Brief Communication

Natural variations in SISOS1 contribute to the loss of salt tolerance during tomato domestication

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Soil salinity is a major constraint on crop cultivability and productivity worldwide (Shabala, 2013). The ion toxicity caused by high salinity is alleviated by the adjustment of cellular Na+ and K+ homeostasis through the functions of ion transporters such as SOS1 (Salt Overly Sensitive 1) and HKT1 (High-Affinity Potassium Transporter 1). SOS1 is a plasma membrane Na+/H+ antiporter mediating Na+ extrusion in root epidermal cells to reduce Na+ accumulation in plants and in the parenchyma cells of root and shoot xylems to promote Na+ translocation from root to shoot, whereas the Na+ transporter HKT1 mediates retrieval of Na+ from the xylem and may contribute to Na+ recirculation from shoot to root (Zhu, 2016). Natural variations in HKT1 have been implicated in salt tolerance in several plant species (An et al., 2017). However, the role of natural variations in SOS1 in adaptation to salt stress has not been reported.

Tomato (Solanum lycopersicum) is one of the most consumed fruit and vegetable crops in the world (Zhu et al., 2018). The wild ancestor of tomato is adapted to highly saline coastal habitats, while cultivated varieties have lost salt tolerance during domestication for larger fruit (Pailles et al., 2020). By using a genome-wide association approach, we recently identified genetic variations in the Na+–K+ transporter SLHAK20 responsible for the variations in root Na+/K+ ratio and the loss of salt tolerance during tomato domestication (Wang et al., 2020). Here, we report that genetic variations in SISOS1 also contribute to the phenotypic variation of salt tolerance in tomato. We collected 326 tomato accessions from the original association population, including 33 wild accessions of S. pimpinellifolium (PIM), 99 domesticated accessions of S. lycopersicum var. cerasiciforme (CER) and 194 improved accessions of S. lycopersicum (BIG). The sequence variations in SISOS1 among the 326 accessions were identified based on the reference genome (Sato et al., 2012). Association analyses revealed two synonymous variations in the coding region and three variations in the promoter region of SISOS1 significantly associated with root Na+/K+ ratio (Figure 1a). The SNP-659 variation in the promoter was found to be the most significantly associated with root Na+/K+ ratio (P = 1.40 × 10−12), and the other two adjacent variations in the promoter, SNP-334 (G/A) and SNP-335 (C/T), were completely in linkage disequilibrium (LD, r2 = 1) with SNP-659 and thus were also strongly associated with root Na+/K+ ratio (Figure 1a). Sequence analysis identified that SNP-334 and SNP-335 are within a known CRT/DRE cis-element with the core sequence of CCGAC, and the promoters containing this cis-element can be recognized and activated by the CBF/DREB transcription factors in response to stress conditions (Yamaguchi-Shinozaki and Shinozaki, 2005). We thus classified the 326 accessions into two haplotype groups, Hap1 (haplotype group 1; n = 45) and Hap2 (n = 281), according to these two variations. Statistically, the accessions in Hap 1 showed significantly lower root Na+/K+ ratios than those in the larger group Hap2 (P = 1.78 × 10−10). Since root Na+/K+ ratio is negatively correlated with salt resistance in tomato, Hap1 and Hap2 were defined as the tolerant and sensitive alleles of SISOS1, respectively (Figure 1b). The distribution of these two alleles in PIM, CER and BIG groups indicated that salt tolerance was gradually lost during tomato domestication and improvement from PIM to CER and then from CER to BIG for larger fruit (Figure 1c).

We chose SISOS1TS−21 in Hap1 and SISOS1TS−577 in Hap2 as representatives of these two groups for further study. The variant promoter SISOS1pTS−21 with C and G at the position of SNP-335 and SNP-334 maintained the CRT/DRE core cis-element, whereas the SISOS1pTS−577 with T and A at the SNP-335 and SNP-334 disrupted the core cis-element (Figure 1d). To assess whether the variations in this cis-element contribute to SISOS1 expression, we analysed the binding capacity of the CRT/DRE variants with SIDREB2, a known salt-inducible DREB transcription factor in tomato recognizing CRT/DRE motif and inducing the expression of target genes (Hichri et al., 2016). Reciprocal competitive electrophoretic mobility shift assay (EMSA) showed strong and specific binding of SIDREB2 to the CRT/DRE motif in the promoter region of SISOS1TS−21, whereas no binding was observed in the region of SISOS1TS−577 promoter with disrupted CRT/DRE motif (Figure 1e). Gene expression analysis revealed that the transcript levels of SISOS1 are increased in the Hap1 varieties in response to high salinity, and this up-regulation was markedly lower in the Hap2 accessions (Figure 1f and g).

The critical role of SISOS1 in salt tolerance was further validated by analysing the knockout mutants of tomato
Wild SISOS1 variants confer salt tolerance

**Figure Legends**

(a) Distribution (%)

(b) Na+/K+ in roots

(c) Distribution (%)

(d) Na+ in shoots (mg/g DW)

(e) Na+/K+ in roots

(f) Bound probe

(g) Free probe

(h) Mn+ in shoots (mg/g DW)

(i) Na+ in roots (mg/g DW)

(j) NaCl in shoots (mg/g DW)

(k) K+ in roots (mg/g DW)

(l) NaCl in roots (mg/g DW)

(m) K+ in shoots (mg/g DW)

(n) NaCl in shoots (mg/g DW)

(o) Plant height (cm)

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**Table Legends**

| Na+/K+ in roots | Hap1 | Hap2 |
|-----------------|------|------|
| 0 d             | 1.00 | 1.00 |
| 1 d             | 1.00 | 1.00 |
| 2 d             | 1.00 | 1.00 |

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**Notes**

- **NaCl** concentrations: 0 mM, 100 mM, 150 mM
- **Time points**: 0 d, 1 d, 2 d, 7 d
The natural variations in SlSOS1 are associated with root Na\(^+\)/K\(^+\) ratio and salt tolerance in tomato. (a) SlSOS1-based association mapping and pairwise LD analysis. The variants in the promoter and coding region are highlighted in red and blue, respectively. Each variant is connected to the pairwise LD diagram with a dashed line. Red lines indicate strong LD values of SNP-659, SNP-335 and SNP-334 in the promoter region. (b) Haplotypes of SlSOS1 in the tomato population analysed based on SNP-334 and SNP-335. (c) The distribution of SlSOS1 alleles in PIM, CER and BKG groups. The n indicates the number of accessions. (d) A schematic diagram showing the SlSOS1(TS21-2) and SlSOS1(TS21-1) promoter regions. The dots represent nucleotide variations. The nucleotides of SNP-334 and –335 are indicated in red. The sequence of CRT/DRE motif in the SlSOS1(TS21-2) promoter is underlined. (e) Relative binding affinity of SIDERB2 to the CRT/DRE motifs in SlSOS1 promoter. Reciprocal competitive EMSA to determine the binding of recombinant MBP-SIDERB2 to the promoter region containing the CRT/DRE motifs of SlSOS1(TS21-2) was carried out using the indicated Cy5-labelled probes and unlabelled competitors. The same region of SlSOS1 promoter was used for analysis. (f, g) SlSOS1 expression in the Hap1 and Hap2 alleles in shoots (f) and roots (g) without or with salt stress treatment. Eleven accessions from each haplotype were used in this experiment. (h) Genomic sequence showing the mutations in SlSOS1 gene generated using CRISPR/Cas9 system in the TS-21 wild variety. The sgRNA target sites are indicated in blue. The PAM sequence is underlined. (i-k) Na\(^+\)/K\(^+\) ratio (i) and the contents of Na\(^+\) (j) and K\(^+\) (k) in roots of slsos1-1, slsos1-2 and TS-21 wild-type plants. Data are shown as means ± SD (n = 4). (l) The contents of Na\(^+\) (l) and K\(^+\) (m) in the shoots of slsos1-1, slsos1-2 and TS-21 wild-type plants. Data are shown as means ± SD (n = 4). (n, o) Salt tolerance of slsos1 mutants and wild type (TS-21) indicated by plant height (n) and growth phenotype (o). Three-week-old slsos1-1, slsos1-2 and TS-21 plants were treated with 0, 100 or 150 mM NaCl for 1 week, followed by recovery for 1 week, and then, plant height was measured. Values are means ± SD (n = 6 plants of each genotype). Bars, 5 cm. In the box plots of (b), (f) and (g), boxes indicate the range of the percentiles of the total data using Turkey method, centre values are medians, dashed lines indicate variability outside the upper and lower quartiles, and dots denote outliers. n denotes the number of accessions belonging to each haplotype group. Statistical significance was determined by Student’s t-test (*P < 0.05, **P < 0.01).

**Conflict of interest**

The authors declare no competing interests.

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