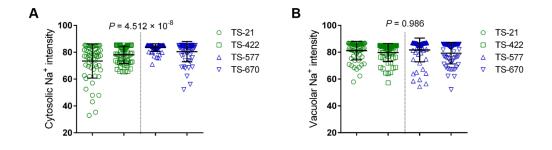
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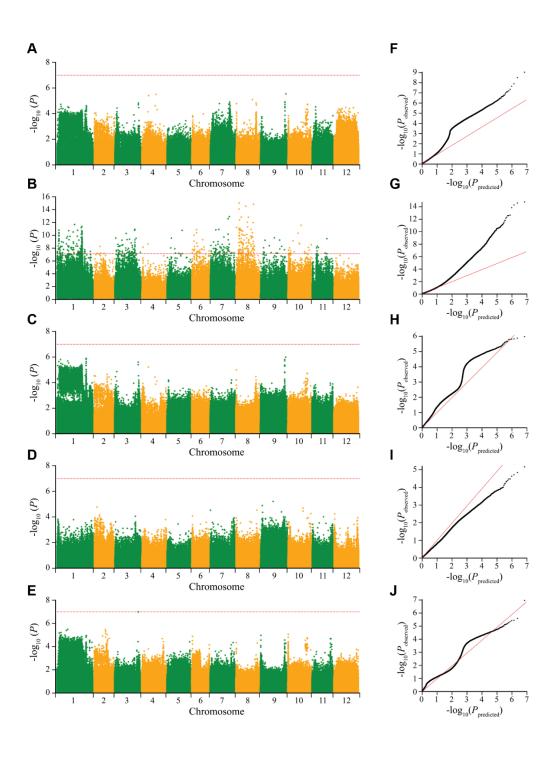


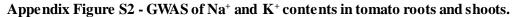
Appendix Figure S1 - The intracellular Na⁺ levels measured by the fluorescence intensities in the elongation zone of tomato roots.

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.

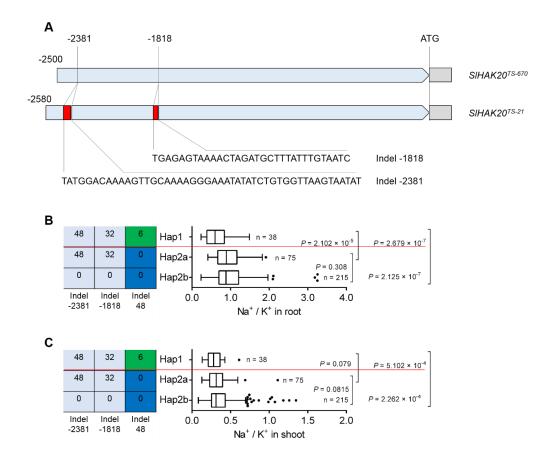
Data information: In (A, B), data are shown as the means \pm SD (n = 60 cells from 5 individual plants for each accession). *P*-value was determined by Student's t-test.





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 ($P = 1.0 \times 10^{-7}$).

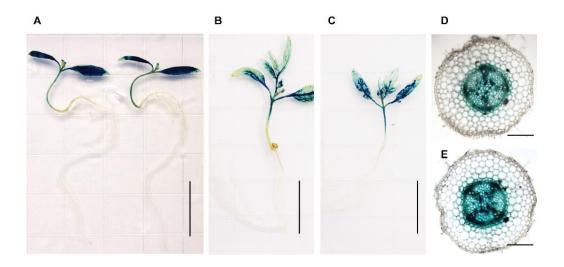
F - J Quantile-quantile plot for the GWAS of root $Na^+(F)$ and $K^+(G)$, shoot $Na^+(H)$ and $K^+(I)$, shoot Na^+/K^+ ratio (J) under a FaST-LMM.



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

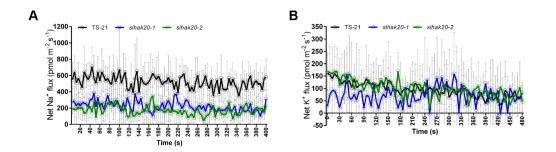
A Schematic diagram of the $SlHAK20^{TS-21}$ and $SlHAK20^{TS-670}$ promoters. Two indels are indicated by red dished lines.

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.



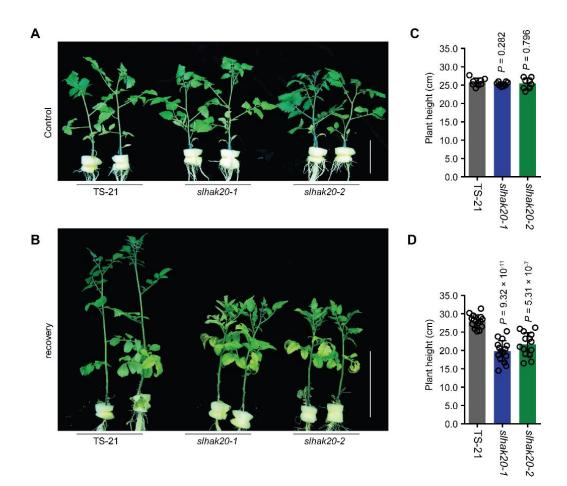
Appendix Figure S4 - Expression pattern of *SlHAK20pro-GUS* in transgenic tomato plants.
A The GUS activity in 7-day-old transgenic tomato of *SlHAK20^{TS-670}pro:GUS* and *SlHAK20^{TS-21}pro:GUS*. Left panel, *SlHAK20^{TS-670}pro:GUS*. Right panel, *SlHAK20^{TS-21}pro:GUS*. Bar, 2 cm.
B, C 14-day-old 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 \pm SD, n = 4 plants for each genotype.



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

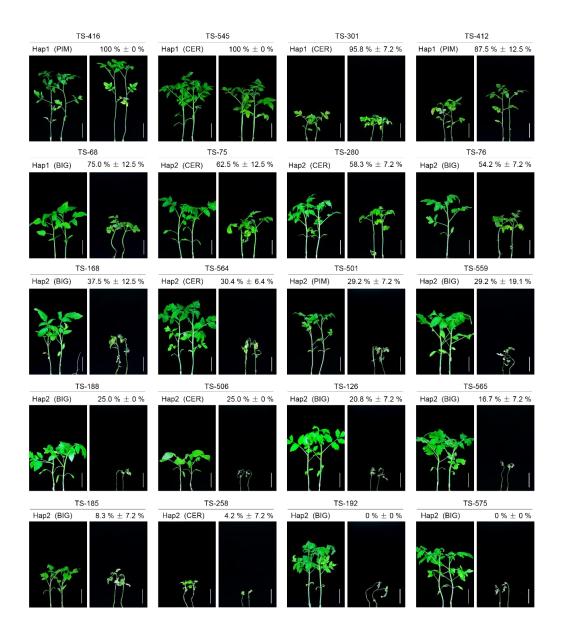
A - D Salinity tolerance assay of *slhak20* mutants grown in liquid 0.25× Hoagland medium. TS-21, *slhak20-1*, and *slhak20-2* grown under normal growth conditions for 34 days (A), and the shoot heights were used as control (B). 24-day-old TS-21, *slhak20-1*, and *slhak20-2* plants treated with 150 mM 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 \pm SD. n \geq 8 plants of each genotype. *P*-value were determined by Student's t-test.

	20		40		60		80		100		120			
OsHAK4	MUROTGRPD	MSSS	HTYTYSMDVE		RWETLVLAYK ISODLILAYK	TLGVV GGLV TLGVV GGLV	TSPLYVYPSM TSPLYVYPSM	PEKSPTEDDY	GIYSIMFWT GIYSIMFWT	LSLIGVVKYA LTLIGVVKYI	T ALQADDOG CLALNADDHG CLALNADDHG			114
				N N N N D N		TLGVVFGGLV	TSPL YVYPSM	NL*SPTE*DY	GING IMENT		CIALNADDHG			
consensus	140		160 IS		-W-DL-LATK	LOWFOOLV	13111111130	NL-SPIE-DT	220	LILIGVVKI	CTALNADDHG	EGGIFAMITSE	260	
	170		100				1				140			
	SSKSASLNSS		KKPSRLGKEC GRPGRLRREI	ERSLIARRYL	LEIAMLGMCM	GOGILTPA	SVL SAMGGL	RARESSVSKS	LVEGLSAIIL	IVLELLOKEG		MGAWT TTP		
	PSKRVYALED		GREGELERE	SSI ARREL		GOGLITPA		RGPEPTVSKP	AVEGESAALE	GENELOKIG	TSKUSELESP	MAASTEATP	GLYSIVHY	
	PSK**Y*FF*		*RPSRLGKF*	E*SLIARRVI	LF*A*LGMCM				*VECLSAALL	ICLELLOKYC	TSKVSFLFSP	IMAAWTETTP	*16**51***	
consensus	PSK	280	- KF 3K LUKF	2 - 3 T TARKYE 300	LT A LONCH	120	ISVESKIDGE	KUFFF3V3KF 340	TEGESAATE	101111100010	TSKVSFLFSF	380	10-131-1	
	YPSIEKALSP HYLALEELEN		1		1		TYPSEVETMA GOTATE RNP		T		1			
			GKOGWIYIGG QTRGWQUIGG							VPRPV YWPME		SOSESATES	VIKOSVVIDY	
	YPGI FKALSP		KROGNOLLIGG							VPRPVYWPME		SOSLISATES	VIKOSVVIDY	
			**OGWOLLGG											
001001000	400		420		440		460		480		500		520	
CILLAN DO	ESSERENCES	SSKEGEWNSP									GGWPFAS			
	EPRVKVVHT S													
		OHKEGEWYSP		VGVILGEGGG		VINVMETTY						TLAMMEGW		
Consensus	FPRVKVVHTS	**KEGEVYSP	E*NYILM*LC	VGVILGFGDG	KDIGNAFGVV	VI*VMLITTI	LETLYMITIW	RTP*VLVGLY	EVPEE*ME**	YVSAVETKIP	EGGW*PFAVS	**LA*IMEGW	YYGRORK*EY	
	540		560		580		600		620		640			
SHAK20		RTITIO	ORVEGUCEEY		PLICHNERNE	DOBUSSTORT	TERVELVOVV	ARCEPTUNC		WIRKOWARKI	SEEGDDEWNO			642
	EAANKYTLER		RRVPGLCEEY					GKORVOAVR			DEEEDDLAGD		GEEEGER	
OsHAK17	EMTNKVSLEH	LGELLARPEV	ORVP G LC FFY	SNIQDGLT	PILSHYIKNM	SSLHTVTIEV	TERSEEVAKV	QSKRILIN-	REGPNGYYGC	TYQYGYADNL	SLEGGDDLAA	OVTSCEOWHI	OMDTDGRESP	644
Consensus	E*TNKV*LER	LGELL**PGL	QRVPGLCFFY	SNIQDG LT	PIL*HYIKNM	RSLH*VTVF*	TLRYLLVAKV	D***R * * * -	R LGP*GVYGC	TIQYGYAD*L	SLEGDD***Q	VV*SLR**V*	*****G*R**	
	660		680		700		720							
SIHAK20	DTEVSELDEA	KEAGWWHERG	KTREVICKOC	GWEDRIMLAE	YEVENSNORS	ALPANGUPEP	ORIEVGMENA	A 713						
	• VEAA			RLFORVELGF	YELLHGACRS	ALPALGIPLO	ORVEIGMLYK							
	EEEMAQLEAA			GWEDKIMLGE		ALPVLGMPLO								
Consensus	* * E * * * L E A A	RLAGVVHVRG	KMR FYVGKD*	GWFDR *MLGF	YE*LHG*CRS	ALPALG*PLQ	QRVEIGMLYK	Α						

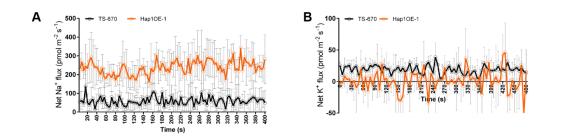
$Appendix\ Figure\ S7-Sequence\ alignment\ of SlHAK20, OsHAK4, and\ OsHAK17\ proteins.$

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 *S1HAK20* 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 \pm 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 CoroNaTM 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 CoroNaTM 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 $Na^{\scriptscriptstyle +}$ and $K^{\scriptscriptstyle +}$ fluxes

The measurement of Na⁺ and K⁺ fluxes were performed using the non-invasive, scanning ionselective 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 $(0.1 \text{ mM CaCl}_2, 0.1 \text{ mM KCl}, 0.5 \text{ mM NaCl}, and 0.3 \text{ mM}$ 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