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Isolation and Characterization of the Sulfate Transporter Gene Family and Its Expression Pattern in Response to Selenium and Abiotic Stress in Walnuts (*Juglans regia* L.)

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Abstract: The sulfate transporter (SULTR) is responsible for the transport and uptake of sulfate, which plays an indispensable role in the growth cycle of plants and adaptation to plant stress. However, there are few reports on the response and regulation of SULTR gene family members in walnuts (*Juglans regia* L.) to sodium selenate, low temperatures, high temperatures, and simulated drought stress. In this study, the whole genome of the *SULTR* genes family in walnuts was identified and analyzed by the bioinformatics method. The results show that the walnut genome contains seventeen *JrSULTR* genes, which are unevenly distributed on eight chromosomes and can be divided into four subfamilies. Cis-acting elements that respond to stress and participate in the regulation of plant hormones were found in the promoter sequence of the *JrSULTR* genes. The analysis of transcriptome data showed that the expression of *JrSULTR1.2b* was significantly upregulated under sodium selenate treatment, and the results of qRT-PCR analysis were basically consistent with the transcriptome data. The expression of *JrSULTR3.1a* and *JrSULTR3.4b* increased with the prolongation of simulated drought stress time. The transcription levels of *JrSULTR1.2b* and *JrSULTR3.1a* were significantly increased after low-temperature treatment. After 9 h of high-temperature treatment, the expression levels of *JrSULTR3.1a* and *JrSULTR3.3* were significantly increased. *JrSULTR1.2b* and *JrSULTR3.1a* showed significant expression specificity under stress treatment. At the same time, we also performed subcellular localization of these two genes, which was consistent with the predicted results and was in the cell membrane, and their regulatory functions need to be further studied. These studies laid the foundation for us to explore the specific function of the *JrSULTR* genes in alleviating abiotic stress in walnuts.

Keywords: *Juglans regia*; sulfate transporter; selenium; abiotic stress; expression analysis

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1. Introduction

Throughout their life cycle, plants face various external environmental stresses that impose significant constraints on their growth and development, thereby reducing their economic value [1]. Sulfur is one of the six essential elements crucial for plant growth, development, and stress response, enhancing the plant's resilience against both biological and abiotic stresses, while simultaneously influencing plant quality and yield [2]. Sulfur is not only a key constituent of proteins, lipids, secondary metabolites, coenzymes, and sulfur-containing amino acids but also plays a crucial role in various physiological and biochemical processes in plants [3–5]. SO_4^{2-} is mainly stored in vacuoles, while organic sulfur is present in various plant tissues and organs [6,7]. Sulfate is involved in the absorption and transport of sulfur in plants through the sulfate transporter (SULTR).

The SULTR protein is characterized by a sulfate transport anti-sigma (STAS) domain at the C-terminus and a sulfur transport domain at the N-terminus, complemented by 12 transmembrane domains (TMDs) [6]. This protein has undergone comprehensive characterization across diverse plant species, including Arabidopsis [7], sorghum [8], wheat [9], apple [10], *Lathyrus sativus* [11], *Camellia sinensis* [12], barley [13], maize [14], tomato [15], radish [16], and others, wherein the *SULTR* genes have been successfully cloned. Group I of the *SULTR* genes functioned as a high-affinity transporter responsible for sulfate absorption, primarily expressed in substantial quantities in the roots [17,18]. In contrast, the functions of Group III *SULTR* genes remained unclear despite their various types. In *Arabidopsis thaliana*, *AtSULTR2;1* has been identified as regulating protein activity, while *AtSULTR3;1* has been found to participate in the transportation of SO_4^{2-} in plants [19,20].

The *SULTR* genes play a positive role in participating in the stress response under heavy metal, drought, and low- and high-temperature environments [8,21–23]. By enhancing the transport of sulfate, the synthesis of glutathione and glucosinolate can be promoted, improving the tolerance of plants in abiotic stress environments [24]. The mutation in *AtSULTR1;1* conferred selenate resistance in Arabidopsis, making it the only gene in the Arabidopsis sulfur transporter family known to have possessed this property [25]. Notably, the high-affinity sulfur transporters *AtSULTR1;1* and *AtSULTR1;2* were functionally equivalent, differing primarily in their capacity to absorb sulfate and their resistance to selenite [26]. Selenium and sulfur, being homologous elements with nearly identical atomic sizes, can exhibit both antagonistic and synergistic effects in their interactions [27]. It is now generally accepted that low selenium concentrations could promote plant growth, whereas elevated concentrations inhibit it [28]. Studies have shown that SeO_4^{2-} could be transported to plants through SULTR protein [29], and the transport and transformation of selenate through the sulfate pathway led to the improvement of plant tolerance to selenium [30,31]. Selenium plays an active role in regulating plant metabolism and nutrient accumulation. An investigation involving 20 wheat lines treated with selenium demonstrated that selenate increased the content of iron, zinc, sulfur, molybdenum, magnesium, calcium, and manganese in wheat seedlings, resulting in substantial gains in both plant growth and nutritional quality [32]. Given the competitive interaction between selenium and sulfur, *SULTR* genes have been found to effectively mitigate selenium stress. This discovery has important implications for optimizing selenium levels in plants and opens up new opportunities for the development of selenium-enriched products and industries.

Walnut (*Juglans regia* L.) belongs to the Juglandaceae family. Due to its rich nutrients, including proteins, fats, unsaturated fatty acids, essential trace elements (Ca, P, Fe), and various vitamins, such as carotene and riboflavin, it is widely cultivated [33]. At present, the functions of the *AtSULTR* members are fully understood, and their roles in sulfur metabolism have been reported in detail, but the research on the SULTR gene in walnuts has been limited. In this study, 17 *JrSULTR* gene members were successfully screened and identified from walnut genome data by bioinformatics technology. Based on the identification of *SULTR* gene family members in walnuts, the phylogenetic tree construction, physicochemical properties prediction, subcellular localization prediction, gene collinearity, gene structure specificity, cis-interacting elements, protein network interaction and its quantitative expression under sodium selenate, low temperatures, high temperatures, and simulated drought stress were analyzed. The results laid the foundation for understanding the mechanism of *JrSULTR* genes involved in alleviating selenium stress and other abiotic stresses.

2. Materials and Methods

2.1. Genome-Wide Identification and Physicochemical Analysis of JrSULTR Genes

Using the model plant Arabidopsis thaliana as the probe sequence, *AtSULTR* protein sequences were downloaded from the Arabidopsis genome database (https://www.arabidopsis.org/, accessed on 15 March 2023), and the walnut protein sequences were downloaded from the walnut genome database (http://www.xhhuanglab.cn/data/juglans.html,

accessed on 15 March 2023). The *JrSULTR* candidate gene (E value < 1×10^{-10}) was initially identified by Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 15 March 2023). The conserved structural domains of *JrSULTR*, as documented in the Pfam database (http://pfam.xfam.org/, accessed on 15 March 2023), were used as HMM files (PF00916 and PF01740) for screening. Then, the candidate genes were further scrutinized using the NCBI-CDD database (https://www.ncbi.nlm.nih.gov/cdd/, accessed on 15 March 2023) and the Clustal Omega program (https://www.ebi.ac.uk/Tools/msa/clustalo/, accessed on 15 March 2023). The feature sequences of the SULTR gene family were analyzed to identify *JrSULTR* genes.

The physicochemical properties of the *JrSULTR* gene family were determined by the online tool Expasy (https://web.expasy.org/protparam/, accessed on 18 March 2023), and subcellular localization predictions were generated with the Plant mPLoc database (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/, accessed on 18 March 2023).

2.2. Phylogenetic Tree and Collinearity Analysis of JrSULTR Genes

The protein sequences of walnut, maize, rice, soybean, cucumber, and Arabidopsis thalliana were used by MEGA 7.0 software using the adjacency method (NJ method, Bootstrap set to 1000, other Parameters are default values), which constructed phylogenetic trees. The modification of the evolutionary tree adopts Evolview Evolution (http://www.evolgenius.info/evolview/#/treeview, accessed on 22 March 2023). To analyze the fragment duplication and tandem duplication of *JrSULTR* genes in walnuts, genome information was downloaded from Arabidopsis and walnut databases, and collinearity analysis and visualization were performed using the One Step MCS-canX module in TBtools.

2.3. Gene structure, Domain, and Motif Analysis of JrSULTR Genes

The gene structure and coding sequence (CDS) of *JrSULTR* genes were analyzed utilizing GSDS2.0 (http://gsds.cbi.pku.edu.cn, accessed on 25 March 2023). A thorough examination of protein-conserved motifs within *JrSULTR* genes was carried out using the online tool MEME (https://meme-suite.org/meme/, accessed on 25 March 2023). Subsequently, the conserved motifs of *JrSULTR* genes were mapped and visualized using Tbtools, with default parameters set to identify up to 10 conserved motifs, while the remaining parameters were maintained at their default settings.

2.4. Analysis of Cis-Acting Elements in the Promoter of JrSULTR Genes

The promoter sequences of 17 *JrSULTR* genes (upstream 2000 base pairs from the ATG start codon) were extracted using Tbtools v2.069 software and submitted to the PlantCARE online program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 27 March 2023) to predict cis-acting elements. Visualization was performed using TBtools software, and further analysis was carried out.

2.5. Transcriptome Data Analysis of the JrSULTR Genes

We opted for the transcriptome data derived from walnuts subjected to varying concentrations of sodium selenate (0.4 mmol·L^{-1} and 0.8 mmol·L^{-1}) to investigate the expression patterns of *JrSULTR* genes under selenium stress. The outcomes of this analysis were subsequently compared with our qRT-PCR results. The walnut transcriptome data utilized in this study were obtained from the NCBI SRA database under accession number PRJNA1067374.

2.6. The Expression Analysis of the JrSULTR Genes under Different Abiotic Stresses

To investigate the expression patterns of the *JrSULTR* genes under various abiotic stresses, the "Qingxiang" walnut was taken as the research object. The seedlings were soil-cultured in artificial greenhouses with the following parameters: 25 °C, full spectrum lamp fill light, light intensity of 600 mol/(m^2·s), light 12 h a day, darkness 12 h, and humidity

60%~70%. After seedling growth for half a year, walnut seedlings with the same growth activity were selected for experimental treatment. Selenium stress was induced by foliar application of varying concentrations of sodium selenate (0.4 mmol·L^{-1} and 0.8 mmol·L^{-1}) to walnut leaves, administered once weekly for a total of five. In addition, sampling was conducted one week after the final application. We also carried out high-temperature, low-temperature, and simulated drought stress on walnut seedlings that were half-year old. Low-temperature stress at 4 °C and high-temperature stress at 38 °C were performed in an intelligent incubator (PGX-350D, Ningbosafe, Ningbo, China). Using a 20% PEG solution to simulate drought stress, the prepared solution was poured on the roots of walnut seedlings with 50 mL, and a tray was placed outside the seedling basin to prevent the loss of the solution. Low-temperature, high-temperature, and simulated drought stress were sampled at 0, 3, 6, 9, 12, 24, and 48 h after treatment, respectively. In each treatment group, 10 seedlings were selected, and the leaves were quick-frozen in liquid nitrogen after collection and stored in a refrigerator at −80 °C for later use.

Total RNA was extracted by an RN38-EASYspin RNA Plant Mini Kit (Aidlab Biotechnology Co., Ltd., Beijing, China) kit. cDNA was then synthesized using a PrimeScriptTM RT kit (TaKaRa, Kyoto, Japan) for qRT-PCR (FQD-96A, Bioer, Hangzhou, China). Three biological replicates were performed for each treatment group, and the reference genes were GAPDH and 18S rRNA. The standard error statistical method was used for qPCR data, and the CT method ($2^{-\Delta\Delta CT}$) was used to determine the relative expression level of genes, which were analyzed for significant differences using IBM SPSS 27 and finally visualized using Graphpad Prism 9.5.

2.7. Subcellular Determination of JrSULTR1.2b and JrSULTR3.1a

To verify the subcellular localization of *JrSULTR1.2b* and *JrSULTR3.1a*, the coding sequence of *JrSULTR1.2b* and *JrSULTR3.1a* was first amplified, and then the green fluorescent protein (GFP) was constructed to fuse with the C-terminus of *JrSULTR1.2b* and *JrSULTR3.1a*. The JrSULTR1.2b-GFP and JrSULTR3.1a-GFP vector was generated. The plasmid pSoup-p19 carried by Agrobacterium tumefaciens GV3101 was injected into the tobacco epidermal cell system, and then the nuclear marker FIB2-mCherry was co-expressed in tobacco cells [34]. Finally, the subcellular localization of *JrSULTR1.2b* and *JrSULTR3.1a* fluorescence was observed under a confocal microscope (Leica, Germany).

3. Results

3.1. Identification and Physicochemical Property Analysis of the JrSULTR Genes

Based on the identification of the walnut genome, 17 *JrSULTR* genes belonging to the *SULTR* genes were identified as *JrSULTR1.1a* to *JrSULTR4.1* (Table 1) based on their phylogenetic relationship with AtSULTR members. The amino acid number distribution of *SULTR* gene members in walnuts was as follows: the longest amino acid *JrSULTR4.1* was 690aa, and the shortest amino acid *JrSULTR3.5b* was 626aa. The maximum molecular weight of the *JrSULTR3.5b* gene was 69.11 kDa, and the minimum molecular weight of *JrSULTR4.1* was 75.70kDa. In the *JrSULTR* protein, the lowest isoelectric point was 8.43 for *JrSULTR2.1* and the highest isoelectric point was 9.41 for *JrSULTR3.4b*, suggesting that they were all alkaline proteins. Additionally, the average hydrophilicity coefficient of the *JrSULTR* genes proteins varied from 0.312 (*JrSULTR1.2b*) to 0.595 *(JrSULTR3.4c*), and the results show that these proteins were mainly hydrophobic (Table 1). According to the prediction analysis of subcellular localization, most members of the *JrSULTR* genes are located in the cytoplasm. Notably, *JrSULTR 4.1* exhibited a relatively unique localization in the chloroplast, suggesting its potential involvement in chloroplastic functions.

Table 1. Analysis of physicochemical properties of *JrSULTR* genes.

Gene ID	Gene Name	Amino Acid Number (aa)	Molecular Weight (kDa)	Theoretical pI	Hydrophilic	Predicted Localization
JreChr06G11410	*JrSULTR1.1a*	646	71.10	9.3	0.376	Cell membrane
JreChr06G11416	*JrSULTR1.1b*	654	72.07	9.3	0.352	Cell membrane
JreChr05G11871	*JrSULTR1.2a*	642	70.85	9.32	0.363	Cell membrane
JreChr06G11781	*JrSULTR1.2b*	658	72.66	9.02	0.312	Cell membrane
JreChr03G10342	*JrSULTR2.1*	683	73.86	8.43	0.537	Cell membrane
JreChr05G11869	*JrSULTR2.2*	661	71.94	9.17	0.488	Cell membrane
JreChr08G10436	*JrSULTR3.1a*	642	70.76	8.53	0.461	Cell membrane
JreChr07G10637	*JrSULTR3.1b*	656	72.31	8.47	0.385	Cell membrane
JreChr01G12210	*JrSULTR3.2a*	649	70.98	8.5	0.455	Cell membrane
JreChr02G10983	*JrSULTR3.2b*	650	71.42	8.89	0.445	Cell membrane
JreChr05G11381	*JrSULTR3.3*	664	72.44	8.87	0.376	Cell membrane
JreChr01G12720	*JrSULTR3.4a*	657	72.05	9.15	0.528	Cell membrane
JreChr01G12712	*JrSULTR3.4b*	639	70.03	9.41	0.538	Cell membrane
JreChr02G11304	*JrSULTR3.4c*	655	71.70	9.1	0.595	Cell membrane
JreChr03G12216	*JrSULTR3.5a*	660	73.84	9.1	0.343	Cell membrane
JreChr04G11407	*JrSULTR3.5b*	626	69.11	8.75	0.423	Cell membrane
JreChr02G11621	*JrSULTR4.1*	690	75.70	8.29	0.359	Chloroplast

3.2. Phylogenetic Tree Analysis of the JrSULTR Genes

We selected the protein sequences of the *SULTR* genes from plants that have been identified and analyzed, such as Arabidopsis, rice, soybean, cucumber, and maize. These sequences were combined with *JrSULTR* gene sequences in walnuts to construct an evolutionary tree and reveal the developmental evolution and functional characteristics of *SULTR* genes among different species.

Multiple alignments were performed on the protein sequences encoded by the selected 17 *JrSULTR* genes, followed by a comprehensive evolutionary analysis. It was found that these *JrSULTR* genes can aggregate into four subgroups (Figure 1). Among these branches, the Group III branch has the most genes, containing 10 *JrSULTR* genes. In contrast, the Group IV branch has fewer genes and contains only one *JrSULTR* gene. The differences in gene distribution across clades highlight potential differences in the evolutionary significance and functional roles of *JrSULTR* genes in different phylogenetic groups. The first subfamily contains four members of *JrSULTR* genes, including *JrSULTR1.1a*, *JrSULTR1.1b*, *JrSULTR1.2a*, and *JrSULTR1.2b*. The second subfamily contains two members of *JrSULTR*, namely *JrSULTR2.1* and *JrSULTR2.2*. The third subgroup contains 10 *JrSULTR* members, including *JrSULTR3.1a*, *JrSULTR3.1b*, *JrSULTR3.2a*, *JrSULTR3.2b*, *JrSULTR3.3*, *JrSULTR3.4a*, *JrSULTR3.4b*, *JrSULTR3.4c*, *JrSULTR3.5a*, and *JrSULTR3.5b*. Finally, the fourth subfamily consists of a single member, *JrSULTR4.1*.

3.3. Chromosomal Localization and Collinearity Analysis of the JrSULTR Genes

The distribution of 17 *JrSULTR* genes was uneven across chromosomes 1, 2, 3, 4, 5, 6, 7, and 8 (Figure 2). Specifically, three *JrSULTR* genes are contained on chromosomes 1, 2, 5, and 6, and only *JrSULTR2.1* and *JrSULTR3.5a* are on chromosome 3. Furthermore, chromosomes 4, 7, and 8 each harbored one *JrSULTR* gene.

Fragment duplication analysis showed that out of the seventeen *JrSULTR* genes, a total of six *JrSULTR* genes were found to form three tandem gene clusters (Figure 2). On chromosome 1, *JrSULTR3.4a* and *JrSULTR3.4b* formed a tandem gene cluster. On chromosome 5, *JrSULTR1.2a* and *JrSULTR2.2* formed a tandem gene cluster. *JrSULTR1.1a* and *JrSULTR1.1b* formed a tandem gene cluster on chromosome 6. *JrSULTR* also had segmental duplication, with a total of eight pairs of fragment duplication gene pairs, including *JrSULTR3.2a* and *JrSULTR3.4c*; *JrSULTR3.4a*, *JrSULTR3.4b*, and *JrSULTR3.4c*; *JrSULTR2.1*, *JrSULTR1.2a*, and *JrSULTR2.2*; *JrSULTR3.5a* and *JrSULTR3.5b*; *JrSULTR3.3* and *JrSULTR1.2b*; *JrSULTR1.2b*, *JrSULTR1.2a*, and *JrSULTR3.4b*; *JrSULTR3.2a* and *JrSULTR3.1b*.

The duplication of the *JrSULTR3.2a* and *JrSULTR3.2b* segments may indicate a similar function of the two genes. Additionally, there was a large fragment duplication between *JrSULTR3.1a* and *JrSULTR3.1b*.

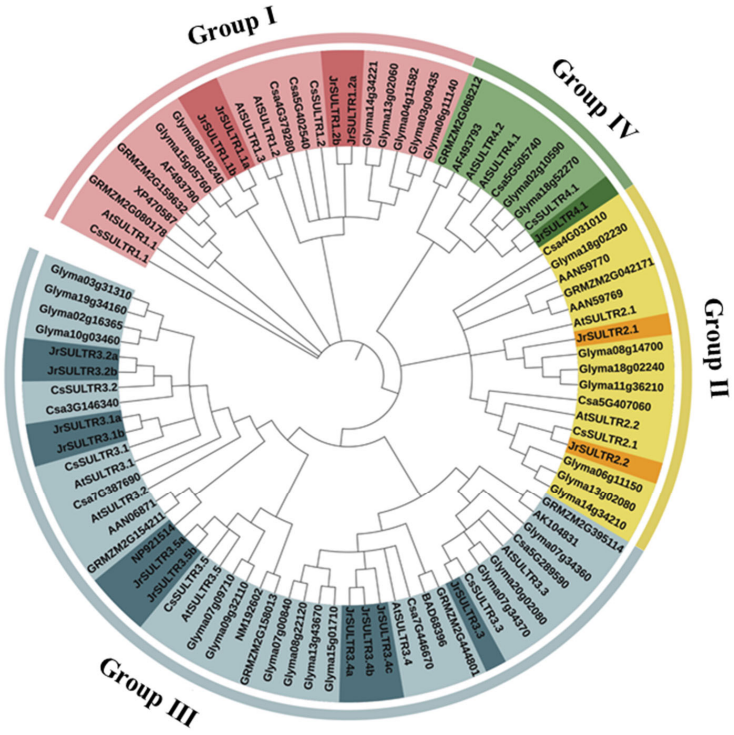

Figure 1. Phylogenetic tree analysis of *SULTR* genes in walnut, Arabidopsis, rice, maize, soybean, and cucumber. Group I is the first subfamily, Group II is the second subfamily, Group III is the third subfamily, and Group IV is the fourth subfamily. The darker part is the *JrSULTR* genes.

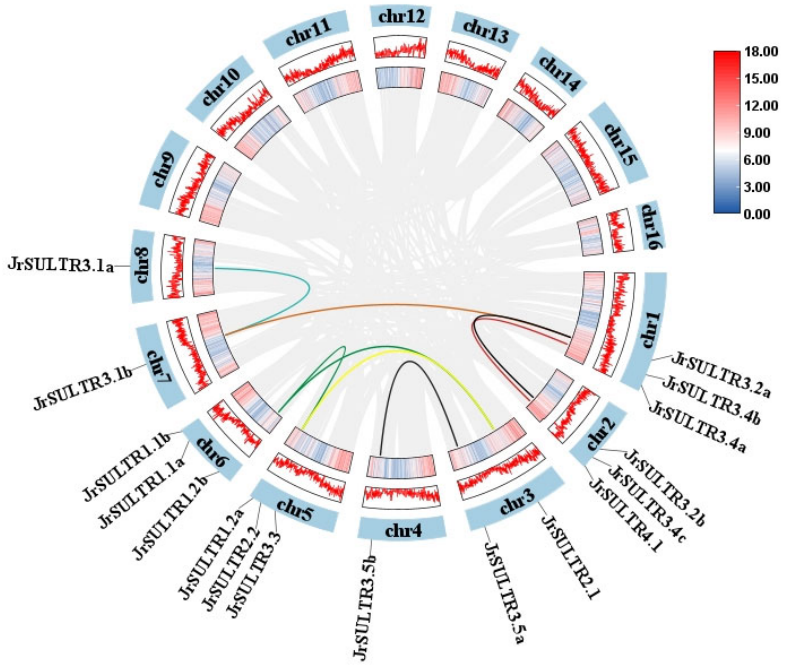

Figure 2. Chromosomal localization and collinearity analysis of the *JrSULTR* genes. The inner line represents the JrSULTR tandem cluster, different colored lines represent clusters of different genes. The heat map of the inner square represents gene density, and the outer blue rectangle represents chromosome position.

Interspecific collinearity analysis of *SULTR* genes in walnut and Arabidopsis thaliana was performed to explore their evolutionary association and differences. There are five orthologous gene pairs between walnut and Arabidopsis (Figure 3). This finding underscores a close homologous relationship between *SULTR* genes in walnut and Arabidopsis, providing valuable information about the evolutionary conservation and divergence of these genes across plant species.

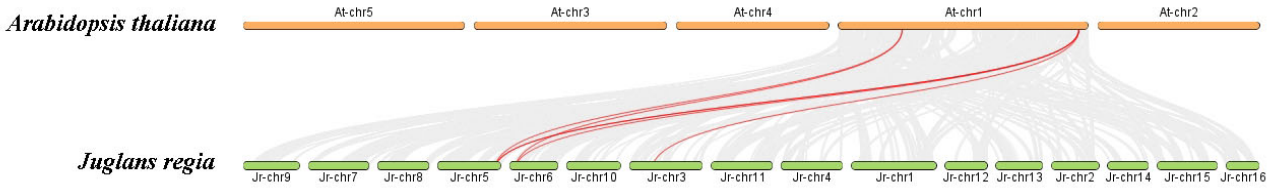

Figure 3. Collinearity analysis of *SULTR* genes in Arabidopsis and walnut. The orange part represents the Arabidopsis chromosome and the green part represents the walnut chromosome. Red lines indicate colinear *SULTR* gene pairs in Arabidopsis and walnut. Gray indicates interspecies collinearity on other genes in walnut and Arabidopsis.

3.4. Gene Structures and Conserved Motif Analysis of the JrSULTR Genes

In the analysis of the *JrSULTR* gene's structural features, it was found that the characteristic regions of motifs 1, 2, 3, 4, 7, and 9 appeared in all 17 SULTR protein sequences, indicating that these regions are highly conserved functional motifs shared by *JrSULTR* genes. (Figure 4A). Domain analysis revealed that all *JrSULTR* genes have the core structure of sulfate transporters, which is characterized by the Sulate_Transp domain (Figure 4B). In addition, a high degree of conservation in domain composition was observed between members of the same subfamily. This finding suggests that *JrSULTR* genes, which are in the same evolutionary branch, have maintained relative stability during evolution. Gene structure analysis revealed that the *JrSULTR* genes had a large number of exons. Specifically, *JrSULTR4.1* comprised 17 exons and *JrSULTR3.5b* comprised 11 exons, while the remaining *JrSULTR* consisted of varying numbers of exons ranging from 12 to 16 (Figure 4C). Furthermore, it was observed that *JrSULTR* genes belonging to the same evolutionary group shared similar gene structures. These results suggest that gene structure is conserved in a given population.

3.5. Analysis of Cis-Acting Elements in the Promoter of JrSULTR Genes

The cis-acting elements within the 2000bp region upstream of the *JrSULTR* genes were analyzed. The promoters of *JrSULTR* genes were found to consistently contain TATA box and CAAT box elements (Figure 5). Beyond the fundamental core promoter elements, the *JrSULTR* genes also identified some action elements related to the regulation of plant hormones in response to abscisic acid, auxin, and gibberellin. Cis-acting elements involved in stress response were also revealed, including MYB transcription factor binding sites associated with low-temperature response and drought induction. Meanwhile, the *JrSULTR* gene promoter region also contains other response elements related to plant growth, stress response elements, MYB regulators, and maize metabolic proteins. The most common among them were photoresponsive elements, which were distributed among 10 *JrSULTR* gene promoters, indicating that their expression may be induced by light. Drought and low-temperature response elements were found at *JrSULTR3.4b* and *JrSULTR3.5b*, and defense and stress-acting elements were identified in *JrSULTR3.4b* and *JrSULTR3.1a*, which suggests that they are highly likely to respond to plant drought, low temperatures, and other stressful environments. It is speculated that *JrSULTR* genes are involved in the hormonal regulatory signaling network in the stress response of walnuts.

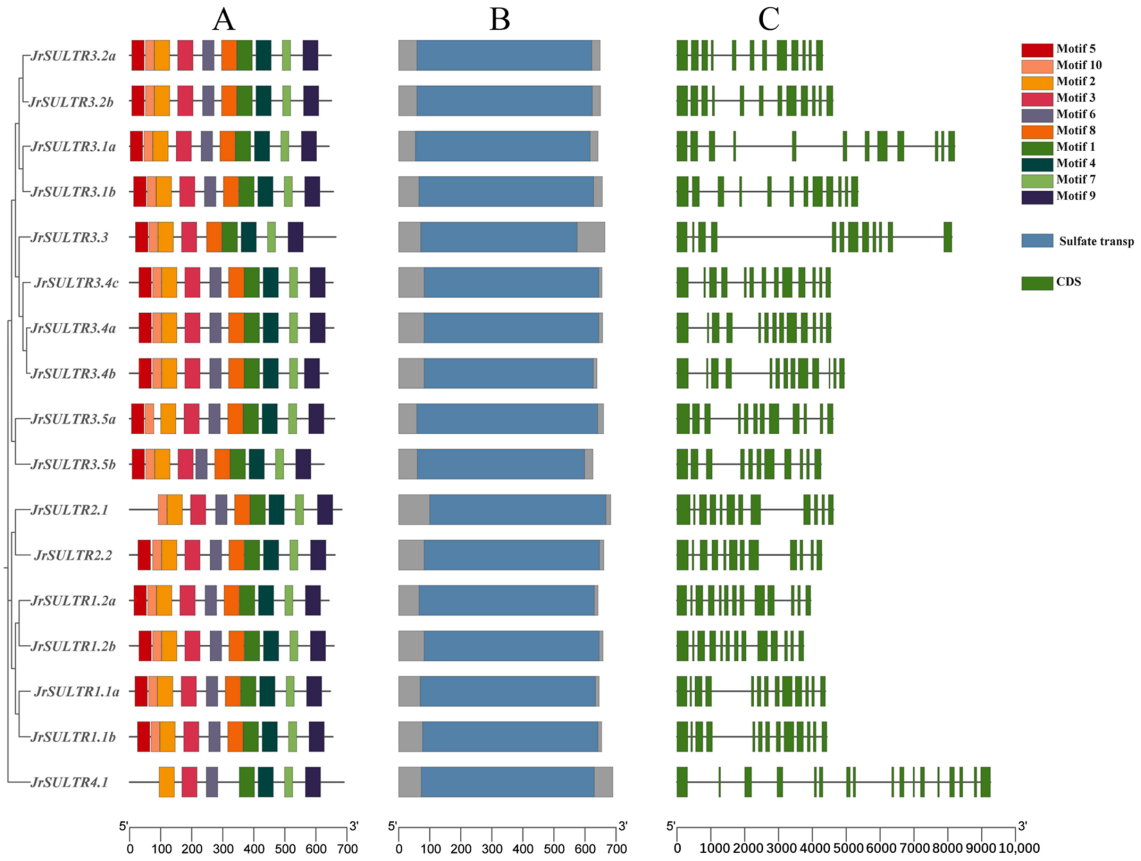

Figure 4. Analysis of conserved motifs, conserved domains, and gene structure of *JrSULTR* genes. (**A**). Conserved motif. (**B**). Conserved domain. (**C**). Gene structure. In (**B**), gray is the exon and blue is the SULTR protein domain.

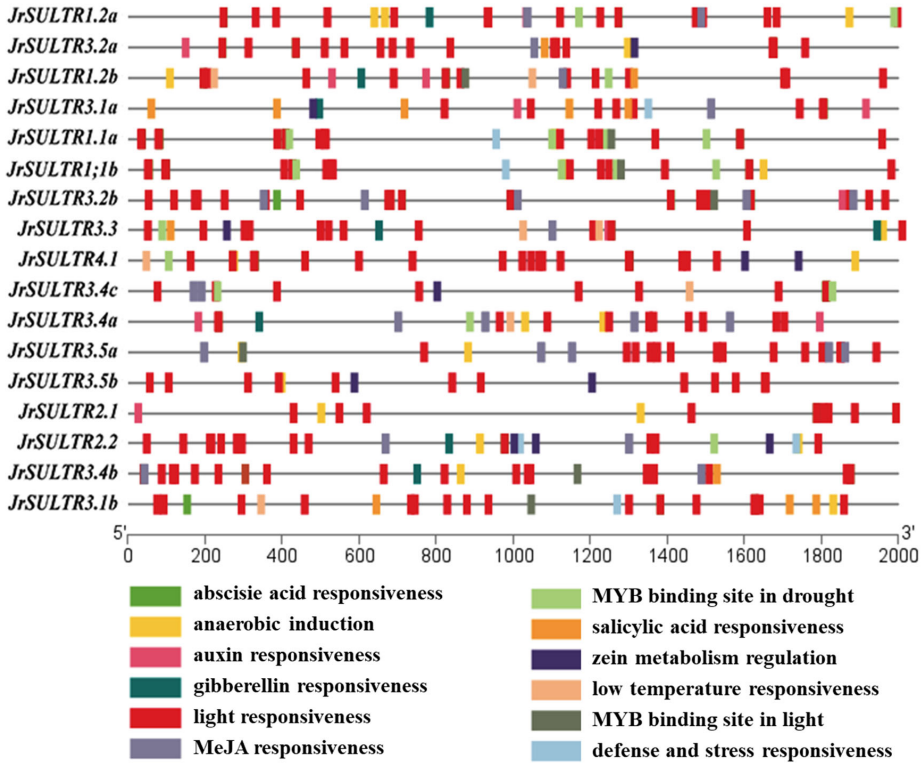

Figure 5. Cis-acting elements analysis of the promoters of *JrSULTR* genes.

3.6. Expression Patterns of the JrSULTR Genes in Transcriptome under Sodium Selenite Treatment

Published transcriptome data were used to explore the regulation of *JrSULTR* gene expression patterns by sodium selenate treatment. When treated with 0.4 mmol· L-sodium selenate, the expression of *JrSULTR1.2b* was significantly increased compared with the control group, and as the sodium selenate concentration increased to 0.8 mmol·L^{-1}, the expression levels of *JrSULTR1.2b*, *JrSULTR1.2a*, and *JrSULTR3.1b* showed a downward trend (Figure 6), indicating that these three genes were more sensitive to low concentrations of sodium selenate than other genes. The expression levels of *JrSULTR2.2*, *JrSULTR3.2a*, *JrSULTR3.3*, *JrSULTR3.5a*, and *JrSULTR4.1* were upregulated with the increase of sodium selenate concentration. However, the expression levels of other genes were less different compared to the control group. Significantly, *JrSULTR1.2b* emerged as the most prominently expressed gene under sodium selenate treatment, signifying its potential pivotal regulatory role in selenium stress.

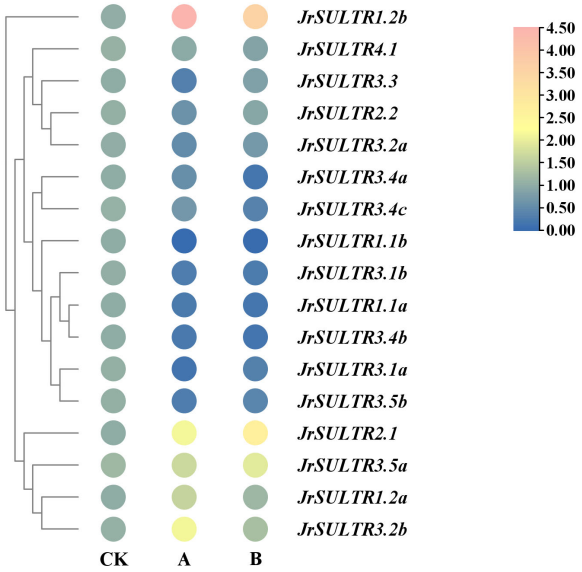

Figure 6. Expression profile of *JrSULTR* genes under sodium selenate in the transcriptome. The color scale indicates the FPKM value. The color of the red block indicates a high stacking level and the light blue block indicates a low stacking level. CK is the control group. A and B represent walnuts under 0.4 mmol·L^{-1} and 0.8 mmol·L^{-1} sodium selenate treatment, respectively.

3.7. The Expression Level of JrSULTR Genes under Different Abiotic Stresses

The expression level of *JrSULTR* genes under sodium selenate treatment was determined by qRT-PCR technology (0.4 mmol·L^{-1}, 0.8 mmol·L^{-1}). The expression level of *JrSULTR1.2b* significantly increased under sodium selenate treatment at 0.4 mmol·L^{-1} compared to the control group, aligning with the transcriptome (Figure 7). The expression of *JrSULTR1.2b*, *JrSULTR1.2a*, and *JrSULTR3.2b* were upregulated under the treatment of 0.4 mmol·L^{-1} sodium selenate, while the expression of *JrSULTR1.2b*, *JrSULTR1.2a*, and *JrSULTR3.2b* were downregulated under the treatment of 0.8 mmol·L^{-1} sodium selenite. This suggested that these genes respond more strongly to treatment with low concentrations of selenium. However, the expression levels of *JrSULTR3.5a*, *JrSULTR3.5b*, *JrSULTR2.1*, *JrSULTR2.2*, and *JrSULTR3.3* showed an upward trend with increasing sodium selenate concentration.

To study the response of *JrSULTR* genes to abiotic stress, the expression levels of *JrSULTR* genes under drought (20% PEG), low temperatures (4 °C), and high temperatures (38 °C) were analyzed. Under simulated drought stress (20% PEG), the expression levels of *JrSULTR3.1a* and *JrSULTR3.4a* showed an upregulation trend with increasing treatment duration, reaching their peaks at 48 h (Figure 8). The expression levels of *JrSULTR1.2b*, *JrSULTR1.2a*, *JrSULTR3.3*, *JrSULTR4.1*, *JrSULTR3.5a*, and *JrSULTR2.1* first increased to

a peak at 9 h and then began to decrease. The expression levels of *JrSULTR3.2a* and *JrSULTR3.2b* increased in the first 6 h post-treatment, stabilizing thereafter. The significant upregulation of *JrSULTR1.2b*, *JrSULTR3.1a*, and *JrSULTR3.4a* under simulated drought stress indicated their crucial regulatory roles in walnuts under drought conditions.

Under low-temperature treatment, the expression level of the *JrSULTR3.1a* gene reached a peak at 9 h after treatment and then began to decrease (Figure 8). *JrSULTR1.2b* showed an expression pattern of an initial increase followed by a decrease, reaching its peak at 6 h. The expression of *JrSULTR3.4c* rapidly increased in the first 3 h post-treatment, indicating a strong early response to low-temperature stress. The expression level of the *JrSULTR2.1* gene increased with treatment duration, reaching its maximum at 48 h. Notably, *JrSULTR3.1a* and *JrSULTR1.2b* exhibited the most pronounced response to low-temperature stress, suggesting their likely involvement in the reaction of the walnut under low-temperature stress.

Under high-temperature treatment, the expression levels of *JrSULTR3.1a*, *JrSULTR2.1*, *JrSULTR4.1*, *JrSULTR3.3*, *JrSULTR3.1b*, and *JrSULTR3.2a* significantly increased at 9 h post-treatment (Figure 8), followed by a subsequent decrease, indicating heightened sensitivity of these genes during the early stages of high-temperature treatment. Notably, the expression level difference of *JrSULTR3.1a* was markedly higher than that of other genes, suggesting that *JrSULTR3.1a* likely played a crucial regulatory role in walnuts under high-temperature stress.

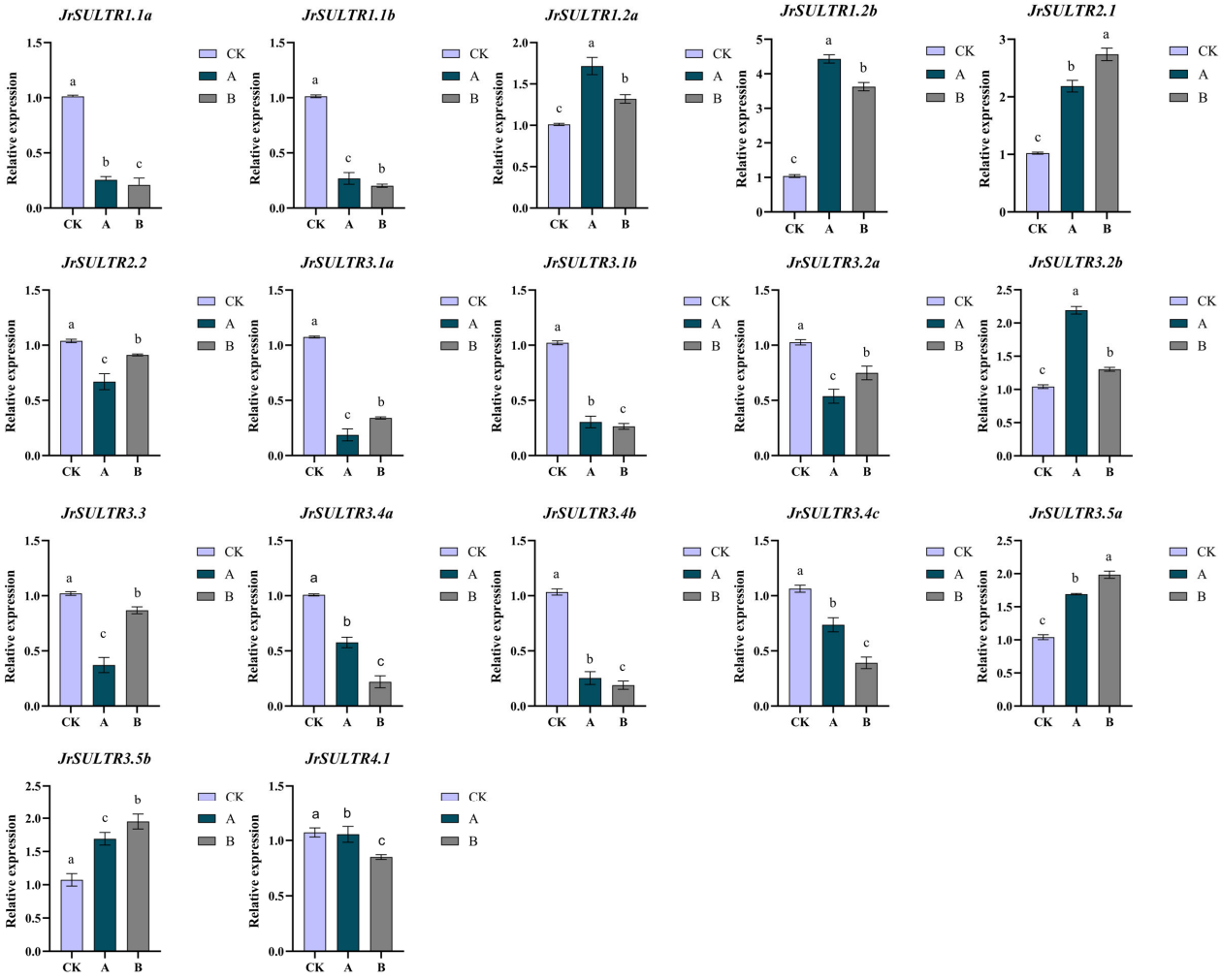

Figure 7. Expression pattern of *JrSULTR* genes in the context of sodium selenate. Different lowercase letters represent significant differences ($p < 0.05$). CK is the control group. A and B represent walnuts under 0.4 mmol·L^{-1} and 0.8 mmol·L^{-1} sodium selenate treatment, respectively.

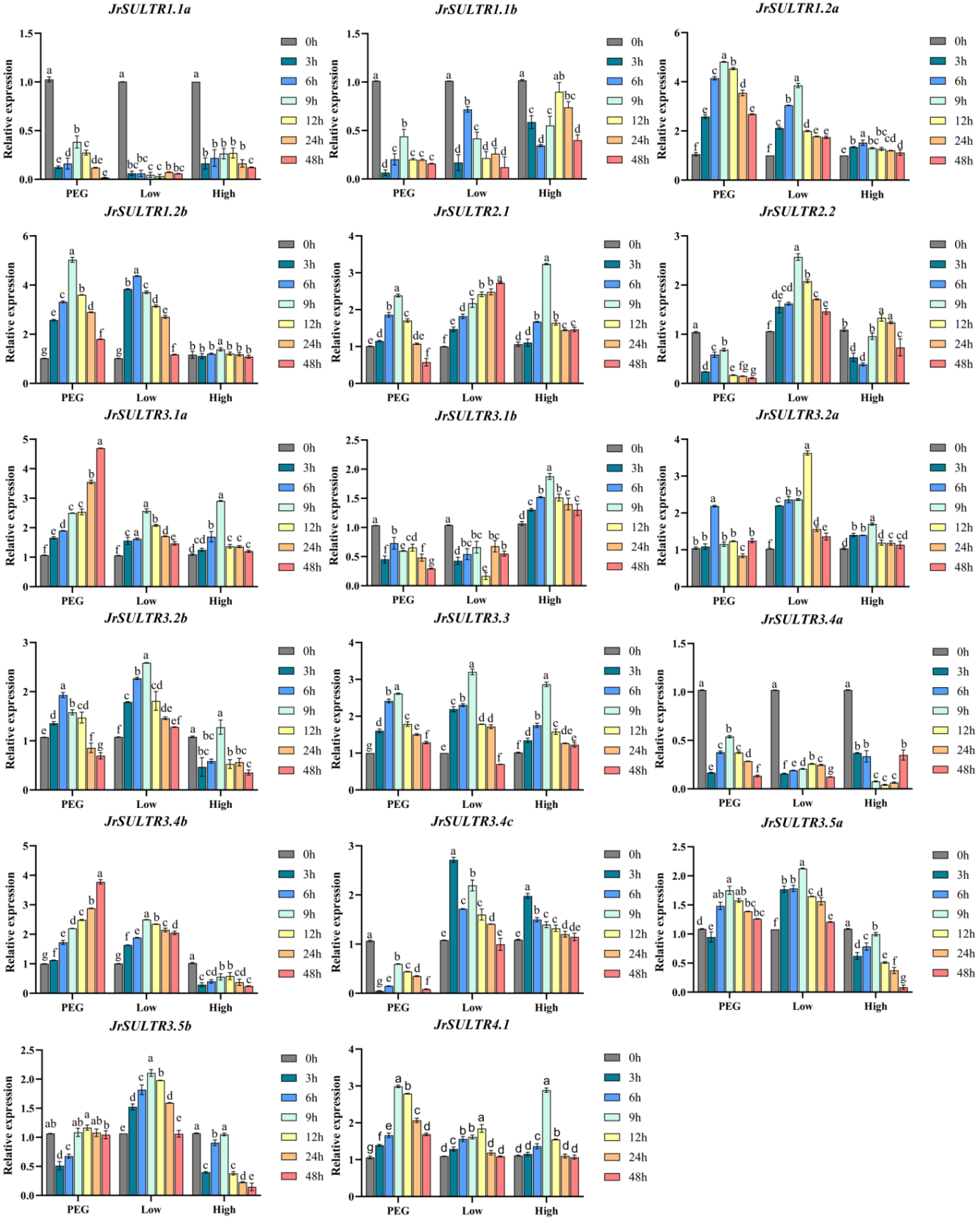

Figure 8. Expression of *JrSULTR* genes in walnuts under drought (20% PEG), low-temperature (4 °C), and high-temperature (38 °C) stress. Different lowercase letters represent significant differences ($p < 0.05$).

3.8. Subcellular Determination of JrSULTR1.2b and JrSULTR3.1a

JrSULTR1.2b and *JrSULTR3.1a* were highly expressed under selenium and abiotic stress, and stress-related cis-acting elements were identified in this gene. To further determine the subcellular localization of *JrSULTR1.2b* and *JrSULTR3.1a*, the fusion construct of JrSULTR1.2b-GTP and JrSULTR3.1a-GTP carried in the pICH86988 vector and the nucleolar marker FIB2:mCherry were transiently co-expressed on the cell membrane of tobacco leaves by the Agrobacterium tumefaciformis mediated method. By confocal fluorescence microscopy, it was found that the fluorescence was mainly on the membrane of protoplasts (Figure 9), indicating that *JrSULTR1.2b* and *JrSULTR3.1a* were localized in the cytoplasmic membrane.

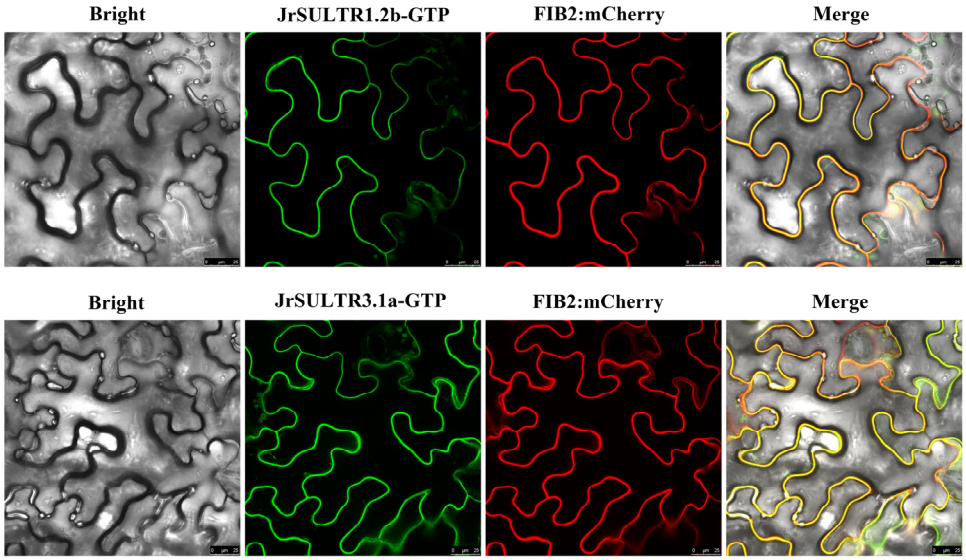

Figure 9. Subcellular localization of *JrSULTR1.2b* and *JrSULTR3.1a* in tobacco epidermal cells. Green fluorescence indicates the location of the GFP fusion protein. Red fluorescence represents FIB2:mCherry staining localization. The overlap of the bright, green, and red channels is shown as a merge. The scale bar represents 25 μm.

4. Discussion

4.1. Functional Diversity and Physicochemical Properties of JrSULTR Genes

Throughout the growth and development of walnuts, they are frequently exposed to diverse abiotic stress conditions, drought, low temperatures, high temperatures, and other adverse environments that can impact their growth [8,21–23]. At present, the research and development of selenium-rich products is more and more extensive; by exogenous spraying, selenium can be rapidly enriched in plants. Studies have shown that foliar spraying of selenate can promote the selenium content of crops, such as potato [35], rice [36], soybean [37], onion [38], buckwheat [39], and carrot [40]. However, an excessive concentration of selenium solution will cause damage to walnut plants, so SULTR was studied to alleviate selenium stress. SULTR proteins play a crucial role in plant stress tolerance, exhibiting regulatory functions across various stress environments [12,41]. This increased activity in the sulfate transport pathway may serve to alleviate selenium stress, enhancing the plant's tolerance to selenium [42,43]. The findings provide a crucial theoretical basis for the treatment of selenium-rich products with exogenous selenium, offering insights into potential strategies to improve plant tolerance and mitigate the effects of selenium stress [44–46].

In recent years, bioinformatics analysis of *SULTR* genes has been performed in a variety of plants; for example, there were twelve *SULTR* genes have been reported in Arabidopsis and rice [10], eleven *SULTR* genes in sorghum [8], ten *SULTR* genes in wheat [9], nine *SULTR* genes in apple [10], and eight SULTR genes in tea [12]. However, there are

few reports on *SULTR* genes in walnuts. In this study, 17 *JrSULTR* genes were retrieved and identified in walnuts. The subcellular localization was predicted and analyzed, and the majority of these JrSULTR proteins were located in the cell membrane. Hence, it can be inferred that these JrSULTR proteins likely exert their transport functions in the cell membrane. The subcellular fluorescence of *JrSULTR1.2b* and *JrSULTR3.1a* was localized on the cell membrane, which was consistent with the prediction that both *JRSulTR1.2b* and *JRSULTR3.1a* were located in the cell membrane. This result aligned with the subcellular localization findings of SULTR proteins in species such as maize [47] and common bean [48], indicating a conserved or similar functionality of SULTR proteins across different species.

4.2. Phylogenetic Relationship and Functional Exploration of JrSULTR Genes

Based on the phylogenetic tree analysis encompassing Arabidopsis, walnut, maize, cucumber, soybean, and rice, the *JrSULTR* genes can be categorized into four groups, with closely related *JrSULTR* genes exhibiting highly similar gene structures. These findings aligned with the clustering results of the *SULTR* gene family reported in previous studies involving Arabidopsis [3], common bean [48], tea, and maize [12,47]. Promoter sequence analysis unveiled a significant presence of light-responsive elements within the *JrSULTR* genes. Additionally, beyond the fundamental core promoter elements, the promoter region of the *JrSULTR* genes includes elements that respond to plant hormones, such as abscisic acid, salicylic acid, auxin, and gibbus. Cis-acting elements involved in stress response were also revealed, including *MYB* transcription factor binding sites associated with low-temperature response and drought induction. Notably, these elements exhibited an uneven distribution among the various *JrSULTR* genes. The most common among them were photoresponsive elements, which were distributed among 10 *JrSULTR* genes, indicating that their expression might be induced by light. ABA-responsive elements were identified in two *JrSULTR* genes, implying a close association with ABA-dependent abiotic stress adaptation processes [20,49]. MeJA-responsive elements were identified in three *JrSULTR* gene promoter regions, indicating their simultaneous involvement in MeJA-related signaling pathways. The presence of low-temperature response elements in the *JrSULTR3.5b* gene indicated that this gene probably responds to the regulation mechanism of low-temperature stress in plants. Similarly, *JrSULTR3.4b* containing drought-responsive elements indicates its potential role in simulated drought stress. Previous studies in maize have shown that except for *ZmSULTR3;3*, other *ZmSULTR* can respond to abiotic stresses, such as drought, high salt, and high temperatures [47]. These findings suggest that *SULTR* genes may respond to various abiotic stress regulatory mechanisms in plants.

4.3. Expression Analysis of JrSULTR Genes to Selenium Stress

Selenate, being structurally similar to sulfate, can be assimilated by sulfate transport systems, leading to its uptake by plant roots [3,29,50]. This common uptake mechanism highlights the interplay of sulfate and selenate transport processes in plants to promote the accumulation of Se elements in Se-rich plants [6]. The consistency between transcriptome data and qRT-PCR analysis revealed a significant upregulation in the expression of *JrSULTR1.2b* in leaves treated with sodium selenate, and the expression of *JrSULTR1.2b* reached its highest level under 0.4 mmol·L^{-1} sodium selenate treatment. When the concentration of sodium selenate increased to 0.8 mmol·L^{-1}, the expression decreased, suggesting that *JrSULTR1.2b* was particularly responsive to or regulated by lower concentrations of sodium selenate. The elevated expression of *JrSULTR* genes indicates that *JrSULTR1.2b* responds more positively when plants encounter selenium stress.

Each subfamily member of the *SULTR* gene family exhibits distinct expression characteristics. The high-affinity subfamily I member was primarily responsible for sulfate absorption [17], whereas *AtSULTR1;2* has been reported to also absorb selenate salts [26]. Studies have shown that when the soil sulfur content is low, the overexpression of *GmSULTR1;2b* in soybean can enhance the tolerance of soybean to sulfur deficiency stress, thereby improving crop yield [51]. According to homology analysis (Figure 2), it was

observed that *JrSULTR1.2b* shares homology with *AtSULTR1;2* and belongs to subfamily I. This suggests that *JrSULTR1.2b* may absorb and accumulate sodium selenate in plants through high-affinity sulfate transporters. Members of the low-affinity subfamily II member are primarily responsible for sulfate transport from the root system to the ground [3]. Under selenium stress, the expression levels of *JrSULTR2.1* and *JrSULTR2.2* increase with the increase of sodium selenate concentration; these two genes may have some selenium transport function. These observations suggest that these specific genes within the sulfate transporter family respond to increasing sodium selenate concentration, which may be related to the adaptive changes of plants to selenium stress [6,26,52]. In particular, *JrSULTR1.2b* exhibits a particularly strong response to selenium, suggesting that it may be a key factor involved in selenium stress in plants.

4.4. Response and Function Analysis of JrSULTR Genes to Drought, Low-Temperature, and High-Temperature Stress

During simulated drought stress, the expression trends of the *JrSULTR3.1a* and *JrSULTR3.4b* genes increased with the prolonged duration of simulated drought stress. The homology analysis between walnuts and Arabidopsis reveals (Figure 2), that both the *JrSULTR3.1a* gene and Arabidopsis SULTR3 members belong to subfamily III. The subfamily III of sulfate transporters, including *SULTR3;1*, *SULTR3;2*, and *SULTR3;3*, is specifically expressed in leaves [19]. Previous studies have shown that SULTR3 members in Arabidopsis and alfalfa exhibit a strong response to abiotic stress, especially *SULTR3;1*, which was upregulated under drought and salt stress [24].

Under low-temperature stress, compared with the normal control group, the expression levels of *JrSULTR3.1a* and *JrSULTR1.2b* were significantly increased, and both showed a trend of initially rising before declining (Figure 8). Under high-temperature stress, the expression levels of *JrSULTR3.1a* and *JrSULTR3.3* exhibited a significant increase after 9 h of stress applications, and then it went down (Figure 8). The expression profile of StSULTR3 members indicated that they were engaged in the oversight of plant stress environments, and the expression of StSULTR3 members was different under conditions such as heat stress, drought, phytohormones, and salt stress [53]. These findings provide insights into the dynamic expression feature of *JrSULTR* under various stresses, highlighting their responsiveness to environmental cues [54].

Moreover, under both low- and high-temperature stress, the expression intensity of *JrSULTR3.4c* rapidly increased in the initial 3 h of the onset of stress and subsequently decreased. Additionally, *AtSULTR3;4* and *MtSULTR3;4a* in Arabidopsis and alfalfa also respond to abiotic stress [49]. Cross-species transformation studies between Arabidopsis and alfalfa further support the notion that *SULTR3* genes exhibit similar characteristics in responding to abiotic stress [53]. These findings highlight the conserved stress-responsive nature of *SULTR3* genes across different plant species [5]. The analysis results reveal that the subfamily III of the *JrSULTR* genes encompasses numerous genes implicated in various abiotic stresses in plants. Notably, *JrSULTR3.1a* displays robust response characteristics under selenium stress, drought, low-temperature, and high-temperature stress. Based on these observations, it is speculated that the *JrSULTR3.1a* plays a potent role in coping with stress defense mechanisms in walnuts.

Furthermore, among closely related *JrSULTR* genes, not only do they exhibit relatively similar gene structures but they also show similarities in expression levels. For instance, the expression of *JrSULTR3.2a* and *JrSULTR3.2b* was upregulated under low-temperature stress, while the expression tended to be downregulated under drought, high temperatures, and selenium stress. The expression intensity of *JrSULTR1.1a* and *JrSULTR1.1b* did not change significantly under drought, low temperatures, high temperatures, and selenium stress, indicating that neither gene responded significantly to abiotic stress. The reaction of these genes to abiotic stress reinforces the idea that the closely related *JrSULTR* genes may have the same function and contribute to different aspects of transport within walnut tissues.

5. Conclusions

This research examined the alterations in *JrSULTR* genes that were analyzed under selenium stress and drought, as well as low- and high-temperature stress environments. The majority of *JrSULTR* genes exhibited upregulation, indicating their involvement in abiotic stress response in walnuts. However, the expression feature varied under different stress conditions, indicating that *SULTR* genes may have both shared transport functions in the same plant tissue and play distinct regulatory roles in different transport processes. Based on the analysis results, it was hypothesized that the *JrSULTR1.2b* gene may be involved in the accumulation of sodium selenate in plants, while *JrSULTR3.1a* serves as a pivotal element in the defense mechanisms of plants against stress. But, there was limited existing literature on the participation of *JrSULTR1.2b* and *JrSULTR3.1a* in selenium stress, drought, low-temperature, and high-temperature stress. Additional studies were required to uncover the specific molecular pathways and regulatory mechanisms involved in the stress responses of *JrSULTR* genes under selenium stress, drought, low-temperature, and high-temperature conditions. This study provided a comprehensive analysis of *JrSULTR* genes and established a theoretical foundation for further investigations into the functions of these genes. The findings laid a foundation for using molecular breeding strategies to innovate walnut germplasm resources and also provided a reference for achieving genetic improvement of walnuts.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f15040702/s1, Table S1: Gene sequences of *JrSULTR* genes. Table S2: Primer sequences used for qRT-PCR. Figure S1: Conserved motifs of *JrSULTR* proteins derived from the MEME Suite.

Author Contributions: Conceptualization, Q.W.; methodology, Q.W.; validation, W.Z.; resources, D.Q. (Danzeng Quzhen) and D.Q. (De Qiong); data curation, S.Z., N.L. and Y.X.; writing—original draft, S.Z.; writing—review and editing, Q.W. and F.X.; visualization, Y.L.; project administration, J.Y. All authors have read and agreed to the published version of the manuscript.

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References

1. Xu, J.; Duan, X.; Yang, J.; Beeching, J.R.; Zhang, P. Coupled Expression of Cu/Zn-Superoxide Dismutase and Catalase in Cassava Improves Tolerance against Cold and Drought Stresses. *Plant Signal. Behav.* **2013**, *8*, e24525. [CrossRef] [PubMed]
2. Buchner, P.; Stuiver, C.E.E.; Westerman, S.; Wirtz, M.; Hell, R.; Hawkesford, M.J.; De Kok, L.J. Regulation of Sulfate Uptake and Expression of Sulfate Transporter Genes in Brassica Oleracea as Affected by Atmospheric H(2)S and Pedospheric Sulfate Nutrition. *Plant Physiol.* **2004**, *136*, 3396–3408. [CrossRef] [PubMed]
3. Takahashi, H.; Watanabe-Takahashi, A.; Smith, F.W.; Blake-Kalff, M.; Hawkesford, M.J.; Saito, K. The Roles of Three Functional Sulphate Transporters Involved in Uptake and Translocation of Sulphate in Arabidopsis Thaliana. *Plant J.* **2000**, *23*, 171–182. [CrossRef] [PubMed]
4. Leggett, J.E.; Epstein, E. Kinetics of Sulfate Absorption by Barley Roots. *Plant Physiol.* **1956**, *31*, 222–226. [CrossRef] [PubMed]
5. Hawkesford, M.J.; Davidian, J.-C.; Grignon, C. Sulphate/Proton Cotransport in Plasma-Membrane Vesicles Isolated from Roots of *Brassica napus* L.: Increased Transport in Membranes Isolated from Sulphur-Starved Plants. *Planta* **1993**, *190*, 297–304. [CrossRef]
6. Shibagaki, N.; Rose, A.; McDermott, J.P.; Fujiwara, T.; Hayashi, H.; Yoneyama, T.; Davies, J.P. Selenate-Resistant Mutants of Arabidopsis Thaliana Identify Sultr1;2, a Sulfate Transporter Required for Efficient Transport of Sulfate into Roots. *Plant J. Cell Mol. Biol.* **2002**, *29*, 475–486. [CrossRef]

7. Chen, Z.; Zhao, P.-X.; Miao, Z.-Q.; Qi, G.-F.; Wang, Z.; Yuan, Y.; Ahmad, N.; Cao, M.-J.; Hell, R.; Wirtz, M.; et al. SULTR3s Function in Chloroplast Sulfate Uptake and Affect ABA Biosynthesis and the Stress Response. *Plant Physiol.* **2019**, *180*, 593–604. [CrossRef] [PubMed]
8. Akbudak, M.A.; Filiz, E.; Kontbay, K. Genome-Wide Identification and Cadmium Induced Expression Profiling of Sulfate Transporter (SULTR) Genes in Sorghum (*Sorghum bicolor* L.). *Biometals Int. J. Role Met. Ions Biol. Biochem. Med.* **2018**, *31*, 91–105. [CrossRef] [PubMed]
9. Buchner, P.; Parmar, S.; Kriegel, A.; Carpentier, M.; Hawkesford, M.J. The Sulfate Transporter Family in Wheat: Tissue-Specific Gene Expression in Relation to Nutrition. *Mol. Plant* **2010**, *3*, 374–389. [CrossRef] [PubMed]
10. Xun, M.; Song, J.; Shi, J.; Li, J.; Shi, Y.; Yan, J.; Zhang, W.; Yang, H. Genome-Wide Identification of Sultr Genes in Malus Domestica and Low Sulfur-Induced MhSultr3;1a to Increase Cysteine-Improving Growth. *Front. Plant Sci.* **2021**, *12*, 748242. [CrossRef] [PubMed]
11. Hong, C.; Peng, C.; Quanle, X. Cloning and Expression Analysis of LsSULTR Gene in Lathyrus Sativus. *Xibei Zhiwu Xuebao* **2021**, *41*, 1954–1961.
12. Zhang, H.; Hao, X.; Zhang, J.; Wang, L.; Wang, Y.; Li, N.; Guo, L.; Ren, H.; Zeng, J. Genome-Wide Identification of SULTR Genes in Tea Plant and Analysis of Their Expression in Response to Sulfur and Selenium. *Protoplasma* **2022**, *259*, 127–140. [CrossRef] [PubMed]
13. Adiputra, I.G.K.; Anderson, J.W. Distribution and Redistribution of Sulphur Taken up from Nutrient Solution during Vegetative Growth in Barley. *Physiol. Plant.* **1992**, *85*, 453–460. [CrossRef]
14. Bolchi, A.; Petrucco, S.; Tenca, P.L.; Foroni, C.; Ottonello, S. Coordinate Modulation of Maize Sulfate Permease and ATP Sulfurylase mRNAs in Response to Variations in Sulfur Nutritional Status: Stereospecific down-Regulation by L-Cysteine. *Plant Mol. Biol.* **1999**, *39*, 527–537. [CrossRef] [PubMed]
15. Howarth, J.R.; Fourcroy, P.; Davidian, J.-C.; Smith, F.W.; Hawkesford, M.J. Cloning of Two Contrasting High-Affinity Sulfate Transporters from Tomato Induced by Low Sulfate and Infection by the Vascular Pathogen Verticillium Dahliae. *Planta* **2003**, *218*, 58–64. [CrossRef] [PubMed]
16. Wang, Z.-W.; Xiang, C.-P.; Mei, S.-Y. Cloning and Characterization of a Novel Sulfate Transporter Gene from Radish (Raphanus Sativus L.). *DNA Seq. J. DNA Seq. Mapp.* **2006**, *17*, 237–241. [CrossRef] [PubMed]
17. Yoshimoto, N.; Takahashi, H.; Smith, F.W.; Yamaya, T.; Saito, K. Two Distinct High-Affinity Sulfate Transporters with Different Inducibilities Mediate Uptake of Sulfate in Arabidopsis Roots. *Plant J.* **2002**, *29*, 465–473. [CrossRef] [PubMed]
18. Yoshimoto, N.; Inoue, E.; Saito, K.; Yamaya, T.; Takahashi, H. Phloem-Localizing Sulfate Transporter, Sultr1;3, Mediates Re-Distribution of Sulfur from Source to Sink Organs in Arabidopsis. *Plant Physiol.* **2003**, *131*, 1511–1517. [CrossRef] [PubMed]
19. Kataoka, T.; Hayashi, N.; Yamaya, T.; Takahashi, H. Root-to-Shoot Transport of Sulfate in Arabidopsis. Evidence for the Role of SULTR3;5 as a Component of Low-Affinity Sulfate Transport System in the Root Vasculature. *Plant Physiol.* **2004**, *136*, 4198–4204. [CrossRef] [PubMed]
20. Cao, M.-J.; Wang, Z.; Wirtz, M.; Hell, R.; Oliver, D.J.; Xiang, C.-B. SULTR3;1 Is a Chloroplast-Localized Sulfate Transporter in Arabidopsis Thaliana. *Plant J.* **2013**, *73*, 607–616. [CrossRef] [PubMed]
21. Farooq, M.; Wahid, A.; Kobayashi, N.; Fujita, D.; Basra, S.M.A. Plant Drought Stress: Effects, Mechanisms and Management. *Agron. Sustain. Dev.* **2009**, *29*, 185–212. [CrossRef]
22. Aslam, M.; Fakher, B.; Ashraf, M.A.; Cheng, Y.; Wang, B.; Qin, Y. Plant Low-Temperature Stress: Signaling and Response. *Agronomy* **2022**, *12*, 702. [CrossRef]
23. Li, Z.; Cheng, B.; Zhao, Y.; Luo, L.; Zhang, Y.; Feng, G.; Han, L.; Peng, Y.; Zhang, X. Metabolic Regulation and Lipidomic Remodeling in Relation to Spermidine-Induced Stress Tolerance to High Temperature in Plants. *Int. J. Mol. Sci.* **2022**, *23*, 12247. [CrossRef]
24. Gallardo, K.; Courty, P.-E.; Le Signor, C.; Wipf, D.; Vernoud, V. Sulfate Transporters in the Plant's Response to Drought and Salinity: Regulation and Possible Functions. *Front. Plant Sci.* **2014**, *5*, 580. [CrossRef] [PubMed]
25. El Kassis, E.; Cathala, N.; Rouached, H.; Fourcroy, P.; Berthomieu, P.; Terry, N.; Davidian, J.-C. Characterization of a Selenate-Resistant Arabidopsis Mutant. Root Growth as a Potential Target for Selenate Toxicity. *Plant Physiol.* **2007**, *143*, 1231–1241. [CrossRef] [PubMed]
26. Barberon, M.; Berthomieu, P.; Clairotte, M.; Shibagaki, N.; Davidian, J.-C.; Gosti, F. Unequal Functional Redundancy between the Two Arabidopsis Thaliana High-Affinity Sulphate Transporters SULTR1;1 and SULTR1;2. *New Phytol.* **2008**, *180*, 608–619. [CrossRef] [PubMed]
27. Terry, N.; Zayed, A.M.; De Souza, M.P.; Tarun, A.S. SELENIUM IN HIGHER PLANTS. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **2000**, *51*, 401–432. [CrossRef] [PubMed]
28. Burnell, J.N. Selenium Metabolism in Neptunia Amplexicaulis1. *Plant Physiol.* **1981**, *67*, 316–324. [CrossRef] [PubMed]
29. White, P.J.; Bowen, H.C.; Parmaguru, P.; Fritz, M.; Spracklen, W.P.; Spiby, R.E.; Meacham, M.C.; Mead, A.; Harriman, M.; Trueman, L.J.; et al. Interactions between Selenium and Sulphur Nutrition in Arabidopsis Thaliana. *J. Exp. Bot.* **2004**, *55*, 1927–1937. [CrossRef]

30. Sors, T.G.; Ellis, D.R.; Na, G.N.; Lahner, B.; Lee, S.; Leustek, T.; Pickering, I.J.; Salt, D.E. Analysis of Sulfur and Selenium Assimilation in Astragalus Plants with Varying Capacities to Accumulate Selenium. *Plant J.* **2005**, *42*, 785–797. [CrossRef] [PubMed]
31. Sors, T.G.; Ellis, D.R.; Salt, D.E. Selenium Uptake, Translocation, Assimilation and Metabolic Fate in Plants. *Photosynth. Res.* **2005**, *86*, 373–389. [CrossRef] [PubMed]
32. de Souza, G.A.; de Carvalho, J.G.; Rutzke, M.; Albrecht, J.C.; Guilherme, L.R.G.; Li, L. Evaluation of Germplasm Effect on Fe, Zn and Se Content in Wheat Seedlings. *Plant Sci. Int. J. Exp. Plant Biol.* **2013**, *210*, 206–213. [CrossRef] [PubMed]
33. Popescu, R.; Ionete, R.E.; Botoran, O.R.; Costinel, D.; Bucura, F.; Geana, E.I.; 'Alabedallat, Y.F.J.; Botu, M. ^{1}H-NMR Profiling and Carbon Isotope Discrimination as Tools for the Comparative Assessment of Walnut (*Juglans regia* L.) Cultivars with Various Geographical and Genetic Origins-A Preliminary Study. *Molecules* **2019**, *24*, 1378. [CrossRef] [PubMed]
34. Liu, N.; Du, H.; Xue, Y.; Liao, Y.; Zhang, W.; Ye, J.; Wang, Q.; Xu, F. Genome-Wide Identification and Expression Analysis of the Walnut C-Repeat Binding Factor Gene Family under Low-Temperature Stress. *Forests* **2023**, *14*, 2274. [CrossRef]
35. Poggi, V.; Arcioni, A.; Filippini, P.; Pifferi, P.G. Foliar Application of Selenite and Selenate to Potato (*Solanum tuberosum*): Effect of a Ligand Agent on Selenium Content of Tubers. *J. Agric. Food Chem.* **2000**, *48*, 4749–4751. [CrossRef] [PubMed]
36. Hu, Q.; Chen, L.; Xu, J.; Zhang, Y.; Pan, G. Determination of Selenium Concentration in Rice and the Effect of Foliar Application of Se-Enriched Fertiliser or Sodium Selenite on the Selenium Content of Rice. *J. Sci. Food Agric.* **2002**, *82*, 869–872. [CrossRef]
37. Yang, F.; Chen, L.; Hu, Q.; Pan, G. Effect of the Application of Selenium on Selenium Content of Soybean and Its Products. *Biol. Trace Elem. Res.* **2003**, *93*, 249–256. [CrossRef] [PubMed]
38. Slekovec, M.; Goessler, W. Accumulation of Selenium in Natural Plants and Selenium Supplemented Vegetable and Selenium Speciation by HPLC-ICPMS. *Chem. Speciat. Bioavailab.* **2005**, *17*, 63–73. [CrossRef]
39. Smrkolj, P.; Stibilj, V.; Kreft, I.; Germ, M. Selenium Species in Buckwheat Cultivated with Foliar Addition of Se(VI) and Various Levels of UV-B Radiation. *Food Chem.* **2006**, *96*, 675–681. [CrossRef]
40. Kápolna, E.; Hillestrøm, P.R.; Laursen, K.H.; Husted, S.; Larsen, E.H. Effect of Foliar Application of Selenium on Its Uptake and Speciation in Carrot. *Food Chem.* **2009**, *115*, 1357–1363. [CrossRef]
41. Irish, K.P.; Harvey, M.-A.; Harris, H.H.; Aarts, M.G.M.; Chan, C.X.; Erskine, P.D.; van der Ent, A. Micro-Analytical and Molecular Approaches for Understanding the Distribution, Biochemistry, and Molecular Biology of Selenium in (Hyperaccumulator) Plants. *PLANTA* **2023**, *257*, 2. [CrossRef] [PubMed]
42. Schiavon, M.; Pilon, M.; Malagoli, M.; Pilon-Smits, E.A.H. Exploring the Importance of Sulfate Transporters and ATP Sulphurylases for Selenium Hyperaccumulation—A Comparison of *Stanleya Pinnata* and *Brassica Juncea* (*Brassicaceae*). *Front. Plant Sci.* **2015**, *6*, 2. [CrossRef] [PubMed]
43. Rao, S.; Yu, T.; Cong, X.; Lai, X.; Xiang, J.; Cao, J.; Liao, X.; Gou, Y.; Chao, W.; Xue, H.; et al. Transcriptome, Proteome, and Metabolome Reveal the Mechanism of Tolerance to Selenate Toxicity in *Cardamine Violifolia*. *J. Hazard. Mater.* **2021**, *406*, 124283. [CrossRef] [PubMed]
44. Zheng, Q.; Guo, L.; Huang, J.; Hao, X.; Li, X.; Li, N.; Wang, Y.; Zhang, K.; Wang, X.; Wang, L.; et al. Comparative Transcriptomics Provides Novel Insights into the Mechanisms of Selenium Accumulation and Transportation in Tea Cultivars (*Camellia sinensis* (L.) O. Kuntze). *Front. Plant Sci.* **2023**, *14*, 1268537. [CrossRef] [PubMed]
45. Heidari, P.; Hasanzadeh, S.; Faraji, S.; Ercisli, S.; Mora-Poblete, F. Genome-Wide Characterization of the Sulfate Transporter Gene Family in Oilseed Crops: *Camelina Sativa* and *Brassica Napus*. *Plants* **2023**, *12*, 628. [CrossRef]
46. Yuan, Z.; Long, W.; Hu, H.; Liang, T.; Luo, X.; Hu, Z.; Zhu, R.; Wu, X. Genome-Wide Identification and Expansion Patterns of SULTR Gene Family in Gramineae Crops and Their Expression Profiles under Abiotic Stress in *Oryza Sativa*. *Genes* **2021**, *12*, 634. [CrossRef] [PubMed]
47. Huang, Q.; Wang, M.; Xia, Z. The SULTR Gene Family in Maize (Zea Mays L.): Gene Cloning and Expression Analyses under Sulfate Starvation and Abiotic Stress. *J. Plant Physiol.* **2018**, *220*, 24–33. [CrossRef] [PubMed]
48. Zhao, Q.; Geng, J.; Du, Y.; Li, S.; Yuan, X.; Zhu, J.; Zhou, Z.; Wang, Q.; Du, J. The Common Bean (*Phaseolus vulgaris*) SULTR Gene Family: Genome-Wide Identification, Phylogeny, Evolutionary Expansion and Expression Patterns. *Biotechnol. Biotechnol. Equip.* **2022**, *36*, 724–736. [CrossRef]
49. Cao, M.-J.; Wang, Z.; Zhao, Q.; Mao, J.-L.; Speiser, A.; Wirtz, M.; Hell, R.; Zhu, J.-K.; Xiang, C.-B. Sulfate Availability Affects ABA Levels and Germination Response to ABA and Salt Stress in Arabidopsis Thaliana. *Plant J. Cell Mol. Biol.* **2014**, *77*, 604–615. [CrossRef] [PubMed]
50. ARVY, M.P. Selenate and Selenite Uptake and Translocation in Bean Plants (*Phaseolus vulgaris*). *J. Exp. Bot.* **1993**, *44*, 1083–1087. [CrossRef]
51. Ding, Y.; Zhou, X.; Zuo, L.; Wang, H.; Yu, D. Identification and Functional Characterization of the Sulfate Transporter Gene *GmSULTR1;2b* in Soybean. *BMC Genom.* **2016**, *17*, 373. [CrossRef] [PubMed]
52. Rouached, H.; Wirtz, M.; Alary, R.; Hell, R.; Arpat, A.B.; Davidian, J.-C.; Fourcroy, P.; Berthomieu, P. Differential Regulation of the Expression of Two High-Affinity Sulfate Transporters, SULTR1.1 and SULTR1.2, in Arabidopsis. *Plant Physiol.* **2008**, *147*, 897–911. [CrossRef] [PubMed]

53. Vatansever, R.; Koc, I.; Ozyigit, I.I.; Sen, U.; Uras, M.E.; Anjum, N.A.; Pereira, E.; Filiz, E. Genome-Wide Identification and Expression Analysis of Sulfate Transporter (SULTR) Genes in Potato (*Solanum tuberosum* L.). *Planta* **2016**, *244*, 1167–1183. [CrossRef] [PubMed]
54. Khan, M.I.R.; Nazir, F.; Asgher, M.; Per, T.S.; Khan, N.A. Selenium and Sulfur Influence Ethylene Formation and Alleviate Cadmium-Induced Oxidative Stress by Improving Proline and Glutathione Production in Wheat. *J. Plant Physiol.* **2015**, *173*, 9–18. [CrossRef] [PubMed]