Expanded View Figures

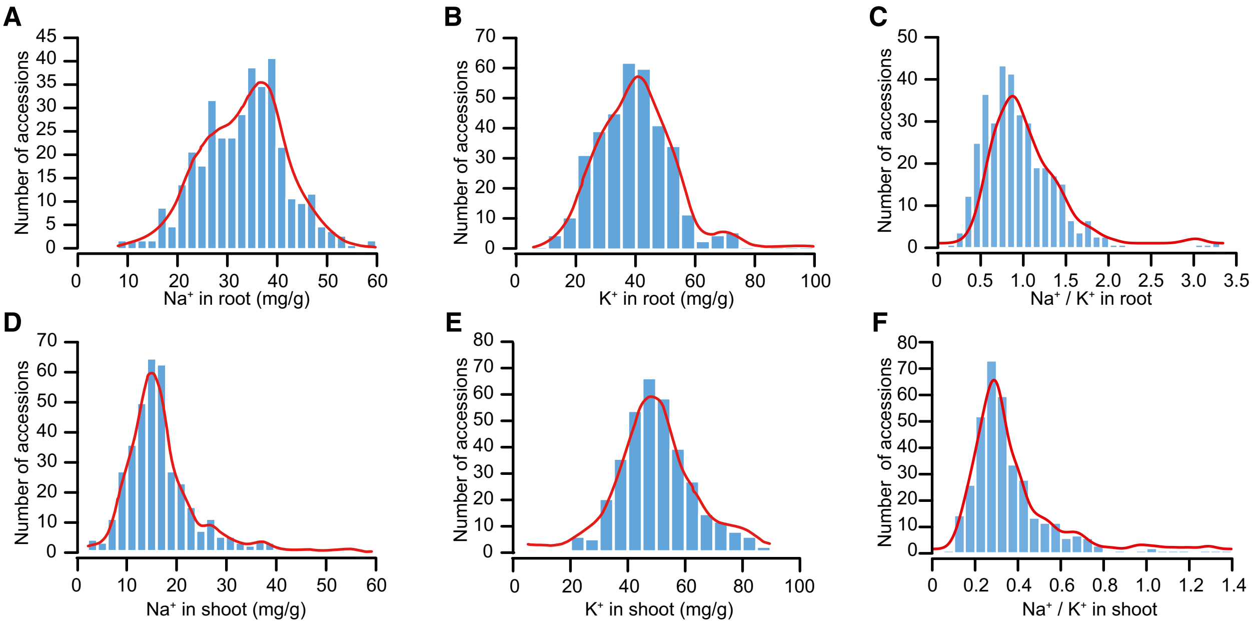


Figure EV1. Frequency distribution of Na^+ and K^+ contents and Na^+/K^+ ratio in root and shoot of 369 accessions.

A–C Frequency distribution of Na^+ (A), K^+ (B), and Na^+/K^+ ratio (C) in roots of the tomato population.
D–F Frequency distribution of Na^+ (D), K^+ (E), and Na^+/K^+ ratio (F) in shoots of the tomato population.

Data information: Red curve indicates the fitted line of the distribution.

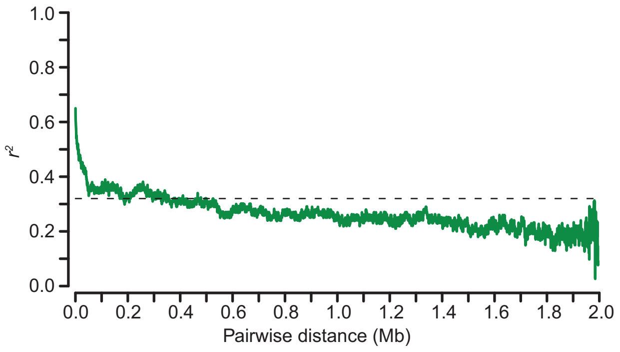


Figure EV2. Average decay of linkage disequilibrium decay for the associated signal region.

Linkage disequilibrium decay was estimated by squared correlation of allele frequencies (r^2) against distance (Mb) among the SNPs. These SNPs were extracted from 2-Mb interval surrounding the leading SNP (ch04:2156747), which was determined by the ratios of Na^+/K^+ in root.

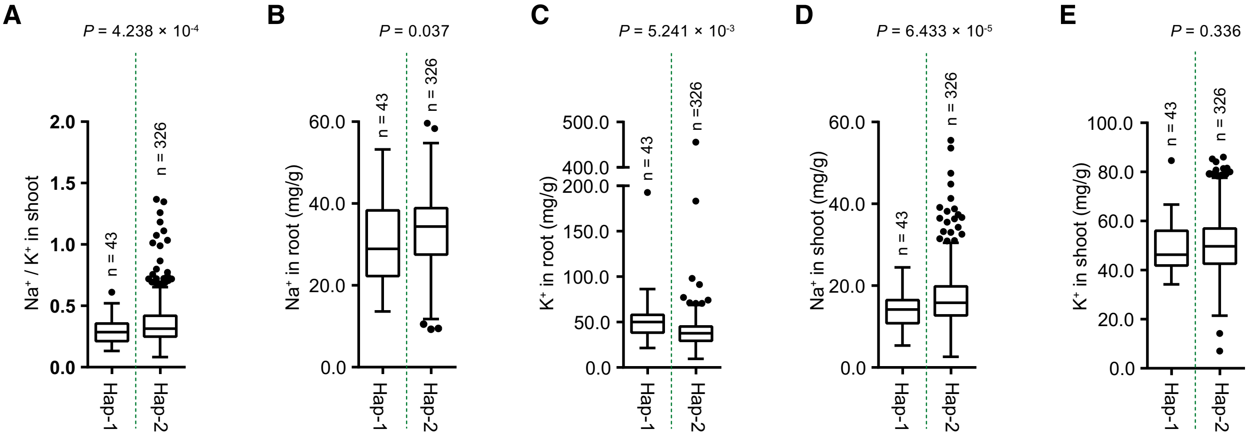


Figure EV3. Analysis of Na^+ and K^+ contents in two *SlHAK20* haplotypes in the population.

A–E Haplotype analysis of the *SlHAK20* gene based on shoot Na^+/K^+ ratios (A), root Na^+ (B) and K^+ (C), and shoot Na^+ (D) and K^+ (E) contents. Box plots represent the interquartile range using Tukey's method, the line in the middle of each box represents the median, the whiskers represent the interquartile range, and the dots represent outlier points. *n* indicates the number of accessions belonging to each haplotype. Significant difference was determined by Student's *t*-test.

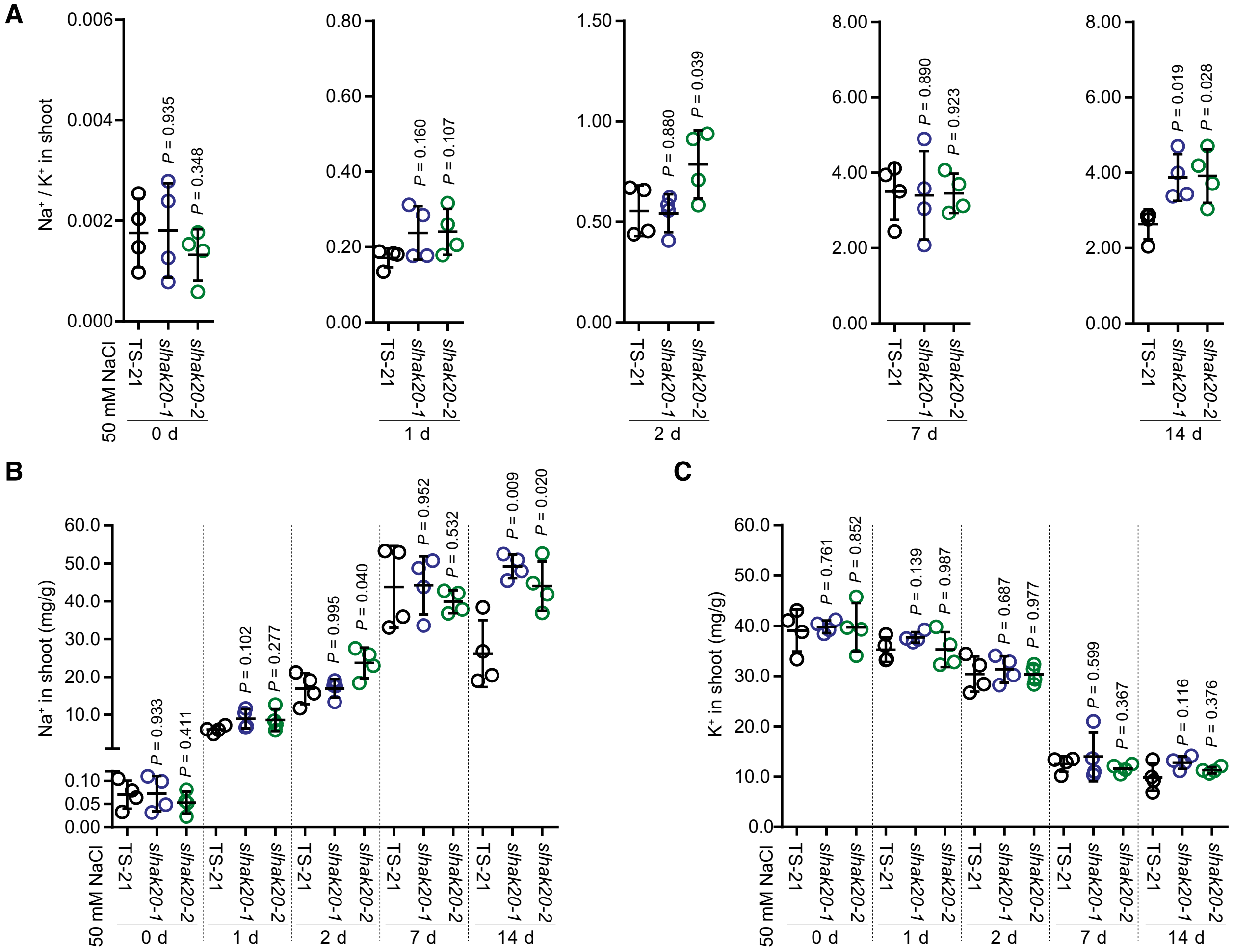
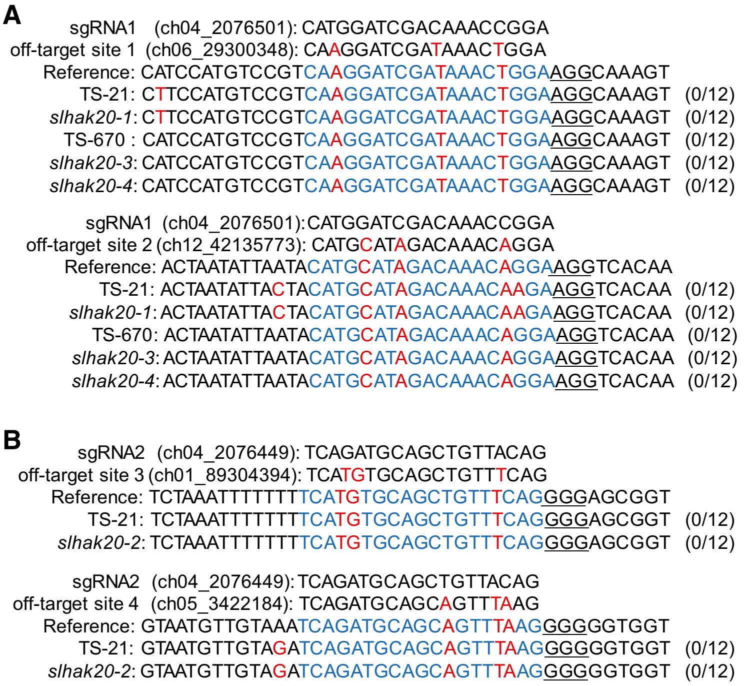


Figure EV4. The Na⁺ and K⁺ contents and Na⁺/K⁺ ratio in the leaves of *slhak20-1* and *slhak20-2* mutant plants.

A Shoot Na⁺/K⁺ ratio of TS-21 and *slhak20* mutant plants after salt treatment.

B, C Shoot Na⁺ (B) and K⁺ (C) contents of TS-21, *slhak20-1*, and *slhak20-2* under normal and salt stress conditions. 24-day-old plants were treated with 50 mM NaCl for the indicated time and used for analysis of Na⁺ and K⁺ contents.

Data information: In (A–C), data are shown as means ± SD (n = 4). *P*-value was determined by Student's *t*-test.



A

sgRNA1 (ch04_2076501): CATGGATCGACAAACCGGA
off-target site 1 (ch06_29300348): CAAGGATCGATAAACTGGA
Reference: CATCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT
TS-21: CTTCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT (0/12)
slhak20-1: CTTCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT (0/12)
TS-670 : CATCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT (0/12)
slhak20-3: CATCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT (0/12)
slhak20-4: CATCCATGTCCGTCAAGGATCGATAAACTGGAAGGCAAAGT (0/12)

sgRNA1 (ch04_2076501): CATGGATCGACAAACCGGA
off-target site 2 (ch12_42135773): CATGCATAGACAAACAGGA
Reference: ACTAATATTAATACATGCATAGACAAACAGGAAGGTCACAA
TS-21: ACTAATATTACTACATGCATAGACAAACAAGAAGGTCACAA (0/12)
slhak20-1: ACTAATATTACTACATGCATAGACAAACAAGAAGGTCACAA (0/12)
TS-670: ACTAATATTAATACATGCATAGACAAACAGGAAGGTCACAA (0/12)
slhak20-3: ACTAATATTAATACATGCATAGACAAACAGGAAGGTCACAA (0/12)
slhak20-4: ACTAATATTAATACATGCATAGACAAACAGGAAGGTCACAA (0/12)

B

sgRNA2 (ch04_2076449): TCAGATGCAGCTGTTACAG
off-target site 3 (ch01_89304394): TCATGTGCAGCTGTTTCAG
Reference: TCTAAATTTTTTTTCATGTGCAGCTGTTTCAGGGGAGCGGT
TS-21: TCTAAATTTTTTTTCATGTGCAGCTGTTTCAGGGGAGCGGT (0/12)
slhak20-2: TCTAAATTTTTTTTCATGTGCAGCTGTTTCAGGGGAGCGGT (0/12)

sgRNA2 (ch04_2076449): TCAGATGCAGCTGTTACAG
off-target site 4 (ch05_3422184): TCAGATGCAGCAGTTTAAG
Reference: GTAATGTTGTAAATCAGATGCAGCAGTTTAAGGGGGGTGGT
TS-21: GTAATGTTGTAGATCAGATGCAGCAGTTTAAGGGGGGTGGT (0/12)
slhak20-2: GTAATGTTGTAGATCAGATGCAGCAGTTTAAGGGGGGTGGT (0/12)

Figure EV5. Analysis of potential off-target sites.

A Two potential off-target sites of sgRNA1 in the T1-independent lines of *slhak20-1*, *slhak20-3*, and *slhak20-4*.

B Two potential off-target sites of sgRNA2 in the T1-independent lines of *slhak20-2*.

Data information: In (A, B), the mismatch bases are shown in red. The potential off-target sites are indicated in blue. The PAM sequences are underlined. The numbers of edited/tested lines are shown between parentheses.