# Salinity Tolerance Evaluation of Asian Barley Accessions (*Hordeum vulgare* L.) at the Early Vegetative Stage

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Abstract: Salinity is one of the major constraints on world barley production. Exploitation of natural variation of salinity tolerance in existing germplasm and the development of tolerant cultivars are potentially effective approaches for minimizing yield losses. In this study, seedlings of 146 accessions were assessed in a hydroponic system. The experiment was arranged in a completely randomized design with the treatment of 0 and 22 ms/cm<sup>2</sup> NaCl, three replications and four plants in each accession per replication. After 17 days of treatment, salinity tolerance index (STI) was calculated for the traits: number of leaves (NL), shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW) and plant dry weight (PDW). There was a wide variation in salinity tolerance among the barley accessions. All traits were affected by salinity. SDW was highly affected by salinity and correlated with the NL, RDW and PDW, with the correlation coefficients of 0.43, 0.84 and 0.99, respectively. Based on a multivariate analysis, 19 tolerant and 14 susceptible accessions were identified.

Key Words: Barley, Multivariate analysis, Salinity tolerance, Seedling

## 1. Introduction

Salinity stress is a major constraint to agricultural food production because it decreases crop yield and restricts the use of agricultural land. It was estimated that salinity affected nearly 950 million ha (6%) in the world (Babu *et al.*, 2007). Approximately 20% of agricultural land in the world and nearly half of all irrigated land suffer from salinity (Flowers and Yeo, 1995; Munns, 2005). The area affected by salinity in South, South East, North and Central Asia covered 25% of the total soil. As a result, the development of salinity-tolerant crops is an important option for maintaining crop production in saline soil.

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop worldwide and is an important source for feed, malt, and human food. Its importance is derived from the ability to grow and produce in marginal environments, such as drought, low temperature and salinity (Hayes *et al.*, 2003). The genetic diversification and the adaptability to a broad range of ecological conditions have highly strengthened the salinity tolerance in barley. These factors might have raised a rich gene pool with a large variation in adaptation to salinity.

Salinity tolerance of barley germplasm collections was evaluated by different parameters including relative water content, germination rate, coleoptile length, stem and radicle length, as well as dry and fresh weight of roots and shoots (Mano and Takeda, 1997; Xue *et al.*, 2009). The screening was done at germination and the vegetative stage according to previous reports (Mano and Takeda, 1997-1998; Ellis *et al.*, 2002; Farhad and Mohammad, 2008) and at late stage in field by Xue *et al.* (2009).

The primary objectives of this work were to 1) investigate the genetic variation in salinity tolerance of Asian barley accessions at the early vegetative stage and 2) select the highly tolerant and susceptible accessions using the multivariate analysis.

## 2. Methods and Materials

## 2.1. Materials and hydroponic culture

A total of 146 barley accessions from six Asian countries were used in this study: Bhutan, China, India, Japan, Korea and Nepal (**Table 1**). Barley accessions were provided by Institute of Plant Science and Resources, Okayama University with support in part by the National Bio-Resource Project of the MEXT, Japan.

The accessions were screened for salinity tolerance at seedling stage in a growth chamber with natural light, and  $24/20^{\circ}$ C day/night temperature. The experiment was laid out in a randomized design with three plots for untreated and 22.04 ms/cm<sup>2</sup> of NaCl (250 mM). A total of 12 seedlings per accession were evaluated (4 seedlings per treatment).

Uniform seedlings were selected based on the evaluation of

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Table 1. Asian accessions used in this study.

| Origin | Categories | Number of accessions |
|--------|------------|----------------------|
| Bhutan | Landrace   | 7                    |
| China  | Improved   | 10                   |
|        | Landrace   | 25                   |
| India  | Improved   | 4                    |
|        | Landrace   | 10                   |
| Japan  | Improved   | 10                   |
|        | Landrace   | 20                   |
| Korea  | Improved   | 10                   |
|        | Landrace   | 20                   |
| Nepal  | Landrace   | 30                   |
| Total  |            | 146                  |

root length, shoot length and root aspect, then transplanted into containers filled with hydroponic solution of the following proportion (%): total N (10%), P<sub>2</sub>O<sub>5</sub> (8%), K<sub>2</sub>O (27%), MgO (4.0%), MnO (0.10%), B<sub>2</sub>O<sub>3</sub> (0.1%), CaO (23%), Fe (0.18%) with Cu, Zn and Mo traces. The salinity treatment was started when two leaves were completely developed. To reduce the effect of osmotic shock, initial salinity concentration was 13.81 ms/cm<sup>2</sup> and then increased within 3 days to reach the final concentration 22.04 ms/cm<sup>2</sup> of NaCl. Then, the treatment was maintained for 14 days. The solution was changed every week. The pH of solution was maintained at 6.5.

#### 2.2. Salinity Tolerance Evaluation

After 17 days of salinity treatment, seedlings were harvested and root length (RL) and shoot length (SL) were recorded. Then, plants were separated into roots and shoots, dried in an oven at 85°C for 72 hours and weighed. Salinity tolerance index (STI) was estimated according to the relative ratio of trait under treated condition by control based on shoot and root data.

$$\mathsf{STI} = \frac{\text{Trait under treated condition}}{\text{control condition}} \times 100$$

#### 2.3. Statistical analysis

The growth parameters, such as shoot and root dry weight, the GLM analysis, the principal component analysis and the cluster analysis were subjected to statistical analysis using software JMP V10 software, ver. 9 (SAS Institute Inc., 2010).

## 3. Results and Discussion

#### 3.1. Salinity tolerance evaluation

As shown in **Table 2**, there was a significant variation among accessions in all traits under control conditions except the SDW. No significant variation among replications was

 
 Table 2.
 GLM statistical analysis (Mean square) for Asian barley under control and salt treatment.

| VS             | ddl | NL      | SL     | RL    | SDW   | RDW  | PDW  |  |
|----------------|-----|---------|--------|-------|-------|------|------|--|
| Control<br>Acc | 145 | 238.6** | 266**  | 432** | 16NS  | 41** | 15** |  |
| R              | 2   | 1384NS  | 2163** | 619** | 26 NS | 93*  | 21NS |  |
| Acc*R          | 290 | 1415**  | 51**   | 51**  | 15NS  | 32*  | 11** |  |
| SALT<br>Acc    | 145 | 1080**  | 174**  | 99**  | 386** | 21** | 7**  |  |
| R              | 2   | 3995**  | 142**  | 797** | 438** | 29*  | 19** |  |
| Acc*R          | 290 | 1991**  | 50**   | 25**  | 264** | 19** | 7**  |  |
|                |     |         |        |       |       |      |      |  |

Acc: Accession; R: Replication; NS: Not significant; **\*\*** and **\***: Significance at 5% and 1%, respectively.



Fig. 1. The frequency distribution of the STI for the traits number of leaves (A), shoot length (B), root length (C), shoot (D), root (E) and plant (F) dry weight.

observed for the NL, SDW and PDW. SDW was not affected by the environment changes (Acc\* R) and within replications. Under salt treatment, a significant variation among the accessions, replications and interactions was observed for all traits.

There was a wide difference in the response to salinity stress among the 146 accessions indicating a great difference in salinity tolerance among Asian landraces and improved cultivars (**Fig. 1**). On the average, NL, SL, RL, SDW, RDW

 Table 3.
 Mean, standard deviation, coefficient of variance and 95% upper and lower confidence interval for STI of all traits.

| STI    | Mean | Sdtv | CV<br>(%) | Upper<br>% | Lower<br>% | Range<br>% |
|--------|------|------|-----------|------------|------------|------------|
| STINL  | 66   | 11.4 | 17.3      | 67.9       | 64.1       | 35- 95     |
| STISL  | 68.7 | 8.1  | 11.8      | 70         | 67.3       | 50- 95     |
| STIRL  | 79.7 | 16.5 | 20.7      | 82.5       | 78.1       | 50-140     |
| STISDW | 55.1 | 19.6 | 35.8      | 58.3       | 51.8       | 30-107     |
| STIRDW | 70.1 | 20.2 | 28.8      | 73.5       | 66.8       | 20-117     |
| STIPDW | 58.7 | 19.5 | 33.3      | 62         | 55.5       | 20-107     |



Fig. 2. Principal component analysis for Asian barley accessions according to the salt tolerance index (+: Bhutan; °: India; v: China; ▼: Japan; z: Korea ■: Nepal).

and PDW of the treated plants were 65, 68, 79, 55, 70 and 58% of the control. The standard deviation ranged from 8 to 20%.

The SDW coefficient variation was the highest compared with the other traits with a CV = 35.8%. This indicates that SDW is more affected than others morphological traits when plants are exposed to salinity stress in hydroponic culture at an early stage (**Table 3**). In addition, SDW was correlated to the NL, RDW and PDW with the correlation coefficients of 0.43, 0.84 and 0.99, respectively.

#### 3.2. Multivariate analysis

#### 3.2.1. Principal component analysis

The principal component analysis (PCA) based on the STI of all traits was used for discrimination between accessions (**Fig. 2**). PCA represented 75% of variability. The biomass (SDW, RDW and PDW) explained 55.1% of the variability whereas component 2 explained 20% of the variability and was influenced by SL and RL. The accessions originated from Bhutan and Japan maintained the highest biomass production compared to others accessions. The Nepalese followed by the Indian accessions are the least productive under salt treatment. The Korean and Chinese accessions were



Fig. 3. Cluster analysis using STI for all the traits of the 146 Asian accessions.

classed as moderately tolerant to salinity.

## 3.2.2. Cluster analysis

Cluster group rankings were obtained based on average linkage (between groups) method using the squared Euclidian distance on the means of the salinity tolerance indexes for all traits at early vegetative stage (**Fig. 3**). The rescaled distance cluster ranged from 1 to 25.

The accessions were divided into two groups consisting of tolerant accessions and both moderately tolerant and highly susceptible at the similarity level 18. The most tolerant accessions were the landrace from Bhutan, improved cultivars from China and landraces from Japan. Chinese and Korean landraces with the improved cultivars from Japan were ranked as moderately tolerant accessions. The most susceptible accessions were the Indian and Nepalese landraces. By multivariate analysis, 19 susceptible and 14 tolerant accessions were identified and used for further research.

### 3.3. Discussion

Screening for salinity is the basic and primordial step for plant breeding needed to make crosses between susceptible and tolerant accessions, to get inbred lines, and to identify the quantitative traits loci responsible for salinity tolerance. Several previous studies have been reported (Eleuch et al., 2008; Qui et al., 2011; Wu et al., 2011; Sbei et al., 2012; Long et al., 2013) that the variation in salt tolerance exists among European, African and Asian Hordeum vulgare. Ellis et al. (2002) used a variety of barley germplasm accessions from the Middle East for breeding. In the current study, East Asian accessions were screened for salinity tolerance under 22 ms/cm of NaCl in hydroponic culture. The results revealed a wide variation in salinity tolerance among the accessions. In particular, most of the accessions originated from Nepal were highly susceptible, whereas Japanese and Bhutan landraces were highly tolerant to salinity. The difference in response to salinity stress among accessions could offer elite alleles conferring salinity tolerance.

## 4. Conclusion

The variation in salinity tolerance in Asian barley accessions could be exploited and used for the barley breeding program. Tolerant and susceptible accessions were selected, according to the STI of each trait, for further study on detection of QTL associated with salinity tolerance.

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