03) | – (0.03) |
| 3      | 1           | 5 Apr 2011  | 20.8 (2.3) | 21.5 (2.8) | 20.6 (3.0) | 510 (18) | 44 (130) | 392 (51) | 113 (22) | 6.39 (0.14) | 6.15 (0.11) | 6.39 (0.09) | 5.66 (0.05) | 6.01 (0.07) |
|        | 2           | 9 Apr 2011  | 25.1 (4.0) | 22.4 (2.1) | 23.2 (2.5) | 487 (56) | 16 (32)  | 47 (133) | 89 (12) | 6.36 (0.04) | 6.04 (0.09) | 6.37 (0.05) | 5.70 (0.08) | 6.03 (0.08) |

\(^a\) Control (non-flooded).
\(^b\) Flooded conditions.
\(^c\) Flooded and reducing soil conditions.
\(^d\) 1 day after treatment.
\(^e\) Minimum pH values corresponding to 7 and 5 days after treatment in period 3 of rep. 1 and rep. 2, respectively.
\(^f\) No data.
Measurement of Eh and pH

The effect of starch treatment on soil condition was analyzed by measuring Eh and pH. Eh (5 cm below the soil surface) and pH (2 cm below the soil surface) were measured with platinum-tipped electrodes and a millivolt meter (Model PRN-41, Fujiwara Scientific Company, Tokyo, Japan). Eh was recorded from three pots from each treatment group (FL and RD) in Experiment 1 and four pots in Experiment 2. pH was measured only in Experiment 2, from 4 pots in each treatment group (FL and RD).

Statistical analyses

The statistics package Microsoft Excel Statistics 2010 for Windows was used for analysis of variance (ANOVA), followed by Tukey’s test when ANOVA revealed a significant difference.

Results

Comparison of soil types (Experiment 1)

With FL treatment (flooding without the addition of soluble starch), Eh decreased from 443 ± 26 mV (just after treatment) to 316 ± 65 mV (14 days after treatment) in the Gr-soil and from 475 ± 46 mV to 295 ± 27 mV in the M-soil (n = 3 for each). Under these conditions, marked growth inhibition or leaf injury (chlorosis) was not observed in the three varieties (data not shown). Therefore, we attempted to use 0.1% starch solution to mimic reducing conditions.

With the addition of soluble starch (RD group), Eh decreased from 476 ± 16 mV to –112 ± 32 mV in the Gr-soil and from 504 ± 47 mV to –99 ± 8 mV in the M-soil, suggesting that reducing conditions had been induced in both types of soil. In the tolerant genotypes OUA301 and OUE265, marked leaf injury was not observed in either the Gr-soil or the M-soil. In contrast, leaf injury in OUJ251 was more severe in the M-soil than in the Gr-soil (Fig. 1), although the Eh reductions were nearly the same in these soils. In the ANOVA, genotype and type of soil were significant at the 1% level and also showed a significant effect (at the 5% level) in the genotype x soil interaction (Table 4). On the basis of these results, we used the M-soil in the subsequent experiments.

Variations in flooding tolerance (Experiment 2)

We measured the experimental conditions in detail, because the varietal ranking was likely to change with changes in these conditions. Accurate data on soil temperature, Eh and pH were therefore important for use by future researchers.

Soil Eh values under flooded conditions (FL group) decreased gradually and varied among the test periods. The reduction in Eh is tended to increase with increasing temperature (period 3, Table 3), and the variation among the pots was large under these conditions (Fig. 2A). Under reducing conditions (RD group), soil Eh rapidly decreased within 2 or 3 days after the start of treatment and then gradually increased (Fig. 2B), but by the end of the experiment the Eh values had not exceeded 0 mV. The variation in Eh values among pots under RD conditions was small, except from 1 to 3 days after the start of treatment (Fig. 2B), suggesting that the reduction effect in the M-soil was uniform. Soil pH values had generally decreased by the end of the flooding period in the FL group (Table 3). In the RD group, pH decreased rapidly for the first 5 to 7 days after the start of treatment and then gradually increased especially at the higher soil temperatures (Table 3).

We performed ANOVA for six traits (leaf injury [LI] in group RD; shoot dry weight [SDW] in groups C, FL and RD; SDW [FL/C]; and SDW [RD/C]) in the 14 barley varieties (Table 5). Genotype effects and seasonal effects were significant at the 5% or 1% level for all traits with the exception of seasonal effects in LI (RD). In particular, LI (RD) showed larger genotypic differences than the other five traits.

Fig. 3A is an example of plant growth in Experiment 2 in
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In the greenhouse. Under reducing soil conditions (RD group), shoot growth in barley seedlings was considerably inhibited, whereas leaf injury was not observed in the FL group. There was extremely wide variation among varieties in flooding tolerance, as evaluated by leaf injury, under reducing soil conditions (LI (RD); Fig. 3B and Table 6). Varietal ranking for flooding tolerance, as evaluated by using LI (RD), generally corresponded to that in the study of Takeda (1989) (Table 1 vs. Table 6). There was no clear varietal variation in SDW (FL/C), which is an index widely used to evaluate stress tolerance.

In the case of the trait LI (RD), varietal ranking and degree of leaf injury were generally stable among the three periods, and highly significant correlation coefficients ($r = 0.900$ to $0.939$, significant at the 1% level) were observed between the periods. Also in SDW (RD/C), significant correlation coefficients ($r = 0.693$ to $0.878$, significant at the 1% level) were found. For SDW (FL/C), the correlation coefficients between the periods were not significant in some combinations ($r = 0.218$ to $0.572$), suggesting that the criterion used was unstable for the purposes of trait evaluation.

By using the reliable and highly repeatable trait LI (RD), the two uzu (semi-brachytic growth) varieties OUJ820 (score 4.6) and N-37 (4.5) and the non-uzu variety OUA301 (4.4) (Table 6) were classified as having highly tolerant genotypes. For the trait SDW (RD/C), OUK121 exhibited the highest value. Using SDW (RD), which is essential for cultivation, OUK121 and OUA301 were classified into the tolerant group; the growth of OUA002, in the sensitive group for LI (RD) and SDW (RD), was significantly inhibited compared with the control in the case of all traits (Table 6). Using the traits SDW (FL) and SDW (FL/C), OUJ247 was classified into the tolerant group, even though this variety showed high sensitivity under reducing soil conditions.

Discussion

Breeding for flooding tolerance in upland crops, including barley, is very difficult, because many factors affect phenotype (Setter and Waters 2003, Shabala 2011, Zhou 2010); thus, heritability is generally low, particularly under field conditions (Collaku and Harrison 2005, Hamachi et al.)
Moderate to high heritability or repeatability can be obtained in greenhouse conditions (Li et al. 2008, Zhou 2011), but consistency of results cannot be obtained among different laboratories (Mano and Oyanagi 2009). These inconsistencies are a major problem in the development of flooding-tolerant barley varieties. In this study, we overcame this problem: by using the criterion of leaf injury in plants grown in soil treated with soluble starch solution, we obtained relatively high repeatability in uncontrolled greenhouse experiments. In addition, our varietal ranking (Table 6) generally corresponded to that of Takeda (1989), even though the two experiments were conducted in different laboratories.

In the study by Takeda (1989), greenhouse experiments were conducted in winter in Kurashiki, Japan, farther south than our current experimental site; paddy soil with rice straw compost and added oil meal was used. During the experiments, the temperature of the flooded soil was kept at 25°C to induce reducing soil conditions. Therefore, the consistency between our results and those of Takeda (1989) may be due to varietal consistency in the common critical factor of tolerance to reducing soil conditions, as evaluated by the degree of leaf injury, although the chemical components of soils could not be compared between the two studies. In an earlier study in barley, the usefulness of the degree of leaf injury for evaluating flooding tolerance was reported (Hamachi et al. 1990). Recently, in greenhouse experiments, a QTL controlling waterlogging tolerance, as evaluated by leaf injury, was successfully identified in barley (Li et al. 2008, Zhou 2011). Furthermore, in field experiments, the effectiveness of the criterion of leaf injury was reported in QTL mapping in soybean (Reyna et al. 2003). Therefore, the criterion of leaf injury, which has high repeatability, is effective for reliable evaluation of flooding tolerance. Confirmation of the relationship between the greenhouse experiments (this study) and field experiments will be the next step towards practical breeding.

Waterlogging tolerance has been evaluated in malting barley for breeding purposes (Hamachi et al. 1985, Kato et al. 2007); malting barley (usually the two-rowed type) is sensitive to soil flooding. Our study confirmed this finding, in that the malting barley OUJ247, which has a high malting quality profile and is widely used in genome analyses (Saisho et al. 2007, Sato and Takeda 2009, Sato et al. 2011), was classified as sensitive (Table 6). Also, in an earlier study, OUJ247 exhibited much less tolerance compared with other varieties when it was flooded at the internode elongation stage under field conditions (Hamachi et al. 1988). In addition to the difference in flooding sensitivity for this two-row type of barley, we found a possible relationship between flooding tolerance and uzu varieties although using only two uzu varieties in this study.

Uzu, or semi-brachytic growth, is a unique morphological character in barley and is found within East Asia (e.g., in Southern and Central Japan and on the South Korean

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**Table 5. Analysis of variance for six traits in 14 barley varieties**

| Source of variance | df | Mean square |
|--------------------|----|-------------|
|                    |    | LI (RD)     | SDW (C) | SDW (FL) | SDW (RD) | SDW (FL/C) | SDW (RD/C) |
| Genotype           | 13 | 4.3175 **   | 7730.46 ** | 100481.4 ** | 49772.8 ** | 0.0365 *   | 0.0449 **   |
| Period             | 2  | 0.1837      | 608548.6 ** | 558767.2 ** | 4740522 ** | 0.5577 **  | 0.2785 **   |
| Error              | 68 | 0.2220      | 15732.5    | 10719.4   | 7615.4    | 0.0158     | 0.0137      |

LI (RD): Leaf injury under reducing soil conditions.
SDW (C), SDW (FL), SDW (RD): Shoot dry weight under control, flooded and reducing soil conditions, respectively.
*, **: Significant at the 5% and 1% levels.
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Table 6. Variations in the six traits in 14 barley varieties

| Code   | LI (RD) (score) | SDW (C) (mg) | SDW (FL) (mg) | SDW (RD) (mg) | SDW (FL/C) | SDW (RD/C) |
|--------|----------------|--------------|--------------|--------------|------------|------------|
|        | Mean ± SD      | Mean ± SD    | Mean ± SD    | Mean ± SD    | Mean ± SD  | Mean ± SD  |
| 1 OUJ820 | 4.6 ± 0.3 a    | 767.3 ± 149.9 ab | 677.7 ± 154.8 bcde | 501.6 ± 142.5 abcd | 0.877 ± 0.129 ab | 0.661 ± 0.171 a |
| 2 N-37  | 4.5 ± 0.7 ab   | 658.8 ± 118.7 b  | 518.8 ± 117.8 e  | 364.3 ± 117.0 de  | 0.789 ± 0.123 ab | 0.554 ± 0.136 ab |
| 3 OUA301 | 4.4 ± 0.2 ab   | 942.5 ± 177.7 ab | 775.2 ± 119.9 abcd | 602.7 ± 195.0 abc | 0.830 ± 0.104 ab | 0.631 ± 0.142 a |
| 4 OUC034 | 4.1 ± 0.5 abc  | 796.7 ± 183.1 ab | 735.5 ± 184.4 abcd | 504.1 ± 165.5 abcd | 0.936 ± 0.192 a  | 0.637 ± 0.155 a |
| 5 OUJ003 | 4.0 ± 0.5 abcd | 924.2 ± 173.3 ab | 806.3 ± 141.9 abc | 558.0 ± 180.6 abcd | 0.889 ± 0.163 ab | 0.618 ± 0.188 ab |
| 6 OUE265 | 4.0 ± 0.5 abcd | 753.6 ± 160.7 ab | 570.5 ± 124.5 de | 403.1 ± 113.4 bcde | 0.763 ± 0.114 ab | 0.540 ± 0.094 ab |
| 7 OUK121 | 3.6 ± 0.3 bcde | 886.5 ± 146.7 ab | 804.5 ± 147.6 abc | 609.4 ± 156.8 a  | 0.913 ± 0.128 a  | 0.689 ± 0.143 a |
| 8 OUJ623 | 3.2 ± 0.8 cde  | 757.2 ± 160.4 ab | 632.5 ± 144.6 cde | 445.3 ± 101.4 abcde | 0.844 ± 0.179 ab | 0.598 ± 0.133 ab |
| 9 OUB057 | 3.1 ± 0.3 def  | 835.3 ± 164.0 ab | 661.7 ± 174.0 bcde | 490.3 ± 122.2 abcde | 0.824 ± 0.283 ab | 0.607 ± 0.182 ab |
| 10 OUA604 | 3.0 ± 0.3 efg | 959.0 ± 228.3 ab | 749.8 ± 159.7 abcd | 538.1 ± 185.5 abcd | 0.795 ± 0.141 ab | 0.575 ± 0.182 ab |
| 11 OUA610 | 3.0 ± 0.1 efg | 964.0 ± 266.4 a  | 810.5 ± 157.6 abc | 554.9 ± 198.3 abcd | 0.864 ± 0.126 ab | 0.589 ± 0.182 ab |
| 12 OUJ251 | 2.6 ± 0.3 efg  | 1014.9 ± 186.1 a | 878.7 ± 197.5 ab | 529.0 ± 90.0 abc | 0.882 ± 0.200 ab | 0.530 ± 0.093 ab |
| 13 OUJ247 | 2.2 ± 0.7 fg   | 1046.3 ± 176.0 a | 938.5 ± 231.7 a  | 510.2 ± 95.3 abcd | 0.916 ± 0.251 a  | 0.495 ± 0.099 ab |
| 14 OUA002 | 2.0 ± 0.4 g    | 872.2 ± 186.8 ab | 519.0 ± 177.4 e  | 286.0 ± 87.1 e    | 0.637 ± 0.229 b  | 0.338 ± 0.073 b |

LI (RD): Leaf injury under reducing soil conditions, from 1 (sensitive) to 5 (tolerant).
SDW (C), SDW (FL), SDW (RD): Shoot dry weight under control, flooded and reducing soil conditions, respectively.

Values with the same letter within a column are not significantly different (Tukey, P < 0.05).

Peninsula) (Takahashi 1982). The uzu gene has a pleiotropic effect that produces short coleoptiles, short and dark green leaves, and short awns and kernels (Takahashi 1982). Uzu varieties were more tolerant than non-uzu varieties to salt at the seedling stage in a selection test of 5182 barley varieties and an analysis of near-isogenic lines (Mano and Takeda 1995). For flooding tolerance after the young panicle formation stage, uzu lines were more tolerant than non-uzu lines using 6 pairs of isogenic lines (Iwasa et al. 1994). OUJ820 and N-37 are uzu varieties and showed high tolerance to flooding, as evaluated by the criterion of leaf injury (Table 6); this observation may have been the results of a morphological advantage of the uzu type, including small leaf area and dark green coloration. In other traits based on shoot dry weight under reducing conditions (SDW [RD] and SDW [RD/C]), OUJ820 was classified as moderately tolerant to tolerant, but N-37 was classified as moderate to sensitive. Also, in a previous field experiment, N-37 was evaluated as having moderate tolerance by the criterion of decrease in plant height and grain yield (Yoshioka et al. 2005). From these criteria, N-37 would be classified into the moderately tolerant group. Therefore, use of the leaf injury trait is an effective additional parameter that may be needed in evaluating varieties with unique morphological characters such as uzu.

In conclusion, our results revealed clear varietal variation in flooding tolerance, and highly repeatability, with use of the criterion of leaf injury under reducing soil conditions on the contrary to a criterion of shoot dry weight ratio (SDW [FL/C] or SDW [RD/C]). By controlling experimental conditions such as temperature, humidity and daylength in a greenhouse, repeatability can be further increased, as observed in seedling tests of salt tolerance in barley (Mano 1996). A reliable trait evaluation was also observed in the study of Takeda (1989); however, it is difficult to obtain consistent varietal rankings by other researchers. Our experimental procedure can be applied by other researchers, because we used commercial soils; the equivalent soil can be obtained by different laboratories, and reducing soil conditions can be easily mimicked by adding soluble starch solution. We have been developing several mapping populations by using parents representing a wide range of flooding tolerances. Because a QTL for flooding tolerance, as evaluated by leaf injury, has been detected successfully in the maize F2 population (Mano et al. 2006), an effective QTL survey may be possible not only in doubled haploid or recombinant inbred lines but also in F2 individuals by using our mapping populations and the experimental conditions established here.

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