

Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figures

Supplementary Figure 1. Phenotypic identification of salt tolerance of barley accessions. (A) Phenotypes of barley accessions under control (CK; 0 mM) and salt treatment (300 mM NaCl) conditions after 3 weeks. (B-O) Raincloud plots for phenotypic traits. SeH: Seedling height (cm); SL: Shoot length (cm); RL: Root length (cm); SeFW: Seedling fresh weight (g); SFW: Shoot fresh weight (g); RFW: Root fresh weight (g); SeDW: Seedling dry weight (g); SDW: Shoot dry weight (g); RDW: Root dry weight (g); SeWC: Seedling water content (g); SWC: Shoot water content (g); RWC: Root water content (g); LN: numberLeaf; YLN: Yellow leaf number. Bars, 10 cm. *P < 0.05; **P < 0.01.

Supplementary Figure 2. Boxplots depicting the genetic effects of SNPs with significant associations with Salt-Na⁺, Salt-Na⁺/K⁺, R-Na⁺, R-Na⁺/K⁺ and and strength in Chr4. (A) Boxplot diagram depicting the genetic effect of eight significant SNPs to Salt-Na⁺. (B) Boxplot diagram depicting the genetic effect of eight significant SNPs to Salt-Na⁺/K⁺. (C) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺. (D) Boxplot diagram depicting the genetic effect of eight significant SNPs to R-Na⁺/K⁺. The box shows the lower quartile, and the median and upper quartile values, and the whiskers show the range of the phenotypic variation in the population.

Supplementary Figure 3. Boxplots depicting the genetic effects of ten SNPs with significant associations with R-Na⁺ in Chr6. The box shows the lower quartile, and the median and upper quartile values, and the whiskers show the range of the phenotypic variation in the population.

Supplementary Figure 4. Boxplots depicting the genetic effects of SNPs with significant associations with Salt-Na⁺ and Salt-Na⁺/K⁺ in Chr7. (A) Boxplot diagram depicting the genetic effect of eight significant SNPs to Salt-Na⁺. (B) Boxplot diagram depicting the genetic effect of eight significant SNPs to Salt-Na⁺. The box shows the lower quartile, and the median and upper quartile values, and the whiskers show the range of the phenotypic variation in the population.

Supplementary Figure 5. RNA-seq analysis of salt response in barley genotypes. (A) Number of DEGs in CM72 and Gairdner subjected to salt stress for different times. The samples were collected at 0, 3, 12, and 48 h under salt stress. (B) Venn diagrams showing the number of distinct and common salt-responsive genes (DEGs) found in two barley genotypes. 3h_CM72 (CM72-3h vs. CM72-0h), 12h_CM72 (CM72-12h vs. CM72-0h), 48h_CM72 (CM72-48h vs. CM72-0h); 3h_Gairdner (Gairdner-3h vs. Gairdner-0h), 12h_Gairdner (Gairdner-48h vs. Gairdner-0h). (C) Sources of variation in barley gene expression under salt stress by principal component analysis.

Supplementary Figure 6. Expression pattern of three selected genes in barley leaves. (A) Expression pattern validation of *HORVU2Hr1G002690* (receptor kinase 2), *HORVU5Hr1G124800* (GsSRK) and *HORVU5Hr1G124880* (MATE) genes in CM72 and Gairdner leaves by qRT-PCR. The samples were collected at 0, 3, 12, and 48 h under salt stress. (B) Relative expression of 3

candidate genes by quantitative RT-PCR in six salt-sensitive and six salt-tolerant varieties. *HORVU2Hr1G002690* (receptor kinase 2). *HORVU5Hr1G124800* (GsSRK). *HORVU5Hr1G124880* (MATE). Six salt-sensitive varieties, S1: Shan 3, S2: AKHELOL 1, S3: Boyer, S4: Rainbow, S5: Birka, S6: Gairdner; six salt-tolerant varieties, T1: PALLIDUM 043, T2: KIMALUNG, T3: Zhu Damai 5, T4: GURZAN, T5: Kunlun 14, T6: CM72.

1.2 Supplementary Tables

Supplementary Table 1 List of 288 barley accessions.

Supplementary Table 2 Analysis of variance (ANOVA) results of the ion traits.

Supplementary Table 3 Correlation analysis between morphological traits and ion traits related to salt tolerance under salt treatment (300 mM NaCl) conditions after 3 weeks.

Supplementary Table 4 The summary of the number of allele SNPs mapped in barley genome.

Supplementary Table 5 List of 616 candidate genes with salt-tolerance related traits.

Supplementary Table 6 KEGG pathway and Gene Ontology analysis of all candidate genes with salt-tolerance related traits.

Supplementary Table 7 Statistics of RNA-Seq numerical data analysis in CM72 (C) and Gairdner (G) barley seedlings. 0h (salt stressed for 0 h), 3h (salt stressed for 3 h), 12h (salt stressed for 12 h), 24h (salt stressed for 48 h).

Supplementary Table 8 List of six salt-tolerant and six salt-sensitive barley accessions.

Supplementary Table 9 List of primers for qRT-PCR.

Supplementary Table 10 Allelic variation in 8 putative candidate genes using cv. Morex as a standard by RNA-Seq in CM72 and Gairdner.