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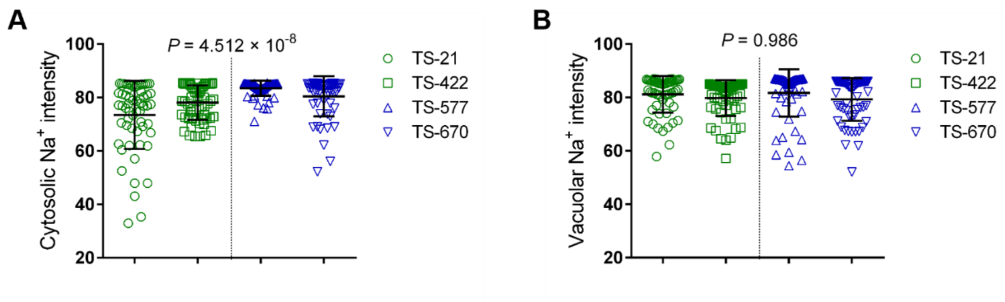
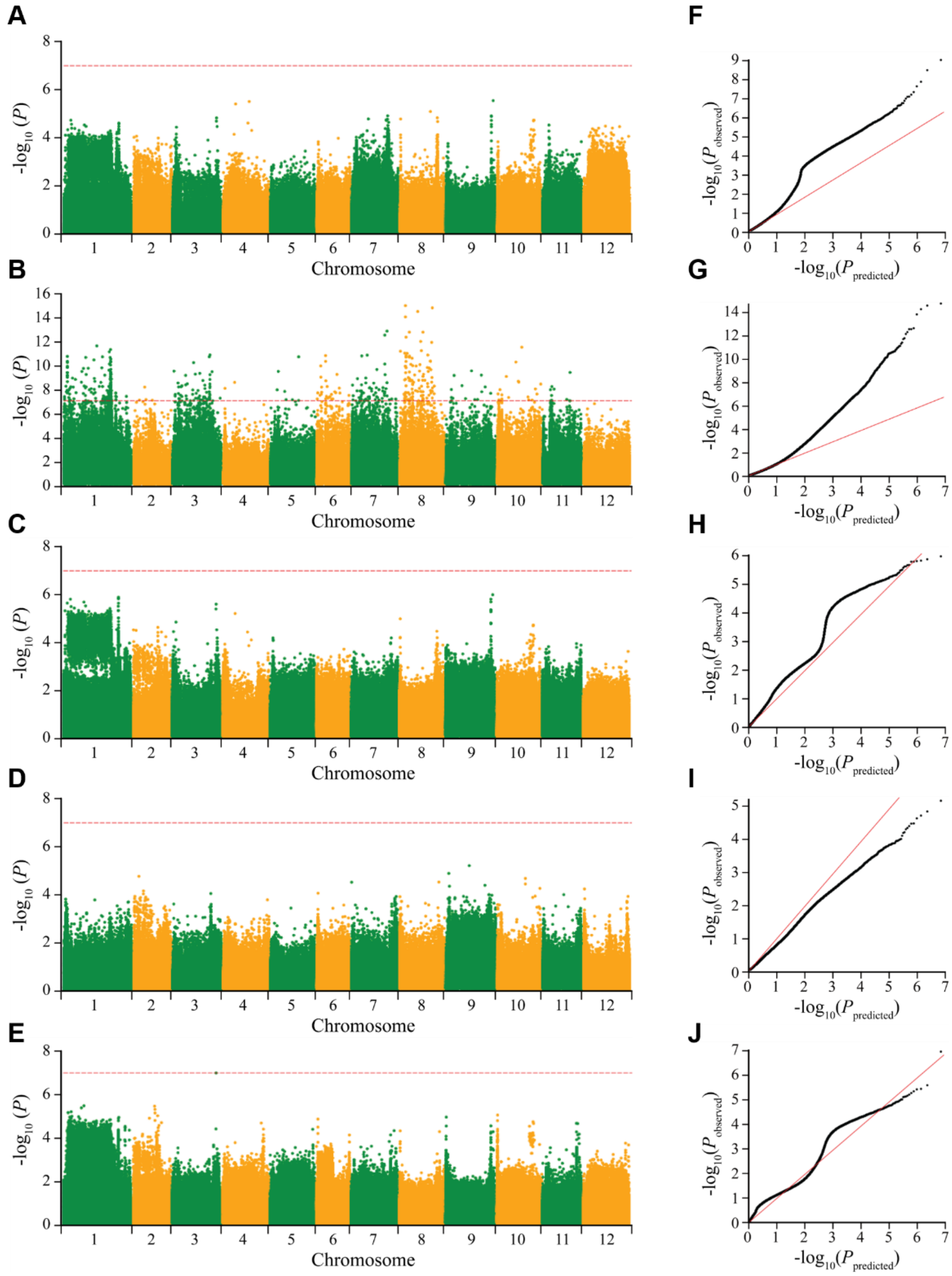


Appendix Figure S1 - The intracellular Na^+ levels measured by the fluorescence intensities in the elongation zone of tomato roots.

A The cytosolic Na^+ level in the root elongation zone of four tomato accessions.

B The vacuolar Na^+ level in the root elongation zone of four tomato accessions.

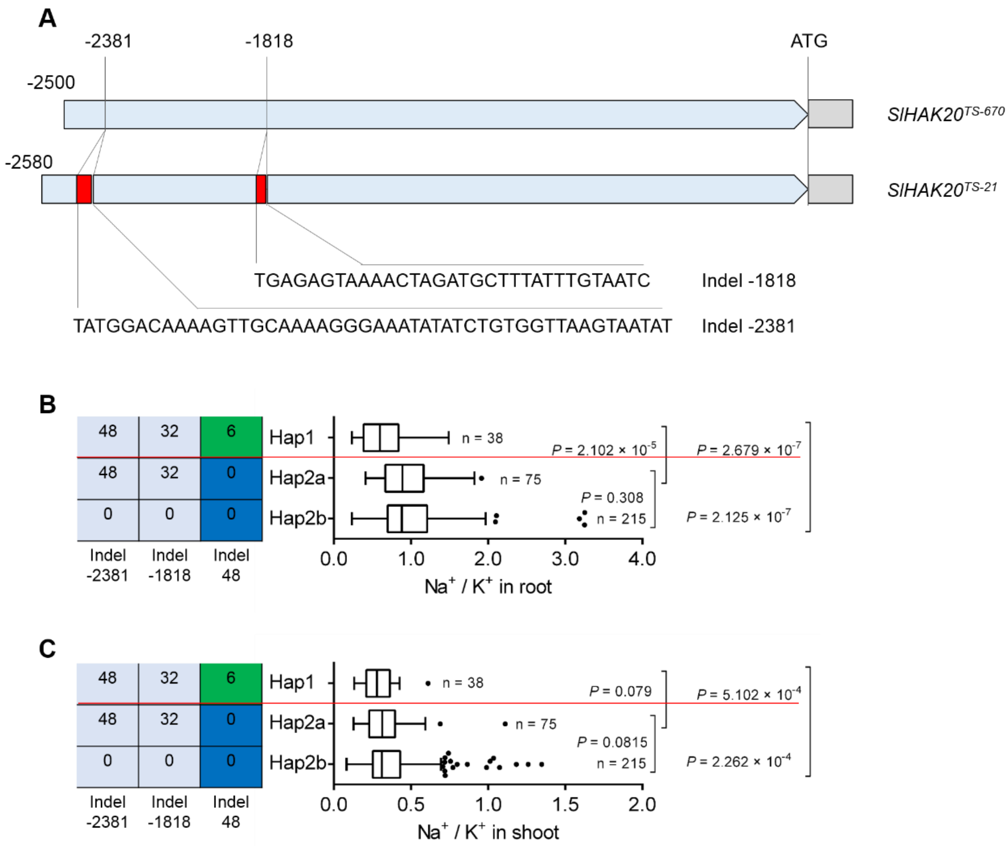
Data information: In (A, B), data are shown as the means ± SD (n = 60 cells from 5 individual plants for each accession). *P*-value was determined by Student's t-test.



Appendix Figure S2 - GWAS of Na^+ and K^+ contents in tomato roots and shoots.

A - E Manhattan plot for GWAS of root Na^+ (A) and K^+ (B), shoot Na^+ (C) and K^+ (D), shoot Na^+/K^+ ratio (E) on chromosomes 1 - 12. The horizontal line shows the Bonferroni-adjusted significance threshold ($P = 1.0 \times 10^{-7}$).

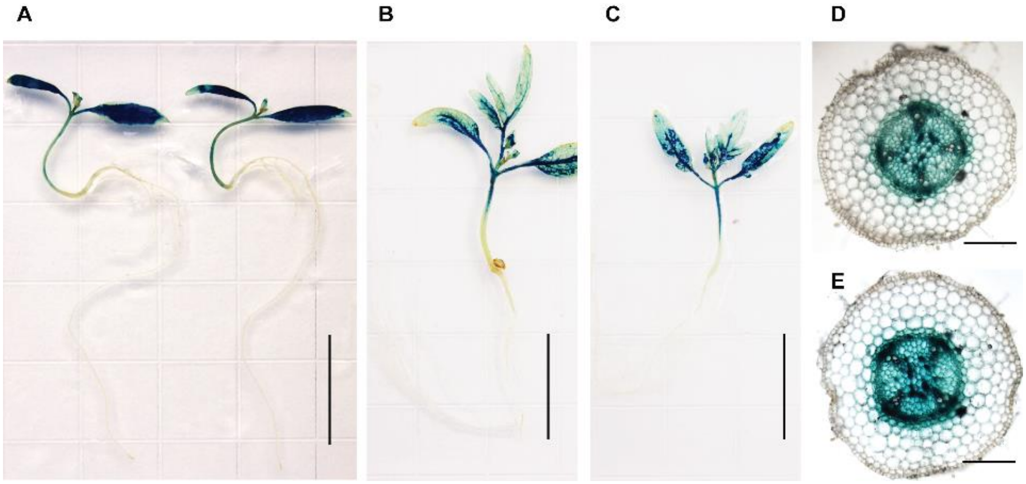
F - J Quantile-quantile plot for the GWAS of root Na^+ (F) and K^+ (G), shoot Na^+ (H) and K^+ (I), shoot Na^+/K^+ ratio (J) under a FaST-LMM.



Appendix Figure S3 - The role of the indels in the promoter region of *SlHAK20*.

A Schematic diagram of the *SlHAK20*$^{TS\text{-}21}$ and *SlHAK20*$^{TS\text{-}670}$ promoters. Two indels are indicated by red dished lines.

B, C Haplotype analysis of the *SlHAK20* gene based on the ratio of Na^+ and K^+ in root (B) and shoot (C). Only haplotypes with total number of accessions ≥ 6 were analyzed. Box plots represent the interquartile range using Turkey method, the line in the middle of each box represents the median, the whiskers represent the interquartile range, and the dots represent outlier points. Significant difference was determined by Student's t-test.

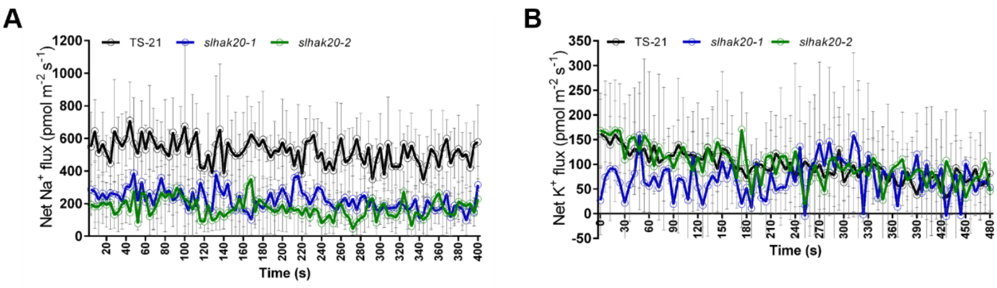


Appendix Figure S4 - Expression pattern of *SlHAK20pro-GUS* in transgenic tomato plants.

A The GUS activity in 7-day-old transgenic tomato of *SlHAK20*$^{TS-670}$*pro:GUS* and *SlHAK20*$^{TS-21}$*pro:GUS*. Left panel, *SlHAK20*$^{TS-670}$*pro:GUS*. Right panel, *SlHAK20*$^{TS-21}$*pro:GUS*. Bar, 2 cm.

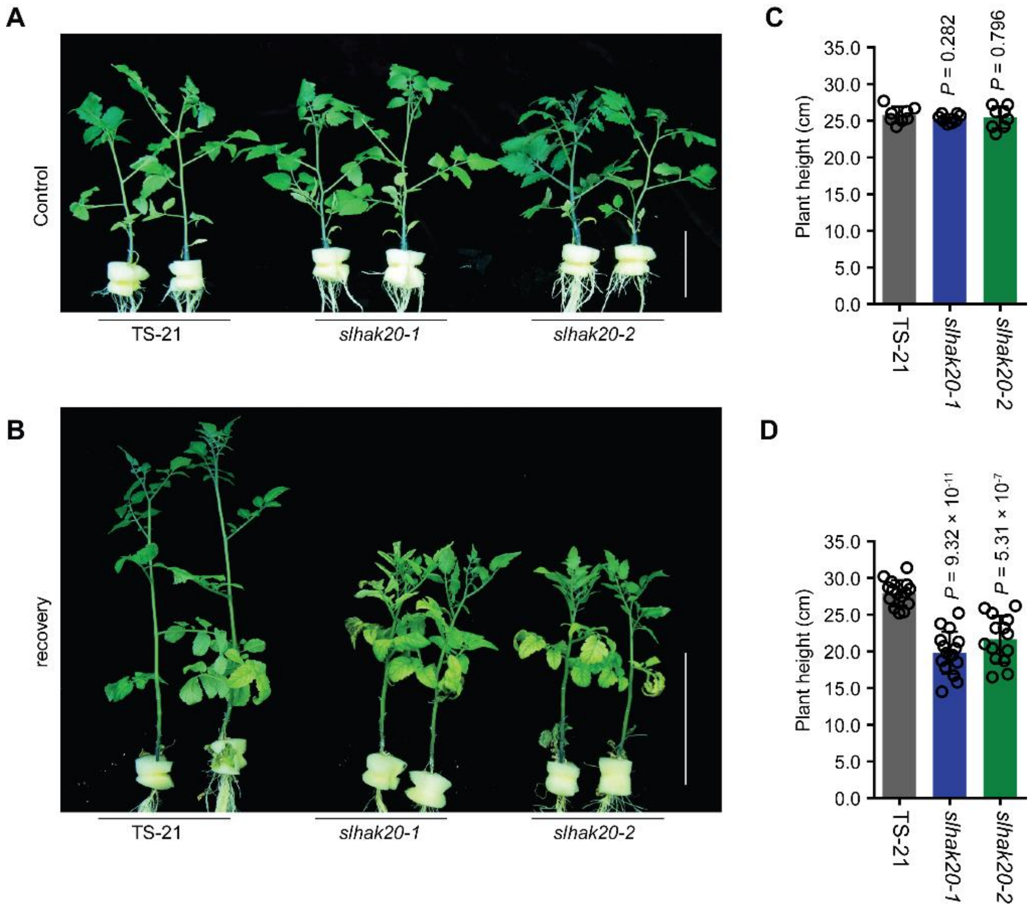
B, C 14-day-old seedlings of *SlHAK20*$^{TS-670}$*pro:GUS* (B) and *SlHAK20*$^{TS-21}$*pro:GUS* (C). Bars, 2 cm.

D, E Cross-sections of hypocotyl of 14-day-old *SlHAK20*$^{TS-670}$*pro:GUS* (D) and *SlHAK20*$^{TS-21}$*pro:GUS* (E) transgenic plants. Bars, 400 μm.



Appendix Figure S5 - Net Na^+ and K^+ flux at the maturation zone of mutant roots.

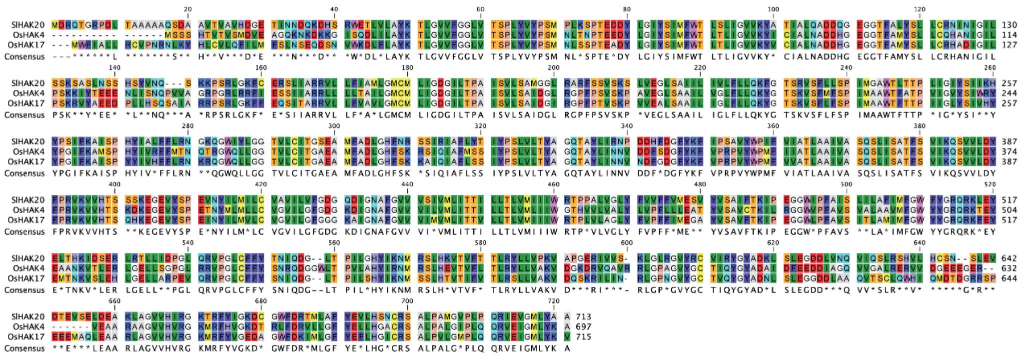
A, B Net Na^+ (A) and K^+ (B) flux analysis of the root maturation zone of *slhak20-1*, *slhak20-2* and TS-21 plants. Four-day-old seedlings were pretreated with 50 mM NaCl for 3 days. The net Na^+ and K^+ fluxes were then detected using SIET system. Error bars represent ± SD, n = 4 plants for each genotype.



Appendix Figure S6 - Salt tolerance of *slhak20* mutant plants in liquid medium.

A - D Salinity tolerance assay of *slhak20* mutants grown in liquid 0.25× Hoagland medium. TS-21, *slhak20-1*, and *slhak20-2* grown under normal growth conditions for 34 days (A), and the shoot heights were used as control (B). 24-day-old TS-21, *slhak20-1*, and *slhak20-2* plants treated with 150 mM NaCl for 18 days, recovered for 14 days (C), and the shoot heights were recorded (D).

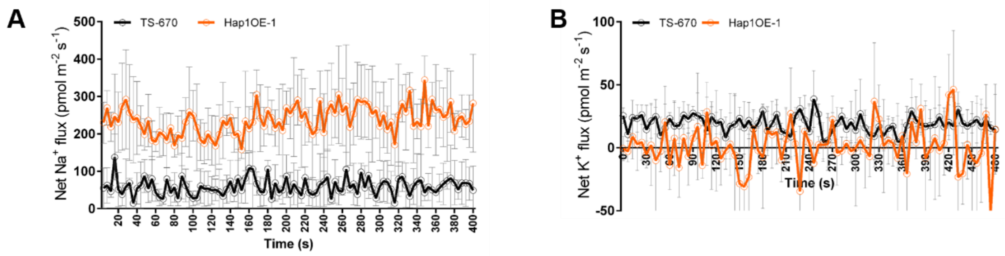
Data information: In (A, B), bar, 10 cm. In (C, D), values are shown as the means ± SD. n ≥ 8 plants of each genotype. *P*-value were determined by Student's t-test.



Appendix Figure S7 - Sequence alignment of SlHAK20, OsHAK4, and OsHAK17 proteins. Color shading indicates identical or similar residues. Sequence alignment was generated using CLASTALW from the website (www.genome.jp).

Appendix Figure S8 - Salt tolerance of tomato varieties grouped by natural variations of *SlHAK20*.

Twenty tomato accessions grown under normal growth conditions for 4 weeks were photographed and shown as control (left). For salt tolerance assay, 2-week-old tomato seedlings were treated with 150 mM NaCl for 3 weeks and the survival rates were calculated. Photographs were taken 1 week after recovery (right). The haplotypes of *SlHAK20* were shown as Hap1 and Hap2, respectively. The groups of accessions were listed as PIM, CER, and BIG, respectively. Survival rates were obtained from three independent experiments, each with eight plants from each accession. Scale bars, 5 cm.



Appendix Figure S9 - Net Na^+ and K^+ flux at the root maturation zone of *SlHAK20*^{Hap1} overexpression plants.

A, B Net Na^+ (A) and K^+ (B) flux analysis at the root maturation zone of TS-670 and Hap1OE-1 transgenic plants. Four-day-old seedlings were pretreated with 50 mM NaCl for 3 days, and then the Na^+ and K^+ fluxes were examined by SIET system. Error bars represent ± SD, n = 4 plants for each genotype.

Appendix Supplementary Methods

Quantification of intracellular Na^+

The Na^+ accumulation in the cytosolic and vacuolar compartments in tomato root cells were determined using fluorescent CoroNa™ Green (ThermoFisher Scientific) as previously described (Wu, Shabala et al., 2019). Briefly, four-day-old TS-21, TS-422, TS-577 and TS-670 were treated with 100 mM NaCl for 3 days, and then the intracellular Na^+ in the root cells at elongation zone was displayed by staining with the Na^+-specific fluorescent dye CoroNa™ Green. The images showing the intracellular Na^+ levels in elongation region of the tomato roots were obtained using a ZEISS laser confocal scanning microscope LSM880. The fluorescence intensities were calculated using the Image J (version 1.5I).

Measurement of net Na^+ and K^+ fluxes

The measurement of Na^+ and K^+ fluxes were performed using the non-invasive, scanning ion-selective electrode technique (Shabala, Ross et al., 2006) with NMT Physiolyzer (Younger USA, LLC, Amherst, MA, USA) and imFluxes software. Four-day-old seedlings were treated in medium with 50 mM NaCl for 3 days. Prior to the flux measurement, the seedling was incubated in the measuring solution (0.1 mM $CaCl_2$, 0.1 mM KCl, 0.5 mM NaCl, and 0.3 mM MES, pH 6.0) for 10 min. Net ion fluxes were then measured at the root maturation zone of equilibrated seedlings under the experimental conditions for additional 10 min to decrease variability due to fluctuations. Experiments were performed with at least 4 biological replicates.

Appendix Supplementary Methods References

Shabala L, Ross T, McMeekin T, Shabala S (2006) Non-invasive microelectrode ion flux measurements to study adaptive responses of microorganisms to the environment. *Fems Microbiol Rev* 30: 472-486

Wu HH, Shabala L, Zhou MX, Su NN, Wu Q, Ul-Haq T, Zhu J, Mancuso S, Azzarello E, Shabala S (2019) Root vacuolar Na+ sequestration but not exclusion from uptake correlates with barley salt tolerance. *Plant J* 100: 55-67