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Identification and Expression Pattern Analysis of the *SOS* Gene Family in Tomatoes

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Abstract: *SOS*s are key genes in the SOS (salt overly sensitive) signaling pathway, which plays an important role in maintaining ion homeostasis in plants under salt stress. Our aim was to clarify the biological function of the *SOS* gene family in tomato plants. We identified 14 *SpeSOS* genes, 10 *SpiSOS* genes, 11 *SpmSOS* genes, 9 *SlmSOS* genes, and 11 *SlySOS* genes from the genomes of "LA0716" (Spe), "LA2093" (Spi), "LA1589" (Spm), "M82" (Slm), and "Heinz 1706" (Sly) separately. The SOS protein family in tomatoes was divided into five subgroups (SOS1, SOS2, SOS3, SOS4, and SOS5) through phylogenetic analysis. The SOS proteins of the same subgroup in tomatoes contained similar conserved domains and motif structures. A subcellular localization prediction showed that the SOS1, SOS3, and SOS5 proteins in tomatoes were located on the cell membrane, while the SOS2 and SOS4 proteins in tomatoes were located on the cytoplasm and chloroplast, respectively. *SlSOS1* contained the most exons and introns (23 and 22, respectively), while *SlSOS5* contained only one exon. Via the analysis of the cis-elements in the promoters of those *SOS* genes in tomatoes, several hormone-, light-, and abiotic stress-related cis-elements were found. In addition, qRT-PCR revealed that the *SpeSOS*, *SpiSOS*, and *SlySOS* genes were induced by salt stress with similar expression patterns. Additionally, the expressions of *SOS1-1*, *SOS1-2*, *SOS2-2*, *SOS3-3*, *SOS4-1*, and *SOS5-2* were higher in salt-tolerant tomatoes compared with salt-sensitive tomatoes under salt stress. In the salt-sensitive "LA1698" tomato and salt-tolerant "LA0516" tomato, most *SOS* genes had the highest expression in the roots. The expressions of *SOS1-1*, *SOS1-2*, *SOS2-1*, *SOS2-2*, *SOS3-2*, *SOS3-3*, and *SOS5-1* in the leaves of salt-tolerant tomatoes were significantly higher than those in salt-sensitive tomatoes. Thereby, the *SOS* genes in tomatoes were induced by salt stress, indicating that they participated in the regulation mechanism of tomato salt tolerance. This study laid the foundation for further study on the function of the *SOS* gene family and revealed the molecular mechanism of tomato salt resistance.

Keywords: tomato; *SOS* gene family; bioinformatics; salt stress; gene expression analysis

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1. Introduction

Soil salinization is prevalent in nature and there are approximately 950 million hm^2 of saline soils globally, which are widely distributed in different countries and regions, including approximately 99.13 million hm^2 in China [1]. The tomato (*Solanum lycopersicum* L.) is one of the most important vegetables worldwide, and it is a moderately salt-sensitive crop [2]. Soil salinization greatly affects the growth and yield of tomato plants. Previous studies have shown that salt stress reduces leaf photosynthetic capacity, induces cell membrane damage, and slows the growth of tomato plants [3].

In order to reduce the damage caused by salt stress, a series of regulatory mechanisms have been developed in plants, such as osmotic regulation, ion homeostasis regulation,

and reactive oxygen species clearance [4]. The molecular mechanisms of plant responses to salt stress involve multiple genes and pathways [5,6]. When plants were subjected to salt stress, the salt oversensitive (SOS) signal transduction pathway was activated to regulate the balance of ions inside and outside the cells to improve plant salt tolerance [7]. Previous studies have discovered and identified five key genes in the SOS signal transduction pathway in *Arabidopsis thaliana*: *SOS1* [8], *SOS2* [9], *SOS3* [10], *SOS4* [11], and *SOS5* [12]. In addition to *Arabidopsis*, Cheng et al. (2019) [13] and Liu et al. (2021) [14] identified twelve and five *SOS* genes in Tuber mustard and *Tamarix hispida*, respectively.

SOS proteins are closely related to the plant salt resistance pathway. When *Arabidopsis* was subjected to salt stress, the intracellular Ca^{2+} concentration increased. The SOS3 gene, which was located in the cytoplasm, sensed the changes in the calcium ion levels and bonded to Ca^{2+} [4]. Subsequently, SOS3 activated the activity of serine/threonine protein kinase SOS2 and combined it with SOS2 to form the SOS3–SOS2 complex [15,16]. The SOS3–SOS2 complex activated the Na^+/H^+ antiporter SOS1 (salt over sensitive 1) on the plasma membrane and excreted the accumulated Na^+ in the cytoplasm, thus regulating the ion balance of K^+ and Na^+ inside and outside the cell [17,18]. Olías et al. (2009) [19] found that *SlSOS1* retains Na^+ in the stem and prevents the transport of Na^+ to the above-ground photosynthetic organs, thereby reducing leaf ion toxicity effects and improving salt tolerance in tomatoes. Park et al. (2016) [20] found that the expression of the *SOS1* gene is also regulated by the circadian cycle and biological clock, which allows plants to effectively predict and respond to dehydration due to transpiration, drought, and saline stress. Huertas et al. [21] cloned and characterized *SlSOS2* from tomato, which maintained intracellular ion homeostasis in plant cells by regulating the activity of ion-transporting proteins such as SlSOS1, LeNHX2, and LeNHX4 in order to enhance salt tolerance in tomatoes. The AtSOS2 protein also regulates the activity of the Ca^{2+} transporter protein CAX1 to modulate calcium ion transport, modulates the activities of NDPK2, CAT2, and CAT3 in H_2O_2 signaling, and reduces ROS injury, thereby mitigating plant damage caused by high-salt environments [22,23]. The SOS3 protein plays an important role in the plastic development of lateral roots through the modulation of auxin gradients and maxima in roots under mild salt stress [24]. The *SOS4* (salt over sensitive 4) gene encodes for pyridoxal kinase, which improves plant salt tolerance by affecting SOS1 protein activity and root hair development under salt stress [11,25]. In comparison, *SOS5* (salt over sensitive 5) was mainly involved in the plant salt tolerance pathway by promoting cell wall development, root elongation, and synergizing with abscisic acid [12,26].

The overexpression of a single gene or the co-expression of multiple genes in the *SOS* gene family enhanced the salt tolerance of plants [27]. The salt tolerance function of the *SOS* genes was demonstrated in rice [28], tomatoes [21], wheat [29], grapes [30], and sugarcane [31]. These indicated that *SOS* genes are involved in plant salt tolerance regulation. However, the bioinformatics characteristics and expression patterns of the *SOS* genes in tomatoes in response to salt stress, especially in different organs, need further investigation.

In the present study, a genome-wide analysis of the identification of *SOS* from the genomes of the "LA0716" (Spe, *Solanum pennellii* L.), "LA2093" (Spi, *Solanum pimpinellifolium* L.), "LA1589" (Spm, *Solanum pimpinellifolium* L.), "M82" (Slm, *Solanum lycopersicum* L.), and "Heinz 1706" (Sly, *Solanum lycopersicum* L.) tomatoes was performed. Additionally, we systematically characterized the protein sequence characteristics, phylogenetic relationships, subcellular localization, gene structure, chromosomal localization, and promoter analysis. Moreover, the expression levels of the *SOS* genes in different tomato genotypes responding to salt stress were examined. The expression of the *SOS* genes of tomatoes in different developmental stages and tissues was detected. These results provide fundamental insights into the genetic improvement of salt tolerance traits and reveal the salt stress response mechanism of tomatoes.

2. Materials and Methods

2.1. Genome-Wide Identification of the SOS Family Genes in Tomatoes

The protein sequences of AtSOS1, AtSOS1b/AtNHX8, AtSOS2, AtCIPK8, AtSOS3, AtSOS4, and AtSOS5 were searched in TAIR (https://www.arabidopsis.org/, accessed on 1 March 2022). The genome sequences, CDS, protein sequences, and genomic annotations for the tomato genotypes, including "LA0716", "LA2093", "LA1589", "M82", and "Heinz 1706" were obtained from SGN (https://solgenomics.net/, accessed on 1 March 2022). Candidate SOS proteins were identified in five tomato genomes through BLastP in TBtools [32] using the AtSOS protein sequences as references. The e-value was set to 1×10^{-5} for BLastP. The candidate protein sequences were submitted to NCBI for BLASTP. The conserved domains of SOS1, SOS2, SOS3, SOS4, and SOS5 were predicted using a CD search [33] (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi, accessed on 1 March 2022) to eliminate the proteins that did not have conserved domains.

2.2. Protein Property Analysis and the Prediction of the Subcellular Localization

The number of amino acids (AAs), molecular weight (MW), and theoretical isoelectric point (pI) of the SOS proteins in tomatoes were predicted using ExPASy [34] (http://web.expasy.org/protparam/, accessed on 15 March 2022). The subcellular localizations of the SOS proteins in the tomatoes were predicted using Cell-PLoc 2.0 [35] (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/, accessed on 15 March 2022). The conserved motifs and conserved domains of the SOS family in tomatoes were identified using MEME (https://meme-suite.org/, accessed on 15 March 2022) and a CD search. The number of motifs was set to 10, while the site distribution was set to any number of repetitions (ANR). The expected value threshold in the CD search was set to 0.01, while the maximum number of hits was set to 500.

2.3. Protein Sequence Alignment and Phylogenetic Analysis of the SOS Family

The sequences of the SOS proteins from *Arabidopsis* and *Oryza sativa* L. were obtained from the TAIR and Phytozome (https://phytozome-next.jgi.doe.gov/, accessed on 1 March 2022). The SOS protein sequences of *Arabidopsis*, *Oryza sativa* L. tomato genotypes, including "LA0716", "LA2093", "LA1589", "M82", and "Heinz 1706" were aligned using MEGA-X version 10.1.7 [36] and visualized using Jalview software version 2.11.3.2. To analyze the evolutionary relationships between AtSOSs, OsSOSs, SpeSOSs, SpiSOSs, SpmSOSs, SlmSOSs, and SlySOSs, a phylogenetic tree was constructed using the maximum likelihood (ML) method in MEGA-X software version 10.1.7, and the BootStrap was set to 1000. The phylogenetic tree model was plotted using EvolView (https://evolgenius.info//evolview-v2/, accessed on 1 April 2022).

2.4. Prediction of SOS Gene Characterization in Tomatoes

The structural intron and exon characteristics and chromosome localization of the *SOS* genes in tomatoes were analyzed using TBtools version 2.080 [36]. The collinearity analysis of the *SOS* genes in tomatoes was conducted using MCScanX [37] in TBtools, and the results were visualized using TBtools. The promoter region (2000 bp upstream of the coding region) of the *SOS* genes in tomatoes was extracted using TBtools, and the promoter cis-element analysis was performed using PlantCARE [38] (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 1 April 2022).

2.5. Spatial and Temporal Expression Analysis of the SOS Genes in Tomatoes

The seven tomato genotypes with different salt tolerance levels are shown in Table S1 from the Laboratory of Vegetable Physiology and Ecology, Nanjing Agricultural University, which were used as the plant materials. The seeds were sown in 50-hole trays with a mixture of peat, vermiculite, and perlite (volume ratio: 2:1:1). The tomato plants with five leaves were transferred into a 32-hole plastic container, and the plants were fixed with quartz sand, cultivated using a half-strength Japanese garden-type nutrient solution

containing 200 mM NaCl [39]. The seedlings were grown in a climate chamber with 16 h of light (360 μmol m^{-2} s^{-1}, LED light source) at 25 °C and 8 h of darkness at 18 °C where the relative humidity was 75%.

We selected three tomato genotypes, "LA0716", "LA2093", and "Heinz 1706", as the materials to verify whether the *SOS* genes in the tomatoes we identified were induced by salt stress. Four tomato genotypes ("LA0516", "LA1698", "LA0012", and "LA1598") with different salt susceptibility levels were used to analyze the *SOS* gene expression at different times of salt treatment. At 0, 4, 8, 12, and 24 h after the NaCl treatment, the 3rd fully expanded leaves from top to bottom of the tomato plants were harvested. Two tomato genotypes ("LA1698" and "LA0516") were used to analyze the expression of the *SOS* genes in different organs of tomato plants. The tomato plants with five leaves were transferred into plastic containers (30 cm height, 35 cm diameter) with a mixture of peat, vermiculite, and perlite (volume ratio: 2:1:1). The plants were cultivated in plastic greenhouses under the same environmental conditions before harvest. The plants were irrigated using a half-strength Japanese garden-type nutrient solution at 1-day intervals during growth. When the 2nd spike of the tomato fruits was ripe, the roots, stems, leaves, open flowers, and the 2nd spike of ripe fruits of the plants were separately taken. Three biological replicates were employed in each treatment, and each replicate included five seedlings. The samples were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

The total RNA was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA), and the cDNA was synthesized using the PrimeScript TM RT reagent kit (Abm, Zhenjiang, China). The qRT-PCR analysis was performed using the TOROGreen® qPCR Master Mix kit (Toroivd, Virigin Islands, UK) and Eppendorf real-time PCR (Thermo Fisher, Singapore). The reaction program included pre-denaturation at 95 °C for 1 min, denaturation at 95 °C for 10 s, and annealing at 60 °C for 30 s for 40 cycles. The lysis curve program included 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 1 s. Three technical and biological replicates were set for each reaction. *SlActin* was selected as the internal standard to normalize the expression. The sequences of primers (Table S2) were designed by GenScript (https://www.genscript.com/, accessed on 1 May 2022). The relative expression level was calculated using the $2^{-\Delta\Delta CT}$ method.

2.6. Statistical Analysis

The data were subjected to a statistical analysis of variance (ANOVA) using the SPSS package (SPSS 25.0). The data were defined as significantly different when $p < 0.05$.

3. Results

3.1. Whole Genome Identification and Analysis of the Tomato SOS Gene Family

3.1.1. Identification of the Tomato *SOS* Gene Family and Physicochemical Properties Analysis

In total, 14, 10, 11, 9, and 11 *SOS* genes were identified from the whole genome of "LA0716" (Spe), "LA2093" (Spi), "LA1589" (Spm), "M82" (Slm), and "Heinz 1706" (Sly), respectively. The characteristics of the protein members in the SOS1 group also differed greatly, while the characteristics of the protein members in the SOS2, SOS3, SOS4, and SOS5 groups differed slightly (Table S3). The number of amino acids in the SOS protein of different tomato genotypes ranged from 173 AAs to 1151 AAs. The molecular weights ranged from 18.31 kDa to 127.5 kDa, and the pI values all ranged from 4.52 to 8.86 (Table S3). These indicated that the sequence characteristics of the SOS protein family members were similar in the different tomato genotypes. The predictions of the subcellular localization showed that some SOS1 and all SOS3 and SOS5 proteins in the tomatoes were localized to the cell membrane, and the SpeSOS1-4, SpiSOS1-2, SpmSOS1-2, SlmSOS1-2, SlySOS1-2, and SlySOS1-3 proteins in the SOS1 subgroup were also located on the vacuole in addition to the cell membrane (Table S3).

3.1.2. Phylogenetic Analysis and Classification of the Tomato SOS Protein Family

The SOS protein family was divided into five subgroups, including SOS1, SOS2, SOS3, SOS4, and SOS5 (Figure 1). The grouping of the SOS protein family in different tomato genotypes was consistent, but the number of members in each group was different. This indicated that the SOS protein may have changed during the evolution of the tomato. The sequence comparison of the SOS proteins using *Arabidopsis*, rice, and five tomato genotypes showed that the SOS protein sequences being categorized into the same subgroup had high homology (Figure 2). It is speculated that their functions among different species and different genotypes of the same species were conservative and similar.

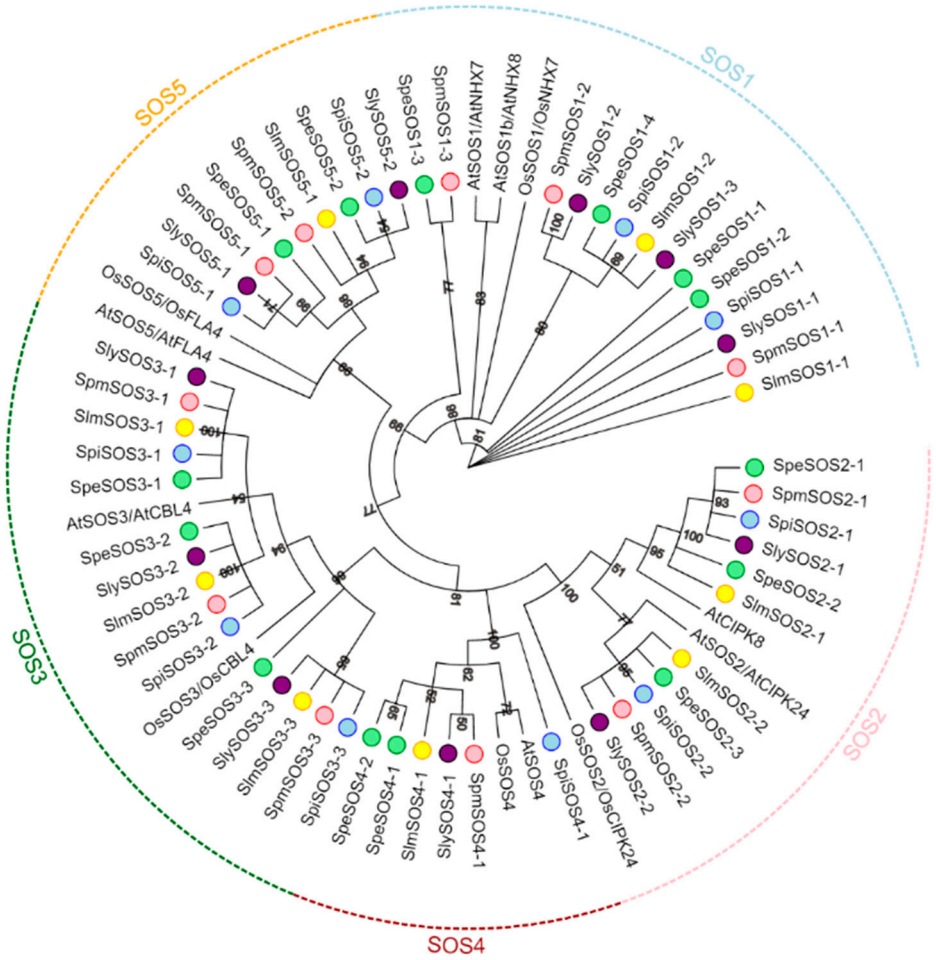

Figure 1. The phylogenic tree of the SOS family *Arabidopsis thaliana*, rice, and tomato.

3.1.3. Analysis of the Conserved Motifs and Conserved Structural Domains of the Tomato SOS Proteins

The *Arabidopsis*, rice, and tomato SOS proteins contained a variety of motif structures, and the motif and conservative domains of the SOS proteins in the same subgroup were uniformly distributed (Figure 3). The SOS1 subgroup contained the Na_H_Exchanger superfamily structural domain, corresponding to motifs 1, 3, 4, 6, and 10. The SOS2 subgroups contained the PKc_like superfamily and CIPK_C structural domains, corresponding to motifs 3, 6, 7, 8, and 9, and motifs 4 and 6, respectively. The SOS3 subgroups contained the FRQ1 superfamily structural domains corresponding to motifs 1, 2, and 5. The SOS4 subgroups contained the PLN02978 structural domains corresponding to motifs 5 and 10. The SOS5 subgroups contained the Fasciclin superfamily structural domains corresponding to motif 6.

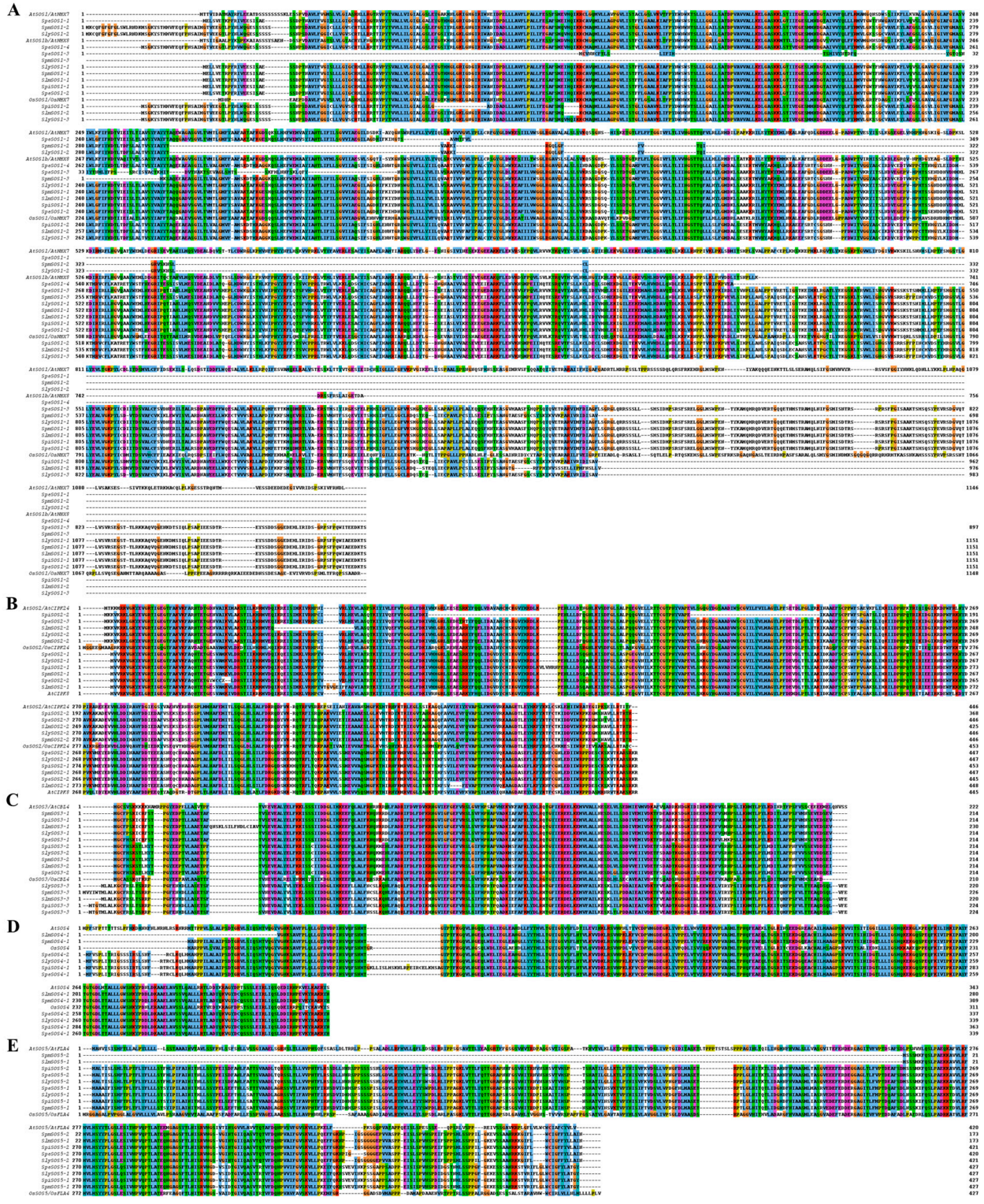

Figure 2. The protein sequence alignment of the SOS protein in *Arabidopsis thaliana*, rice, and tomato. (**A**) The protein sequence alignment of SOS1s. (**B**) The protein sequence alignment of SOS2s. (**C**) The protein sequence alignment of SOS3s. (**D**) The protein sequence alignment of SOS4s. (**E**) The protein sequence alignment of SOS5s.

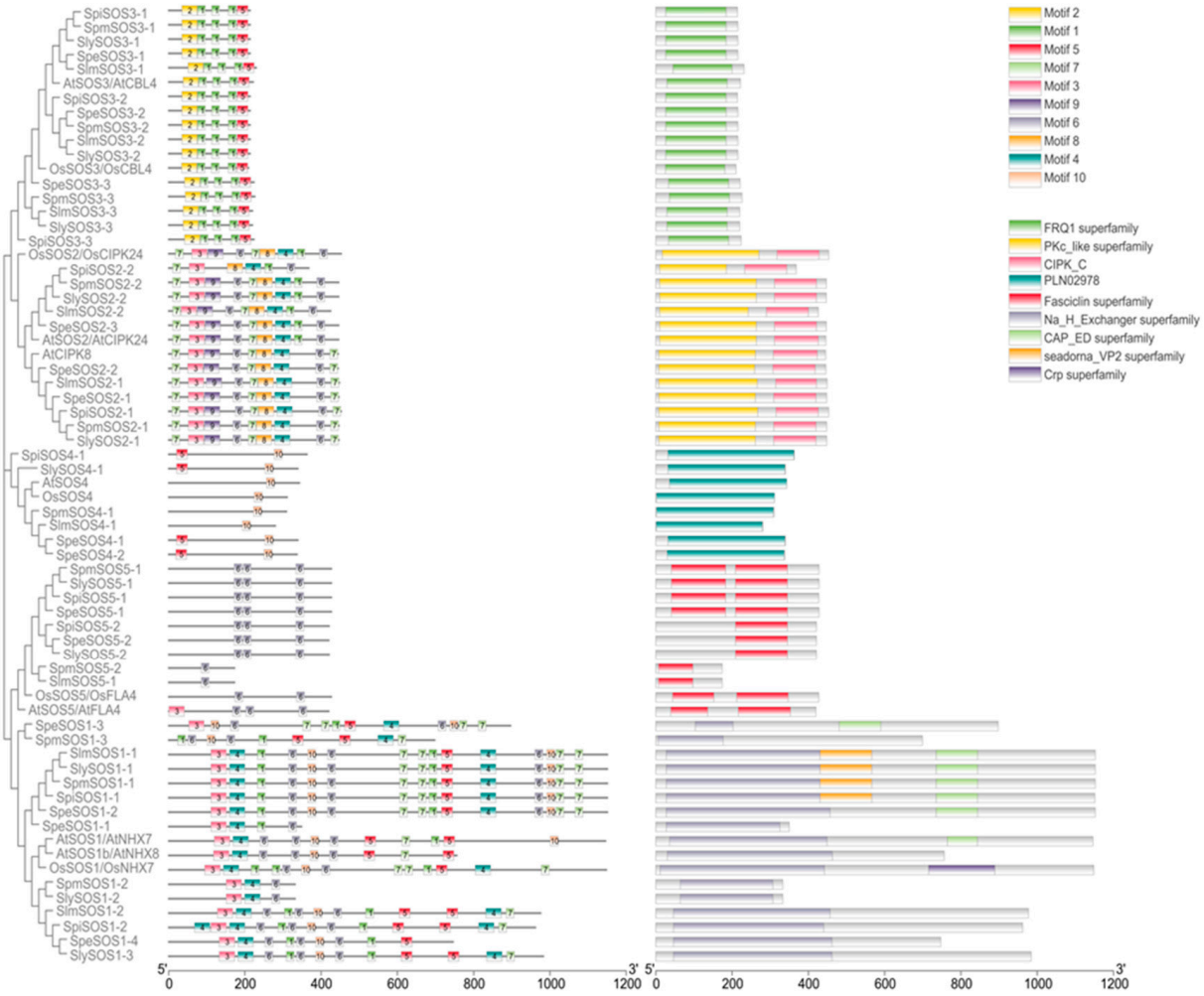

Figure 3. The conserved motifs and domains of the SOS homologs proteins. The left is the conserved motif of the SOS protein, and the right is the conserved domain of the SOS protein.

3.1.4. Chromosomal Localization and Collinearity and Structural Analysis of the Tomato SOS Genes

The SOS genes were distributed on chromosomes 1, 2, 3, 4, 6, and 12 of the tomato (Figure 4). For instance, the *SOS1* genes were distributed on tomato chromosomes 1 and 4, and the *SOS2* genes were distributed on chromosomes 4 and 12. In order to explore the evolutionary relationship of the *SOS* genes among different genotypes of tomatoes, the genomes of the "LA0716", "LA2093", "LA1589", "M82", and "Heinz 1706" tomatoes were analyzed (Figure 5). The results showed that in *SOS1*, *SpeSOS1-1*, *SpiSOS1-1*, *SpmSOS1-1*, *SlmSOS1-1*, and *SlySOS1-1* had a collinear relationship, and *SpeSOS1-4*, *SpiSOS1-2*, *SpmSOS1-3*, *SlmSOS1-2*, and *SlySOS1-3* had a collinear relationship. However, *SpeSOS1-2* and *SpeSOS1-3* had no collinear relationship with the other four genotypes. These indicated that they may be unique to wild tomatoes.

The gene structure of *SOS* genes in the same subgroup was similar (Figure 6). For example, among the *SOS1* genes, *SpeSOS1-2*, *SpiSOS1-1*, *SpmSOS1-1*, *SlmSOS1-1*, and *SlySOS1-1* contained 23 exons and 22 introns. Among the *SOS2* genes, *SpeSOS2-2* and *SlmSOS2-1* contained 15 exons and 14 introns, and *SpeSOS2-1*, *SpeSOS2-3*, *SpiSOS2-1*, *SpmSOS2-1*, and *SlySOS2-1* contained 14 exons and 13 introns.

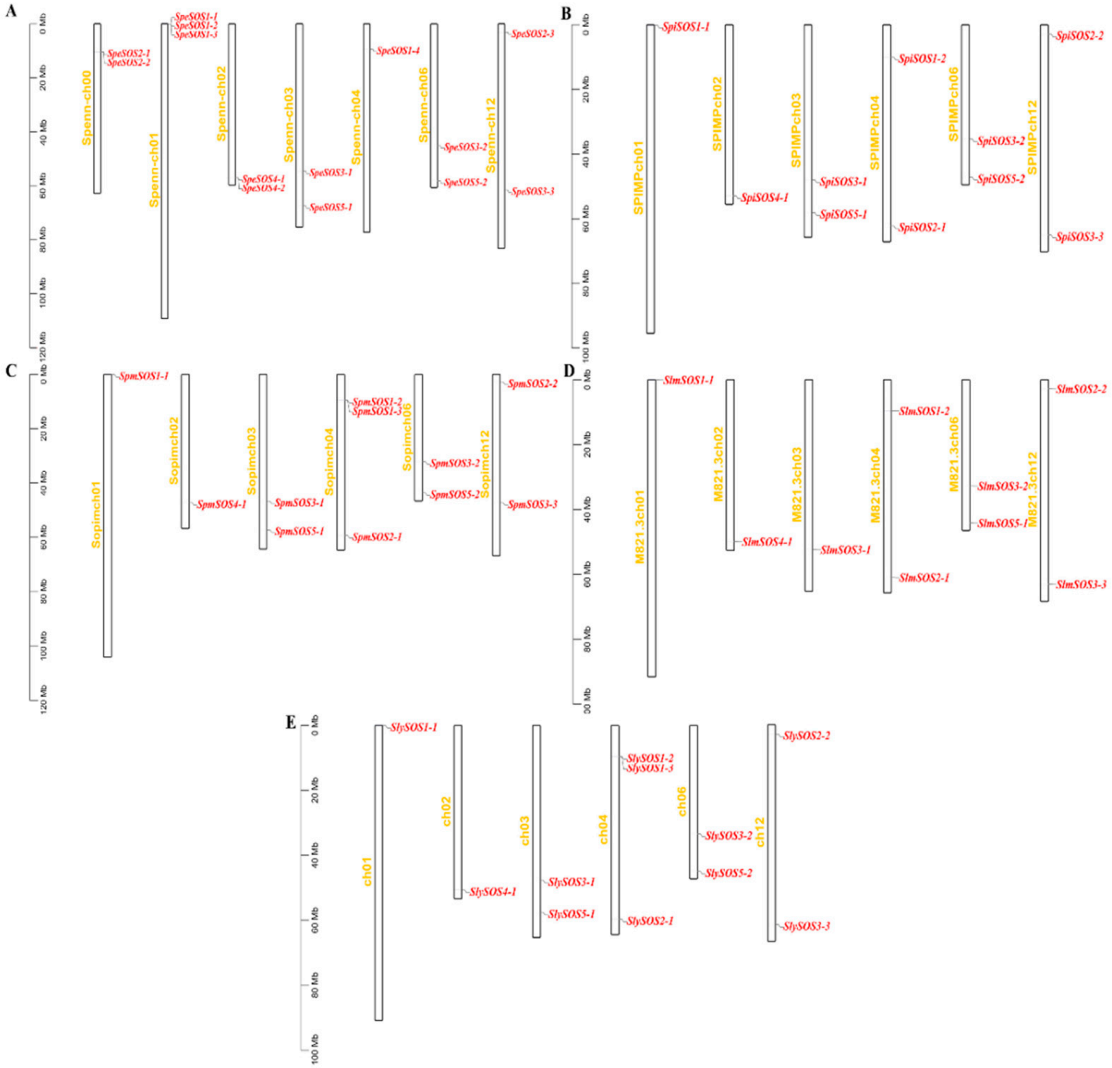

Figure 4. Positions of the *SOS* gene family members on the tomato chromosomes: (**A**) LA0716 (*Solanum pennellii* L.); (**B**) LA2093 (*Solanum pimpinellifolium* L.); (**C**) LA1589 (*Solanum pimpinellifolium* L.); (**D**) M82 (*Solanum lycopersicum* L.); (**E**) Heinz 1706 (*Solanum lycopersicum* L.).

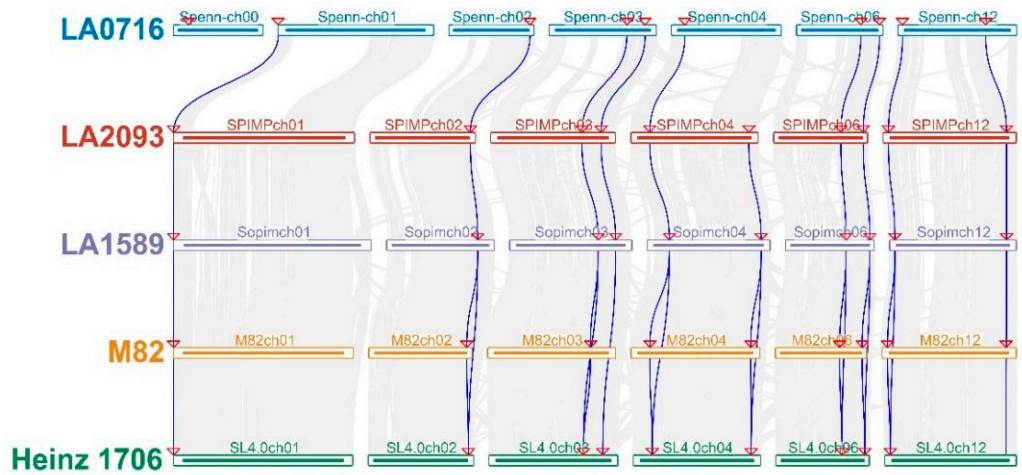

Figure 5. Collinearity analysis of the *SOS* genes in tomatoes.

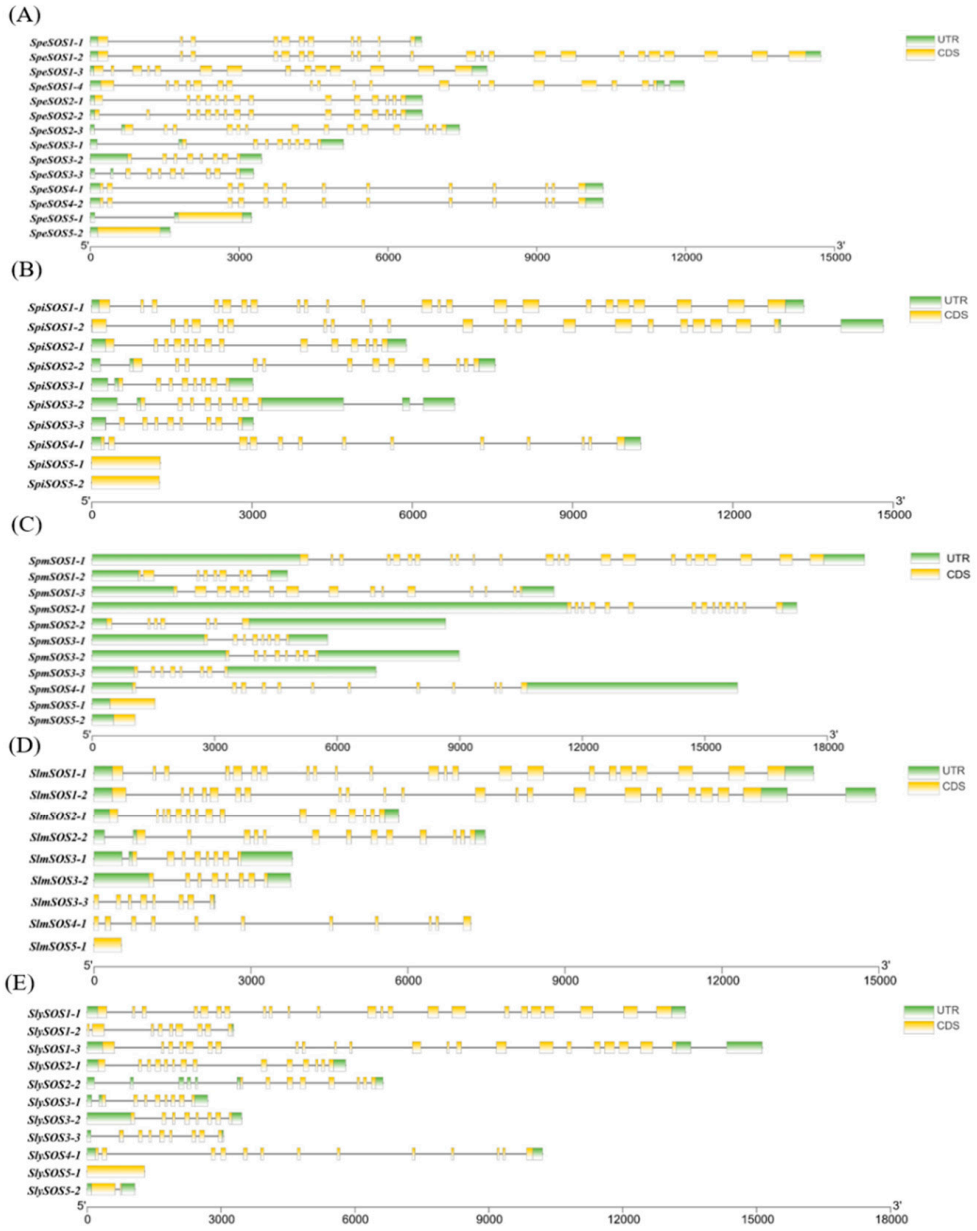

Figure 6. The gene structures of the *SOS* family genes in tomatoes: (**A**) LA0716 (*Solanum pennellii* L.); (**B**) LA2093 (*Solanum pimpinellifolium* L.); (**C**) LA1589 (*Solanum pimpinellifolium* L.); (**D**) M82 (*Solanum lycopersicum* L.); (**E**) Heinz 1706 (*Solanum lycopersicum* L.).

3.1.5. Analysis of the Cis-Acting Element in the Promoter Regions of the Tomato SOS Gene Family

There were many types of response elements 2000 bp upstream of the CDS sequence, such as hormone response, light response, abiotic stress response elements, and elements related to the growth and development of tomatoes (Table S4). All promoter regions of the tomato *SOS* genes contained light response elements and the MYB and MYC transcription factor binding sites that respond to abiotic stress (Figure 7). The members of the *SOS* gene family with similar genetic relationships contained similar element structures. For instance the promotor region in *SlmSOS1-1* from M82, *SlySOS1-1* from Heinz 1706, *SpmSOS1-1* from LA1589, *SpiSOS1-1* from LA2093, and *SpeSOS1-2* from LA0716 all contained drought, gibberellin, stress, and wound-response elements distributing to similar locations.

Figure 7. The promoter cis-elements analysis of the *SOS* family genes.

3.1.6. Spatio-Temporal Expression Pattern of the *SOS* Gene Family in Different Tomato Genotypes under Salt Stress

Except for the *SpeSOS2-2*, *SpeSOS3-1*, *SlySOS1-3*, and *SlySOS3-1* genes, the other members of the *SpeSOS*, *SpiSOS*, and *SlySOS* genes responded to salt stress. In "LA0716", the expression levels of the *SpeSOS1-3* and *SpeSOS4-1* genes reached their peak earlier, with the highest expression at the 4 h of salt treatment time point (Figure 8). The expression levels of the *SpeSOS2-3*, *SpeSOS3-3*, and *SpeSOS5-2* genes were significantly upregulated under salt stress, with the highest expression being 4.39, 3.55, and 4.21 times higher than the control (Figure 8). In "LA2093", the expression levels of the *SpiSOS3-2* and *SpiSOS3-3* genes were higher than the other members, which were 14.06 and 33.25 times higher than the control 12 h after the salt treatment (Figure 9). In "Heinz 1706", the expression levels of the *SlySOS2-1*, *SlySOS3-3*, *SlySOS4-1*, and *SlySOS5-1* genes at the highest point were 3.38, 3.51, 3.74, and 8.43 times higher than those at 0 h, respectively (Figure 10).

Salt-sensitive tomatoes "LA1698" and "LA0012" and salt-tolerant tomatoes "LA0516" and "LA1598" were well grown under normal growing conditions (Figure S1). After 7 days of salt stress, the plant growth of all four tomato genotypes was inhibited, with wilted and chlorotic leaves and significantly decreased plant heights (Figure S1). Compared with the other tomato genotypes, the leaf area of "LA1698" was significantly reduced, and the leaf abscission was serious, showing that this genotype was more sensitive to salt stress (Figure S1C).

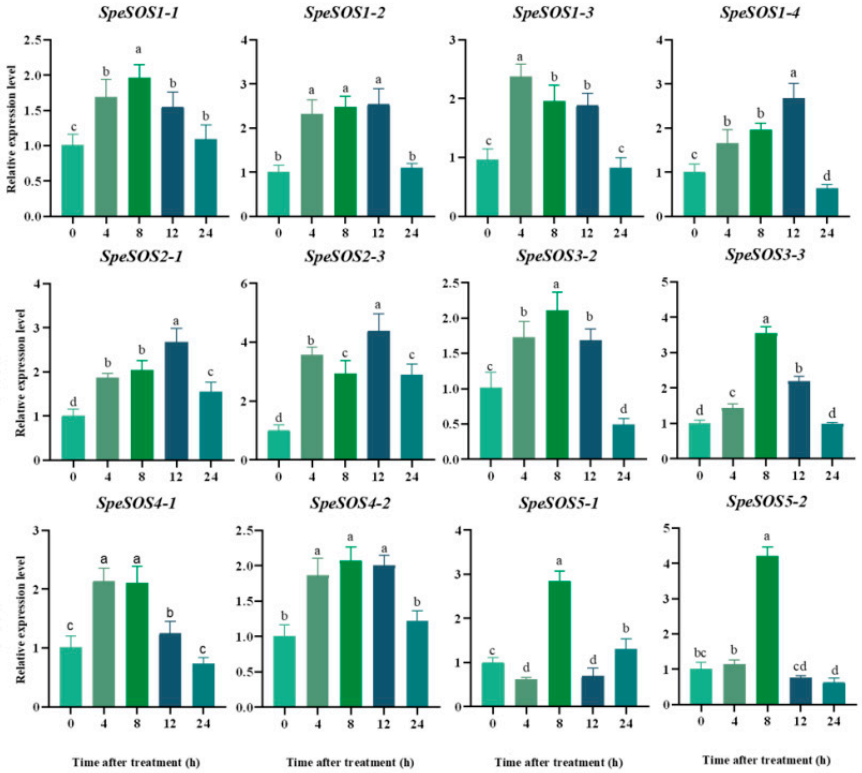

Figure 8. The expression patterns of the *SOS* family genes under salt stress in "LA0716" (*Solanum pennellii* L.). The different letters represent significant differences ($p < 0.05$, the same as below).

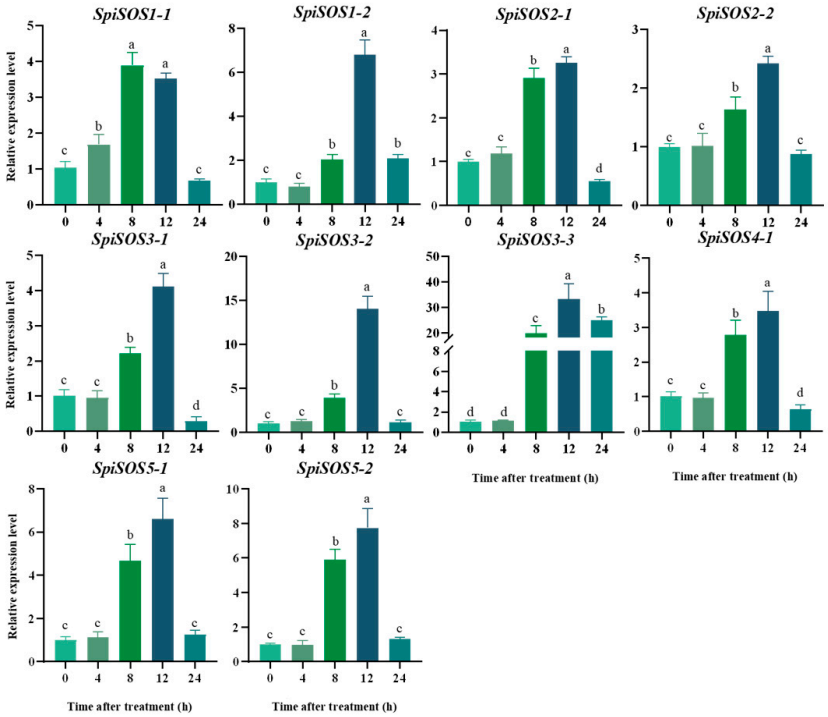

Figure 9. The expression patterns of the *SOS* family genes under salt stress in "LA2093" (*Solanum pimpinellifolium* L.). The different letters represent significant differences ($p < 0.05$, the same as below).

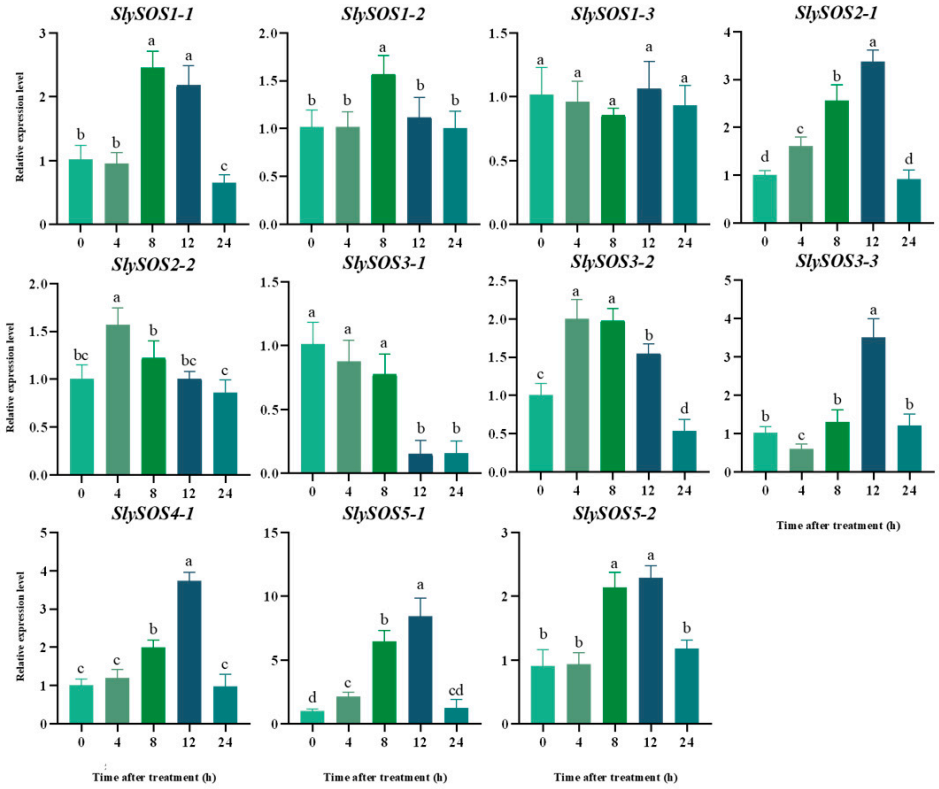

Figure 10. The expression patterns of the *SOS* family genes under salt stress in "Heinz 1706" (*Solanum lycopersicum* L.). The different letters represent significant differences ($p < 0.05$, the same as below).

Salt stress significantly increased the expression of some members of the *SOS* gene family in the four tomato genotypes (Figure 11). The expression pattern of *SOS1-1* in the four tomato genotypes was similar within 24 h of the salt treatment (Figure 11). The expression of *SOS1-2* in "LA1698", "LA0012", and "LA1598" under salt stress increased and then decreased, reaching a peak at 12 h. In comparison, the expression of *SOS1-2* in "LA0516" continued to rise, reaching a peak at 24 h. The expression level of *SOS2-1* in the four tomato genotypes under salt stress first increased and then decreased. Within 24 h of the salt treatment, the expression of *SOS2-2* in "LA1698" and "LA0516" first increased and then decreased, reaching a peak at 12 h. The expression of *SOS3-2* in "LA1698", "LA0012", and "LA0516" under salt stress first increased and then decreased. The expression of *SOS3-3* in "LA1698" and "LA1598" reached the peak at 8 h after the salt treatment, which was 1.64 and 4.05 times higher than the control, respectively. Within 24 h of the salt treatment, the expression of *SOS4-1* increased and then decreased in "LA1698", "LA0516", and "LA1598". Moreover, the expression level of the *SOS* gene was distinct in different tomato genotypes (Figure 11). For instance, at 12 h and 24 h of salt stress, the expression levels of *SOS1-1* and *SOS1-2* in "LA0516" were significantly higher than in "LA1698" and "LA0012". At the 4, 8, 12, and 24 h time points of salt treatment, the expression of *SOS2-2* was significantly higher in "LA1598" than in "LA1698" and "LA0012".

All members of the *SOS* gene family were expressed in the roots, stems, leaves, flowers, and mature fruits of the salt-sensitive tomato "LA1698" and salt-tolerant tomato "LA0516" (Figure 12). In "LA1698" and "LA0516", the expression levels of *SOS1-1*, *SOS2-2*, *SOS3-2*, *SOS4-1*, *SOS5-1*, and *SOS5-2* were the highest in the roots, followed by the leaves or stems. The expression levels of *SOS1-2*, *SOS2-1*, and *SOS3-3* were the highest in the leaves, followed by the roots or leaves. The expression levels of *SOS1-2*, *SOS2-3*, *SOS4-1*, and *SOS5-2* were the lowest in the flowers, and the expression levels of *SOS3-2*, *SOS3-3*, and *SOS5-1* were the lowest in the mature tomato fruits. The expression levels of *SOS1-1*, *SOS1-2*, *SOS2-1*, *SOS2-2*, *SOS3-2*, *SOS3-3*, and *SOS5-2* in the leaves of salt-tolerant tomato

"LA0516" were significantly higher than "LA1698". Based on the expression study, we concluded that the expression levels of *SOS1-1*, *SOS1-2*, *SOS2-1*, *SOS2-2*, *SOS3-2*, *SOS3-3*, *SOS4-1*, and *SOS5-2* were induced by salt stress in tomatoes.

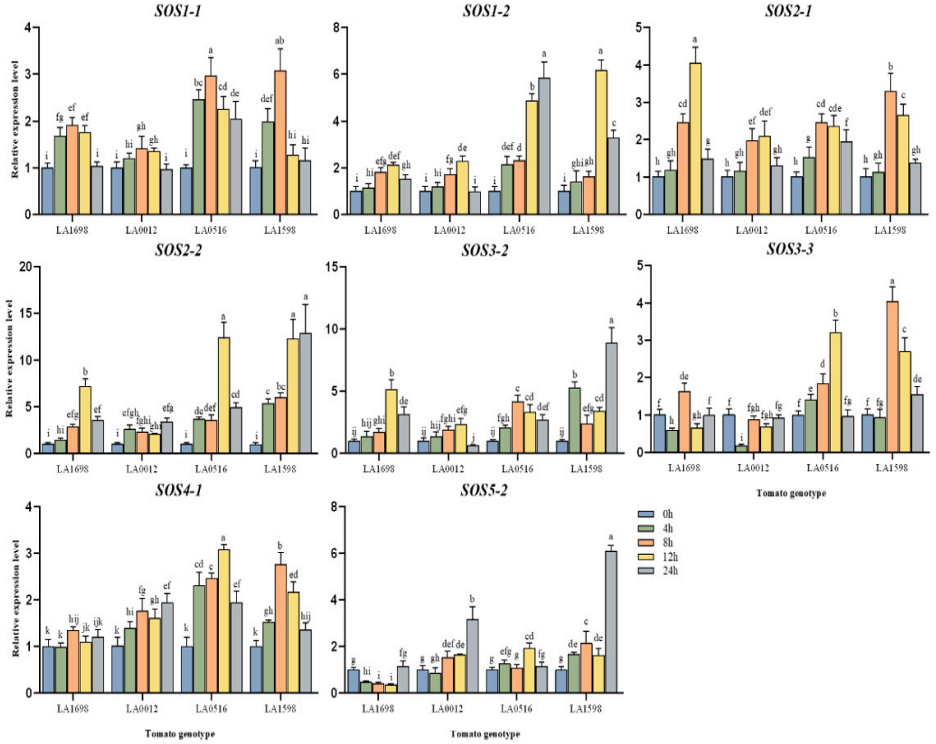

Figure 11. Expression characteristics of the *SOS* genes at different times of salt treatment. The different letters represent significant differences ($p < 0.05$, the same as below).

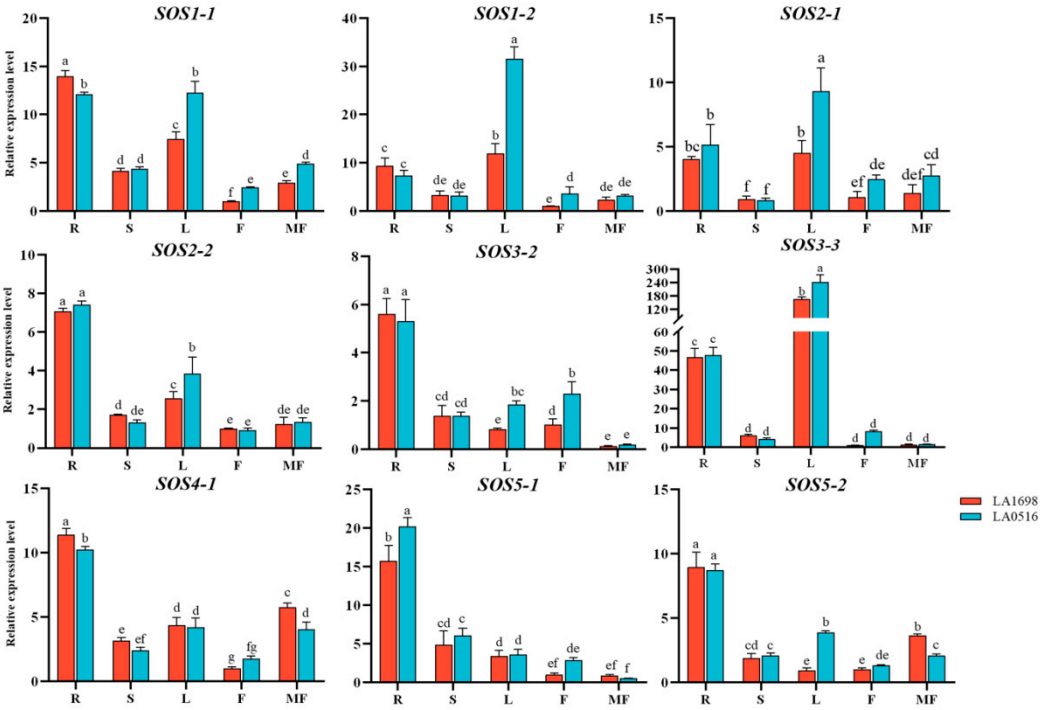

Figure 12. Expression characteristics of the *SOS* genes in different organs of tomato plants. R, S, L, F, and MF represent the roots, stems, leaves, flowers, and mature fruits of tomatoes, respectively. The different letters represent significant differences ($p < 0.05$, the same as below).

In summary, except for the *SpeSOS2-2*, *SpeSOS3-1*, *SlySOS1-3*, and *SlySOS3-1* genes, the other members of the *SpeSOS*, *SpiSOS*, and *SlySOS* gene family responded to salt stress induction with similar expression trends, and their expression tended to increase and then decrease with the prolongation of salt treatment time, peaking at the 4th, 8th, or 12th h of treatment. The expression levels of the *SOS* genes in the salt-sensitive lines, "LA1698" and "LA0012", and salt-tolerant lines, "LA0516" and "LA1598", were induced by salt stress. Under salt stress, *SOS1-2*, *SOS2-1*, *SOS3-2*, *SOS3-3*, and *SOS4-1* responded earlier in salt-tolerant lines, and *SOS1-1*, *SOS1-2*, *SOS2-3*, *SOS3-3*, and *SOS4-1* were upregulated to a greater extent and the response time lasted for a longer period of time in the salt-tolerant lines. The *SOS* genes were distributed in the roots, stems, leaves, flowers, and mature fruits of tomatoes, and the organ distribution was similar in salt-sensitive tomato "LA1698" and salt-tolerant tomato "LA0516", both of which had more than half of their *SOS* genes most highly expressed in the roots.

4. Discussion

4.1. Characteristics of the SOS Gene Family Members in Tomatoes

The *SOS* gene family is closely related to salt tolerance regulation in plants since it can promote Na^+ transport and compartmentalization in plants to achieve intracellular ion homeostasis under salt stress. The classification and characterization of the *SOS* gene family members are important to study *SOS* gene function. Among the five tomato genotypes, wild tomatoes had the largest number of gene family members. The reason why the number of *SOS* gene family members in wild tomatoes is more than that in tuber mustard [13] may be that the genome of the wild tomato (1.2 G) is much larger than that of tuber mustard (784 MB), and its genome function annotation is more detailed [40]. The amino acid number of tomato SOS proteins (173-1151 AAs) were similar to that of tuber mustard (180-1106 AAs) [13] and *Tamarix hispida* (213-1165 AAs) [14], indicating that the SOS proteins were conserved among the different species. Consistent with previous conclusions in other plant species [13,14], the tomato SOS family was divided into five subgroups (SOS1-SOS5). The *Arabidopsis*, rice, and tomato SOS protein sequences belonging to the same subgroup have high homology and similar conserved domains and motif structures (Figure 3). The *SOS* gene members in the same subgroup all have similar exon–intron structures (Figure 6), and the genes with collinear relationships have high homology in their corresponding amino acid sequences (Figure 5). This indicated that they may be orthologous genes, and the functions of the SOS proteins were similar among the different species and among different genotypes within the same species.

Specific cis-acting elements in the promoter region function to enhance or repress gene expression when the plant's growth state is changed or stimulated by the external environment [41]. The prediction of cis-acting elements in the promoter region of the *SOS* gene family has been reported in tuber mustard [13], wheat [42], and so on. Here, there were MYB and MYC transcription factor binding sites in the promoter region of the tomato *SOS* gene, and abiotic stress response elements such as low temperature, drought, and anaerobic conditions. This suggested that the transcriptional regulation of the tomato *SOS* gene could be affected by abiotic stress. In addition, we found that there were hormone-responsive elements, such as abscisic acid, ethylene, and gibberellin, in the promoter region of the tomato *SOS* genes. Shi et al. (2003) [12] found that ABA treatment significantly increased the expression of the *SOS5* gene in *Arabidopsis*. The expression levels of the *BjSOS3-1* and *BjSOS4-1* genes in tuber mustard significantly increased with ABA treatment [13]. Acet and kadioglu (2020) [43] found that *AtSOS5* and ABA synergistically activated the antioxidant system to scavenge reactive oxygen species and affected the expression of related stress genes, thereby enhancing plant salt tolerance. This finding provides theoretical guidance for the in-depth study of the SOS signaling pathway and the molecular mechanism of the tomato response to salt stress.

The expression of the *SOS* gene in grape [30] and spinach [44] significantly increased under salt stress. In *Arabidopsis*, the *SOS* gene alleviated the damage of salt stress on plants

mainly by regulating ion homeostasis [45]. We found that the expression levels of the *SOS* gene family members in LA0716, LA2093, and Heinz 1706 were significantly upregulated under salt stress. These indicated that the *SOS* genes of tomatoes respond to salt stress induction and may play a key role in the regulation mechanism of tomato salt tolerance.

4.2. The Tomato SOS Gene Family May Be Positive Regulators in the Response to Salt Stress, with High Expression in the Roots and Leaves of Salt-Tolerant Tomatoes

Different tomato genotypes have different susceptibilities to salt stress with different degrees of salt damage [46], which may be related to the more actively responsive genes being involved in the regulation of salt resistance of tomato plants [47]. The relative leaf water content, membrane stability index, and chlorophyll content of salt-tolerant mustard varieties under salt stress was significantly higher than salt-sensitive Brassica varieties, being related to the high expression of the *SOS1*, *SOS2,* and *SOS3* genes [48]. Sathee et al. (2015) [29] found that the transcription levels of the *SOS1*, *SOS2*, and *SOS3* genes in salt-tolerant wheat were significantly higher than in salt-sensitive wheat under long-term salt stress. Brindha et al. (2021) [31] found that the expression levels of the *SOS1*, *SOS2,* and *SOS3* genes in salt-tolerant sugarcane genotypes were higher than in salt-sensitive sugarcane genotypes under salt stress. Olías et al. (2009) [19] found that the *SOS* gene was involved in regulating plant salt tolerance since inhibiting the expression of *SlSOS1* made tomato plants more vulnerable to salt stress. The overexpression of *SlSOS2* positively regulated the salt tolerance of tomatoes [21]. Moreover, the overexpression of *BjSOS3* in an *Arabidopsis* mutant allowed plants to accumulate more K^+ and excrete more Na^+, thereby reducing the damage caused by salt stress [49]. We found that the expression levels of the *SOS1-1*, *SOS1-2*, *SOS2-2*, *SOS3-3,* and *SOS4-1* genes in salt-tolerant tomatoes were higher than salt-sensitive tomatoes under salt stress. The expression levels of the *SOS1-2*, *SOS2-1*, *SOS3-2*, *SOS3-3,* and *SOS4-1* genes were lower in salt-tolerant tomatoes in response to salt stress. Compared to traditional breeding methods, molecular plant breeding can improve plant traits more precisely and increase breeding efficiency and success. In our study, we suggested that *SOS* gene members could be positive regulators in tomatoes responding to salt stress. The overexpression of these genes may be beneficial in improving salt tolerance in tomatoes. This finding provides a new idea for the breeding of salt-tolerant varieties of tomatoes and the establishment of salt-tolerant cultivation techniques.

The expression characteristics of genes in various organs are closely related to their functions. In *Arabidopsis thaliana*, *AtSOS1* was mainly expressed in the root tip, which could expel Na^+ from the root cells, prevent Na^+ from being transported to the shoots, and maintain the balance of K^+ and Na^+ concentrations in the cells [50]. The *AtSOS2* gene was expressed in both the roots and stems, and the expression level in the roots increased significantly under salt stress [51]. In spinach, the expression level of the *SoSOS2* gene was higher in the roots, while the expression level of the *SoSOS3* gene was higher in the leaves [44]. In *Arabidopsis thaliana*, *AtSOS5* was distributed in the roots, stems, leaves, flowers, and pods, and the expression level was higher in the leaves and flowers [12]. This study found that the *SOS* gene was expressed in tomato roots, stems, leaves, flowers, and mature fruits. The organ distribution characteristics of the *SOS* gene in the salt-sensitive tomato "LA1698" and salt-tolerant tomato "LA0516" were similar. More than half of the *SOS* gene members were highly expressed in the roots, followed by the leaves, indicating that the *SOS* genes may mainly play a role in the roots and leaves. With the extension of salt stress time, Na^+ will be transported from the root to the shoot tissue and will accumulate in plant leaves, causing leaf necrosis, and reducing the photosynthetic rate of plants [52]. There were differences in *SOS* gene expression among the tomato genotypes with different salt tolerance levels. In accordance with Sun et al. (2010) [47], the higher expression levels of *SOS1-1*, *SOS1-2*, *SOS2-1*, *SOS2-2*, *SOS3-2*, *SOS3-3*, and *SOS5-2* in the leaves of salt-tolerant tomatoes as compared with sensitive tomatoes contribute to the salt tolerance of tomato plants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14040773/s1. Figure S1: Morphological characteristics of different tomato genotypes at control (left) and salt stress (right) for seven days. A. "LA0516"; B. "LA1598"; C. "LA1698"; D. "LA0012". Table S1: Tomato genotypes used in the experiment. Table S2. Primer sequence information for qRT-PCR. Table S3: The characteristics of *SOS* gene family in *S. pennellii*, *S. pimpinellifolium* and *S. lycopersicum*. Table S4: Kinds of cis elements in the upstream regions of *SOS* gene family in tomato.

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