Article

Comprehensive Analysis of StSRO Gene Family and Its Expression in Response to Different Abiotic Stresses in Potato

Yanming Ma 1, Xiangyan Zhou 1,2,*, Ziliang Liu 1 and Bing Wu 1,*

1 College of Life Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
2 Gansu Provincial Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou 730070, China
* Correspondence: zhouxy@gsau.edu.cn (X.Z.); wub@gsau.edu.cn (B.W.)

Abstract: As a highly conserved family of plant-specific proteins, SIMILAR-TO-RCD-ONE (SROs) play an essential role in plant growth, development and response to abiotic stresses. In this study, six StSRO genes were identified by searching the PARP, RST and WWE domains based on the genome-wide data of potato database DM v6.1, and they were named StSRO1–6 according to their locations on chromosomes. StSRO genes were comprehensively analyzed using bioinformatics methods. The results showed that six StSRO genes were irregularly distributed on five chromosomes. Phylogenetic analysis showed that 30 SRO genes of four species were distributed in three groups, while StSRO genes were distributed in groups II and III. The promoter sequence of StSRO genes contained many cis-acting elements related to hormones and stress responses. In addition, the expression level of StSRO genes in different tissues of doubled monoploid (DM) potato, as well as under salt, drought stresses and hormone treatments, was analyzed by RNA-seq data from the online database and quantitative real-time polymerase chain reaction (qRT-PCR) analysis. Furthermore, the expression level of StSRO genes was analyzed by transcriptome analysis under mild, moderate and severe salt stress. It was concluded that StSRO genes could respond to different abiotic conditions, but their expression level was significantly different. This study lays a foundation for further studies on the biological functions of the StSRO gene family.

Keywords: potato; SRO genes; bioinformatics; abiotic stress; gene expression

1. Introduction

When plants are subjected to the stress of abiotic and biotic environments, they can usually adapt to changing and complex environments by inducing the expression of stress genes [1–3]. Many members of the plants’ gene families were involved in plant-specific development and a series of stresses [4,5]. For example, multiple organellar RNA editing factor (MORF) genes regulate the plant–pathogen interaction by controlling the extent of RNA editing; growth regulatory factors (GRF) genes show different expression patterns in different tissues or under drought and salt stress; MaGRAS plays an important role in response to abscisic acid (ABA) and abiotic stress [5–7]. The SIMILAR-TO-RCD-ONE (SRO) plays a vital role in plants coping with abiotic and biotic stresses and participating in growth and development [4,8,9]. Among the plant-specific TF families, the SRO proteins were identified as a plant-specific small protein family, which played a significant role in the process of plant growth, development and coping with stresses, such as heavy metals, salt, cold and drought [1,4,9,10]. The plant SRO proteins usually contain highly conserved poly ADP ribose polymerase (PF00644; PARP) catalytic center and the C-terminal RST (RCD-SRO-TAF4; PF12174) domain. Additionally, some plant SRO proteins may possess the domain of N-terminal WWE (PF02825), which may have functions such as transcriptional regulation and modification [11,12].
The SRO gene family exists in all terrestrial plant genomes, and its composition varies greatly among different species. The SRO protein is a highly conserved plant-specific protein family [13,14]. Previous studies showed that although SRO proteins were usually not regulated by the transcription level, they can interact with transcription factors through the C-terminal RST domain [3,4,11]. The catalytic core of the PARP domain is the most conservative feature of the SRO gene family. Still, the biochemical analysis of AtRCD1 and bioinformatics analysis of PARP domain folding structure showed that the SRO does not possess ADP-ribosyl transferase activity. PARP connected a single ADP ribose unit to a protein and catalyzed the extension and branching of long poly ADP ribose chains [3,13,15]. PARP plays a critical role in chromatin remodeling, telomere stability, DNA repair, transcription and cell death. Therefore, the SRO plays an essential part in plants, and it may be related to the regulation of transcription factors and the formation of complexes [3,4,13]. A total of six SRO homologous genes were identified and obtained from Arabidopsis, and the six members were AtSRO1–5 and AtRCD1 [16,17]. Studies have shown that Arabidopsis Clone eight-one (CEO1) could enhance the ability of yeast activator proteins 1 (Yapl) mutant and wild-type yeast to resist oxidative stress, which showed that AtCEO1 played a vital part in biological oxidative stress response [18,19]. In subsequent studies, it was found that the AtCEO1 gene belongs to the plant’s specific SRO gene family, and its mutation may lead to the rapid death of plant cells, so AtCEO1 was also called AtRCD1 (Radical-induced cell death 1) [17–21]. AtRCD1 was the first member of the SRO gene family identified in Arabidopsis. Generally, AtRCD1 could be involved in drought stress mediated by the ABA signaling pathway through interaction with transcription factors in the nucleus. Additionally, it could also regulate plant growth and development by participating in some hormone signaling pathways, such as ABA, methyl jasmonate (MeJA) and ethylene (ETH) [4,13,18]. AtRCD1 and AtSRO1 are highly homologous, and both of them have three domains. With the common PARP and RST conserved domains, they also have an N-terminal WWE domain and belong to type A, while AtSRO2–5 lack the WWE domain and only contain two domains of PARP and RST, which belong to type B [19–21].

Potato (Solanum tuberosum L.) originated from the Andean regions of Peru and Bolivia and is considered to be one of the most important food crops worldwide [22,23]. However, the yield and quality of potato are severely affected by various abiotic stresses, particularly drought and salt stresses [23,24].

SRO genes have been reported in many plants. The SRO gene family may be a transcriptional regulator in plant response to multiple abiotic stresses [1,4,9,10,12–14,16]. However, little is known about the gene structure characteristics and functions of the SisR0 gene family, especially their functions under stresses in potatoes. Therefore, it is crucial to understand the gene structure of SisR0 and its function under abiotic stress. In this study, the physical, chemical and structural characteristics of the SisR0 gene family were identified using bioinformatics methods. Their conserved motifs, chromosome distribution, evolutionary relationship, cis-acting elements and protein network interaction were studied and validated. The expression patterns of SisR0 genes in different tissues of potato were analyzed. The expression levels of SisR0 genes under exogenous ABA treatment, salt and drought stress treatment were analyzed by RNA-seq data from the online database and qRT-PCR, and their expression levels under salt stress were also analyzed by transcriptomics. The results lay a foundation for further study of the structure or functions of SisR0 genes.

2. Results

2.1. Identification of SRO Genes in Potato

SRO protein coding genes were determined, and a total of six SisR0 genes were obtained by searching the PARP, RST and WWE domains in the potato database DM v6.1. All SisR0 genes were named SisR01–6 according to their distribution on the chromosome. Additionally, ten transcripts of the SisR0 gene family were identified in the potato database DM v4.03/4.04, which correspond to the transcripts of the SisR0 gene family in the potato
In this study, six StSRO genes identified from the genome of potato database DM v6.1 were used as the research object. As listed in Table 1, six StSRO genes have at least one and up to three transcripts. The six StSRO genes were divided into two types according to whether their N-terminal contained the WWE domain or not. Only the StSRO6 gene has three domains: PARP, RST and WWE. While StSRO1–5 lacked the WWE domain, they included only two domains: PARP and RST.

Table 1. StSRO genes in the PGSC database.

| Gene Name | Gene ID (DM v6.1) | PARP | RST | WWE | Transcript ID (DM v6.1) | Transcript ID (DM v4.03/4.04) |
|-----------|------------------|------|-----|-----|-------------------------|-----------------------------|
| StSRO1    | Soltu.DM.03G028360 | +    | +   | -   | Soltu.DM.03G028360.1    | PGSC0003DMT400063250        |
|           |                  |      |     |     | Soltu.DM.03G028360.2    | PGSC0003DMT400063249        |
|           |                  |      |     |     | Soltu.DM.03G028360.3    | PGSC0003DMT400063247        |
| StSRO2    | Soltu.DM.04G031810 | +    | +   | -   | Soltu.DM.04G031810.1    | PGSC0003DMT400012724        |
| StSRO3    | Soltu.DM.05G008850 | +    | +   | -   | Soltu.DM.05G008850.1    | PGSC0003DMT400035039        |
|           |                  |      |     |     | Soltu.DM.05G008850.2    |                             |
|           |                  |      |     |     | Soltu.DM.05G008850.3    | PGSC0003DMT400035057        |
| StSRO4    | Soltu.DM.05G008950 | +    | +   | -   | Soltu.DM.05G008950.1    | PGSC0003DMT400035056        |
|           |                  |      |     |     | Soltu.DM.05G008950.2    |                             |
|           |                  |      |     |     | Soltu.DM.05G008950.3    | PGSC0003DMT400035056        |
| StSRO5    | Soltu.DM.06G018860 | +    | +   | -   | Soltu.DM.06G018860.1    | PGSC0003DMT400043197        |
|           |                  |      |     |     | Soltu.DM.06G018860.2    | PGSC0003DMT400043196        |
| StSRO6    | Soltu.DM.08G022220 | +    | +   | -   | Soltu.DM.08G022220.1    | PGSC0003DMT400038368        |

As shown in Table 2, the lengths of the six StSRO proteins identified were between 316 and 594 AA; the molecular weight (MW) of StSRO proteins varied between 33,719.99 and 67,469.25 KD. The isoelectric point (pI) varied significantly from 5.97 to 9.16; among them, the pI of StSRO2, StSRO5 and StSRO6 were below 7.0, while the pI of StSRO1, StSRO3 and StSRO4 were higher than 7.0. Therefore, it can be inferred that StSRO2, StSRO5 and StSRO6 were acidic proteins, and StSRO1, StSRO3 and StSRO4 were essential proteins.

Since the grand average of hydropathicity (GRAVY) values predicted for StSRO proteins ranging from −0.446 to −0.234 were all negative values, it can be inferred that they were hydrophilic proteins. The instability index (II) of StSRO proteins ranged from 38.53 to 52.78 (only one below 40), that is, most StSRO proteins were unstable. The aliphatic index (AI) of StSRO proteins was between 68.46 and 88.2, and StSRO1 and StSRO4 had the lowest and highest aliphatic indices, respectively. The subcellular localization (SL) predicted that most StSRO proteins were located in the nucleus or cytoplasm, where the number of subcellular localizations of StSRO2 and StSRO3 proteins was the highest, while the number of subcellular localizations of StSRO5 was the lowest.

Table 2. Analysis of physical and chemical properties of StSRO gene family in potato.

| Gene Name | Chr. | Protein Length (AA) | MW (kD) | pI | GRAVY | II | AI | SL |
|-----------|------|---------------------|---------|----|-------|----|----|----|
| StSRO1    | 3    | 376                 | 41,505.63 | 8.52 | −0.446 | 49.19 | 68.46 | N, C, CP, P |
| StSRO2    | 4    | 510                 | 57,088.39 | 5.97 | −0.234 | 38.53 | 87.82 | CP, N, C, V, CY |
| StSRO3    | 5    | 319                 | 33,719.99 | 9.16 | −0.291 | 52.78 | 86.49 | C, N, CP, E, CY |
| StSRO4    | 5    | 316                 | 35,377.56 | 7.64 | −0.260 | 47.20 | 88.20 | C, N, E, V |
| StSRO5    | 6    | 594                 | 67,469.25 | 6.55 | −0.414 | 43.15 | 86.13 | N, C, V |
| StSRO6    | 8    | 589                 | 66,542.67 | 6.63 | −0.431 | 46.06 | 81.19 | N, C, CP, V |

Note: AA. amino acid sequence length; MW. molecular weight; pI. isoelectric point; GRAVY. grand average of hydropathicity; II. instability index; AI. aliphatic index; SL. subcellular localization; N. nucleus; C. cytoplasm; CP. chloroplast; V. vacuole; P. peroxisome; E. extracellular matrix; CY. cytoskeleton.
2.2. Gene Structure and Conserved Motifs of SRO Proteins in Potato

The evolution tree of StSRO gene family members was constructed separately and predicted by TBtools software. Ten conserved motifs of StSRO genes were identified (Figure 1). Generally, the difference in protein function is caused by the different protein motifs [21]. Therefore, the conservative motif analysis of StSRO genes showed that motifs 1, 2, 3, 7 and 8 were highly conserved and existed in the amino acid sequence of each StSRO gene, so it was speculated that these five motifs might play an important role in the StSRO gene family. StSRO1–4 contained motif 4, while motif 5 and motif 6 existed in StSRO2, StSRO5 and StSRO6; motif 9 existed in StSRO3–StSRO6; motif 10 only existed in StSRO5–6.

![Phylogenetic relationship, conserved motifs and gene structure of StSRO genes.](image)

Figure 1. Phylogenetic relationship, conserved motifs and gene structure of StSRO genes. (A) Phylogenetic relationship of StSRO genes, (B) the ten conserved motifs of StSRO genes are displayed in different colors, (C) the exon-intron structure. The coding sequence (CDS) and untranslated region (UTR) are displayed in different colors, and the lines between the boxes represent introns.

The exon-intron structure is not only an essential evolutionary feature of genes but also a critical clue to the diversification of gene functions [1]. Therefore, the exon-intron structure of StSRO genes was analyzed. The results showed that StSRO genes with a close genetic relationship have a similar exon-intron structure, and six members of the StSRO genes had three to seven introns with different lengths, of which StSRO2 had the lowest number of introns (three), while StSRO6 had the highest number of introns (seven).

2.3. Chromosomal Mapping and Secondary Structure Analysis of StSRO Genes in Potato

The chromosome mapping analysis of StSRO genes showed that six StSRO genes were randomly distributed on five chromosomes (Figure 2). Among them, StSRO3 and StSRO4 were distributed on chromosome 5; StSRO1, StSRO2, StSRO5 and StSRO6 were distributed on chromosome 3, 4, 6, 8. Among these six StSRO genes, StSRO3 and StSRO4 were distributed adjacent on chromosome 5, which may be caused by tandem duplicated events of genes in chromosome regions.

There were four main secondary structures of proteins, including α-helix, β-sheet, β-turn and random curl. Because the MW of the protein was relatively large, this may lead to different forms of secondary structure in different peptide segments of a protein [27]. According to the predicted secondary structure of six StSRO proteins (Figure 3), the secondary structures of StSRO proteins were mainly α-helix and random curl structures; the β-turn structure accounted for a small proportion. The random curl structures of
StSRO2 and StSRO6 accounted for 50.59%, and the α-helix structure represented 24.9% and 28.18%, respectively.

**Figure 2.** The chromosomal mapping analysis of StSRO gene family in potato.

**Figure 3.** Secondary structure prediction of StSRO proteins.

### 2.4. Synteny Analysis of SRO Gene Family

In order to identify the duplications of StSRO genes, the segmental duplication of the potato genome during evolution was analyzed. As shown in Figure 4, there was one segmental duplication event (StSRO5/StSRO6) between different chromosomes. The Ka/Ks ratio between duplicate gene pairs was calculated, and the Ka/Ks value was less than 1 (Table 3), which showed that they had undergone purification selection in the process of evolution.

| Duplicated Genes | Ka       | Ks       | Ka/Ks   |
|-------------------|----------|----------|---------|
| StSRO5/StSRO6     | 0.26898  | 0.82258  | 0.32699 |
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It has been shown that the SROs gene has similar expression patterns in response to certain stresses in potato and tomato, such as salt stress [28]. Therefore, to explore the orthologous relationships of SRO genes among potato, tomato and Arabidopsis, the collinearity relationship of them was analyzed by TBtools with MCScanX. The results showed two orthologous gene pairs between potato and Arabidopsis (Figure 5), and seven orthologous gene pairs were identified between potato and tomato. Therefore, the synteny relationships among potato, Arabidopsis and tomato indicated that potato had a closer evolutionary relationship with tomato.

**Figure 5.** Synteny relationships of SRO genes between potato and Arabidopsis, tomato. Gray lines represent collinear blocks between potato and Arabidopsis, tomato genomes. Red lines represent syntenic SRO gene pairs among potato and Arabidopsis, tomato.

### 2.5. Phylogenetic Tree Analysis of SRO Genes

To understand the phylogenetic relationship of the StSRO gene family, the protein sequences of 30 SRO genes of potato, Arabidopsis, *Brassica pekinensis* and *Zea mays* were compared by ClustalW. Then, the phylogenetic trees of four species were constructed (Figure 6). According to the results of evolutionary branches, SRO genes could be divided into three groups. Group II and group III have two subgroups, respectively, in which StSRO1, StSRO3 and StSRO4 were clustered in subgroup II-2; StSRO2 was clustered in subgroup III-1; StSRO5 and StSRO6 were clustered in subgroup III-2. In addition, the SRO genes in group I were only from the *Brassica pekinensis*; the SRO genes in group II were found in potato, *Brassica pekinensis* and Arabidopsis; and the SRO genes included in group III were found in *Brassica pekinensis*, potato, Arabidopsis and *Zea mays*, which showed that potato was closely related to *Brassica pekinensis* and Arabidopsis but far from *Zea mays* in evolutionary relationships.
2.6. Analysis of Cis-Acting Elements of SRO Gene Family in Potato

The cis-acting elements of a promoter are a critical binding region of transcription initiation factors and play a vital role in gene expression regulation [29]. To analyze the biological functions of StSRO genes, 33 main homeopathic regulatory elements were screened from six StSRO genes (Figure 7). They were divided into four categories: seven phytohormone responsiveness elements, nine tissue-specific expression elements, two stress responsiveness elements and fifteen light responsiveness elements. Among them, the light responsiveness elements had the highest number, and the numbers of G-box, G-Box and GT1 motif cis-elements were the highest in the gene family; the stress responsiveness elements had the lowest number, and there were no stress responsiveness elements in StSRO2 and StSRO5. In terms of tissue-specific expression and phytohormone responsiveness, each member of the StSRO genes contained corresponding cis-acting elements, and some cis-acting elements existed only in one gene, such as the RY-element, TATC-box, GARE-motif, TGA-element, P-box and GC motif, and so on. These results showed that StSRO genes participated in the growth and development of potato plants by responding to different cis-acting elements.

Figure 6. Phylogenetic tree analysis of SROs gene family in potato (St), Arabidopsis thaliana (At), Brassica pekinensis (Br) and Zea mays (Zm).

Figure 7. Analysis of cis-elements in the promoter region of StSRO genes. The number in the box represents the number of cis-acting elements.
2.7. Construction of SRO Protein Interaction Network and Functional Annotation in Potato

The protein–protein clustering interaction network is composed of proteins through their interaction with each other, which is involved in various aspects of life processes, such as biological signal transmission, gene expression regulation, energy and material metabolism and cell cycle regulation [30–33]. The clustering of proteins can analyze the interaction between proteins in biological systems, which is of great significance for understanding how proteins work in a biosystem, the reaction mechanisms of biological signals and energy metabolism under special physiological conditions and the functional relationship between proteins [30,32–34]. Connecting unknown functional proteins to a protein–protein interaction network through protein network interaction is helpful to further understand the rich protein biological functions through protein network interaction and the dynamic network regulation between various biomolecules in cells [34,35]. This study was based on the model plant Arabidopsis to predict the physical and chemical properties of StSRO proteins and their potential interacting proteins related to their functions (Figure 8). Three functional SROs and five potential interacting proteins directly associated with the StSRO proteins were identified. They were SRO2, SRO5, RCD1 and ALDH12AL, NAC13, NAC046, DREB2A, SOS1. SRO2 and SRO5 have similar a structure and function, encoding a protein similar to RCD1 but without the WWE domain. The protein had a PARP signature upstream of the C-terminal protein interaction domain. Additionally, the PARP signature may bind NAD$^+$ and attach the ADP-ribose moiety from NAD$^+$ to the target molecule. Its existence proved that SRO2 and SRO5 proteins played a role in ADP ribosylation [4,8]. RCD1 encoded a protein belonging to the (ADP-ribosyl) transferase domain-containing subfamily of the WWE protein–protein interaction domain protein family. Superoxide radicals were necessary and sufficient to propagate cell death or lesion formation in RCD1 mutants. RCD1 was localized in the nucleus without stress treatment. However, RCD1 was found not only in the nucleus but also in the cytoplasm under high salt or oxidative stress [4,8]. Therefore, the functions of the six StSRO proteins were similar to the above three Arabidopsis transcription factors. StSRO3 and StSRO4 may have similar functions to SRO2; StSRO1 may have similar functions to SRO5, while StSRO2, StSRO5 and StSRO6 may have similar functions to RCD1.

![Figure 8. StSRO protein–protein clustering interaction network diagram.](image)

2.8. Functional Annotation of SRO Genes in Potato

The gene ontology (GO) annotation of StSRO genes was carried out to demonstrate the possible functional classifications. As Figure 9 and Table 4 show, StSRO genes were annotated and classified into 40 functional groups in the categories of “Biological Process, BP”, “Cell Component, CC” and “Molecular Function, MF”. In terms of the MF, it primarily focused on catalytic activity (GO:0003824) and transferase activity (GO:0016740).
Among the CC, the reaction mainly occurred in the nucleus (GO:0009987), cytoplasm (GO:0005737), intracellular organelle (GO:0043229) and intracellular membrane-bounded organelle (GO:0043231), which was also highly consistent with subcellular localization prediction. In BP, it mainly focused on responding to biological stimulus (GO:0050896), responding to stress (GO:0006950), responding to stimulus (GO:0050896) and so on. The GO data showed that StSRO genes were essential in regulating gene expression.

**Figure 9.** Gene ontology (GO) annotation of StSRO genes in potato. The results were divided into three categories: “BP”, “CC” and “MF”. The x-axis and y-axis were the GO term and the number of StSRO genes, respectively.

**Table 4.** The GO classification of the annotated StSRO genes in potato.

| Class | GO Term            | Annotation             | StSRO Genes                  |
|-------|--------------------|------------------------|------------------------------|
| MF    | GO:0003824         | catalytic activity     | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0005488         | binding                | StSRO1, StSRO5, StSRO6       |
|       | GO:0016740         | transferase activity   | StSRO4, StSRO5, StSRO6       |
|       | GO:0005515         | protein binding        | StSRO1, StSRO5, StSRO6       |
| CC    | GO:0005634         | nucleus                | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0110165         | cellular anatomical entity | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0005622         | intracellular anatomical structure | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0005737         | cytoplasm              | StSRO1, StSRO5, StSRO6       |
|       | GO:0043226         | organelle              | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0043227         | membrane-bounded organelle | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0005739         | mitochondrion          | StSRO1, StSRO5, StSRO6       |
|       | GO:0043229         | intracellular organelle | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
|       | GO:0043231         | intracellular membrane-bounded organelle | StSRO1, StSRO2, StSRO3, StSRO4, StSRO5, StSRO6 |
Class GO Term Annotation StSRO Genes

**BP**

| Class        | GO Term                          | Annotation             | StSRO Genes |
|--------------|----------------------------------|------------------------|-------------|
|              | GO:0009987                       | cellular process       | StSRO1, StSRO5, StSRO6 |
|              | GO:0000003                       | reproduction           | StSRO2, StSRO5, StSRO6 |
|              | GO:0051716                       | cellular response to stimulus | StSRO5, StSRO6 |
|              | GO:0009605                       | response to external stimulus | StSRO5, StSRO6 |
|              | GO:0009607                       | response to biotic stimulus  | StSRO5, StSRO6 |
|              | GO:0009416                       | response to light stimulus | StSRO2 |
|              | GO:0020502                       | signaling              | StSRO5, StSRO6 |
|              | GO:0050896                       | response to stimulus    | StSRO1, StSRO2, StSRO5, StSRO6 |
|              | GO:0008152                       | metabolic process       | StSRO1, StSRO5, StSRO6 |
|              | GO:0048856                       | anatomical structure development | StSRO2, StSRO5, StSRO6 |
|              | GO:0008219                       | cell death              | StSRO5, StSRO6 |
|              | GO:0009628                       | response to abiotic stimulus | StSRO1, StSRO2, StSRO5, StSRO6 |
|              | GO:0009058                       | biosynthetic process    | StSRO5, StSRO6 |
|              | GO:0009314                       | response to radiation   | StSRO2 |
|              | GO:0050789                       | regulation of biological process | StSRO5, StSRO6 |
|              | GO:0006950                       | response to stress      | StSRO1, StSRO2, StSRO5, StSRO6 |
|              | GO:0050794                       | regulation of cellular process | StSRO5, StSRO6 |
|              | GO:0007275                       | multicellular organism development | StSRO2, StSRO5, StSRO6 |
|              | GO:0042221                       | response to chemical    | StSRO2, StSRO5, StSRO6 |
|              | GO:0065007                       | biological regulation   | StSRO5, StSRO6 |
|              | GO:0007154                       | cell communication      | StSRO5, StSRO6 |
|              | GO:0032501                       | multicellular organismal process | StSRO2, StSRO5, StSRO6 |
|              | GO:0032502                       | developmental process   | StSRO2, StSRO5, StSRO6 |
|              | GO:0009719                       | response to endogenous stimulus | StSRO5, StSRO6 |
|              | GO:0007165                       | signal transduction     | StSRO5, StSRO6 |
|              | GO:0009790                       | embryo development      | StSRO2, StSRO5, StSRO6 |
|              | GO:0009791                       | post-embryonic development | StSRO2, StSRO5, StSRO6 |

2.9. Effects of Abiotic Stresses and Hormone Treatments on Potato Plantlets In Vitro

In order to understand the effect of salt stress, drought stress and ABA treatment on potato plantlets, this study observed the phenotype changes of in vitro potato plantlets with different treatment times (2 h, 6 h, 12 h, 24 h, 48 h) under 200 mM chloride (NaCl), 20% polyethylene glycol-6000 (PEG-6000), 100 µM ABA and the control (0 h) (Figure 10). Under salt stress, the results showed that the changes of in vitro potato seedlings were not obvious from 0 to 6 h, and the leaves appeared to wither slightly from 12 h. The degree of withering increased with the treatment time from 24 to 48 h. The leaves obviously withered and showed chlorosis under severe salt stress (48 h).

![Figure 10. Phenotype changes in potato plantlets cultured for 30 d and treated for different durations (2 h, 6 h, 12 h, 24 h, 48 h) under salt stress (200 mM NaCl), drought stress (20% PEG-6000), hormone ABA stress (100 µM) and the control (0 h).](image_url)
Under drought stress, the changes of potato plants were not significant from 0 to 6 h treatment, and the leaves showed slight wilting after 6 h. The degree of wilting increased with the treatment time from 12 to 48 h. The leaves began to show chlorosis at 24 h. The leaves appeared wilted, shrunken and browning under severe drought stress (48 h).

Under the ABA treatment, in vitro potato plants did not change significantly from 0 to 2 h treatment, and the leaves showed slight wilting after 2 h. The degree of wilting increased with the treatment time from 6 to 48 h. Chlorosis appeared on the leaves at 12 h, and the plants withered, and leaves were completely withered under severe ABA treatment (48 h).

2.10. Analysis of SRO Gene Expression in Different Tissues and Treatments in DM Potato

The analysis of tissue-specific gene expression patterns can provide clues for the possible function of genes in development [36,37]. Therefore, the DM potato RNA sequencing data published on the online database were downloaded to analyze the changes in SISRO gene expression in different tissues and stresses. The results showed that (Figure 11A) the expression of SISRO1 was higher in the petiole, mature whole fruit, roots and whole in vitro plant, while the expression was lower or absent in other tissues. The expression of SISRO2 was only present in immature whole fruit of potato but not in other tissues. SISRO3 was highly expressed in tubers and immature whole fruits, but its expression level was low in other tissues. The expression of SISRO4 was higher in leaves and petiole but lower in other tissues. The expression patterns of SISRO5 and SISRO6 were similar, both being expressed only in the mature whole fruit, and the expression level was low or absent in other tissues. All SISROs expressed in flowers, stems and shoots were at a low level. Thus, most SISRO genes play a vital role in the growth and development of potatoes, and SISRO genes were highly expressed in petiole and immature fruit. Therefore, SISRO genes may have indispensable biological functions in potato petiole and immature whole fruit.

In addition, by analyzing the expression patterns of SISRO genes under six different treatments at 24 h (Figure 11B), SISRO3, SISRO5 and SISRO6 were highly expressed under salt treatment. SISRO4 and SISRO5 were upregulated under ABA treatment. SISRO4 and SISRO6 were highly expressed under gibberellic acid (GA3) treatment. SISRO1, SISRO2, SISRO5 and SISRO6 were upregulated under bone alkaline phosphatase (BAP) treatment. Under indoleacetic acid (IAA) and mannitol treatments, the expression of SISRO genes was low or even absent.
2.11. Expression Levels of StSRO Genes under NaCl, PEG and ABA Treatments

In addition, the expression levels of StSRO genes under salt, exogenous ABA treatment and drought stress were analyzed by qRT-PCR, with 0 h treatment as control (Figure 12). Under each treatment, most StSRO genes induced expression levels, which varied at different treatment times. Under 200 mM NaCl stress treatment, the expression levels of StSRO1 were significantly higher than the control. StSRO1, StSRO3 and StSRO5 had the same expression pattern. However, the expression of StSRO1 and StSRO3 reached a peak at 6 h, while the expression of StSRO5 peaked at 2 h. The expression levels of StSRO2 showed a trend of increasing and then decreasing and reached a peak at 6 h. The expression of StSRO4 showed a trend of decreasing at first, rising at 6 h and then falling. Meanwhile, the expression level of StSRO6 was lower than that of control at four different treatment times.

![Figure 12. Expression analysis of StSRO genes under (A,B) NaCl, (C,D) PEG stress and (E,F) ABA treatment.](image-url)
Under the 20% PEG treatment, the expression patterns of StSRO1, StSRO2 and StSRO5 were similar. StSRO3, StSRO4 and StSRO6 showed a similar expression pattern, and the expression of all StSRO genes peaked at 12 h. The peak expression of StSRO1–6 genes was 3.5-fold, 1.2-fold, 2.8-fold, 12.9-fold, 1.9-fold and 1.9-fold that of the control, respectively.

Under exogenous 100 μM ABA, the expressions of StSRO1, StSRO2, StSRO3, StSRO4 and StSRO6 were upregulated at each treatment time. The expression levels of StSRO1–5 genes peaked at 6 h. The relative expression at the peak was 14.2-fold, 15.2-fold, 18.1-fold, 13.8-fold and 16.5-fold that of the control, respectively. While the expression of StSRO6 reached a peak at 12 h, it was 4.7-fold that of the control. Compared with the control (0 h), the StSRO genes, except StSRO1 and StSRO4, could respond positively to severe ABA treatment (48 h). Compared with moderate ABA treatment (24 h), only StSRO5 and StSRO6 were upregulated. The qRT-PCR results were basically consistent with RNA-seq data from the online database.

Overall, these results indicated that StSRO genes could respond to salt stress, drought stress and exogenous hormone ABA treatment to different degrees, except StSRO6, which could not respond to salt stress. Among them, StSRO1 could positively respond to salt stress, while StSRO6 did not. The expression of the StSRO gene reached a peak at 12 h under drought stress. The expressions of StSRO2, StSRO3 and StSRO6 were significantly upregulated with different treatment times.

2.12. Transcriptome-Based Expression Profiling of StSRO Genes in Potato Cultivar “Zihuabai” under Salt Stress

Furthermore, the transcriptome analysis of potatoes under salt stress was not published in our research (the transcriptome data were validated) [38], in which the FPKM values were used to draw the heatmap (Table S1 and Figure 13). The results showed that six StSRO genes were differentially expressed under 200 mM NaCl at different treatment times. StSRO1 and StSRO6 were downregulated under control (0 h) and moderate salt stress (24 h) while being highly expressed under severe salt stress (48 h). StSRO2 was upregulated under control (0 h) and moderate salt stress (24 h) but downregulated under severe salt stress (48 h). By comparing the gene expression levels of control (0 h) and moderate salt stress (24 h), it was found that the expression levels of StSRO2 and StSRO6 were downregulated. In contrast, the expression levels of StSRO1 and StSRO3–5 were upregulated. The comparison of gene expression between control (0 h) and severe salt stress (48 h) showed that the expression levels of StSRO2 were significantly downregulated, and the expressions of the other five StSRO genes were significantly upregulated. The comparison of gene expression between moderate (24 h) and severe (48 h) salt stress showed that the expressions of StSRO2 and StSRO3 were downregulated, while the expressions of StSRO1 and StSRO4–6 were upregulated. The expression levels of all StSRO genes under moderate salt stress (24 h) were consistent with the published RNA-seq data from the online database (Figure 11B) and our qRT-PCR results (Figure 12). Therefore, StSRO genes not only respond to mild and moderate salt stress (Figures 11B and 13) but also notably respond to severe salt stress (Figure 13).

Figure 13. StSRO genes expression based on transcriptome analysis.
3. Discussion

As a highly conserved plant-specific protein family, the SRO protein plays a crucial role in the response of plants to abiotic and biological stresses. The SRO family exists in all terrestrial plant genomes, but its composition varies greatly among different species. The number of SRO gene family members identified in other species (tomato, maize, wheat, rice, Chinese cabbage, apple, banana, tea plants, upland cotton) were different, including 6, 6, 30, 5, 12, 6, 6, 9 and 12 SRO members, respectively [1,4,8,12,13,28,39–41]. In this study, a total of six StSRO genes were identified based on conserved domains. They were divided into two categories according to the presence or absence of the WWE domain: type A and type B. StSRO6 had three domains of PARP, RST and WWE, which belonged to type A. Meanwhile, StSRO1–5 had only two domains of PARP and RST, and it lacked the WWE domain, which belonged to type B.

The length range of the six StSRO proteins was 316–594 AA; the variation range of pI was 5.97–9.16; and the MW range of the six StSRO proteins was 33,719.99–67,469.25 KD. Subcellular localization of StSRO proteins showed that most StSRO proteins mainly existed in the nucleus or cytoplasm. Chromosome analysis showed that the six StSRO genes were irregularly distributed on five chromosomes. The exon-intron structure provides additional information for phylogenetic analysis and plays an important role in the evolution of gene families [41–44]. By analyzing the gene structures, conserved motifs and phylogenetic relationships of StSRO genes, it was revealed that there were similar gene structures and conserved motifs in the same group, indicating a significant correlation between the phylogenetic relationships of StSRO genes and StSRO gene structures. At the same time, the secondary structure of StSRO proteins also predicted and found that there were α-Helix and random curl, two main secondary structures of StSRO proteins. By constructing the phylogenetic tree of potato, Arabidopsis, Brassica pekinensis and Zea mays, the results showed that potato was closely related to Brassica pekinensis and Arabidopsis, while potato was far from Zea mays in evolutionary relationships.

Previous studies have shown that gene duplications were supposed to be one of the primary driving forces of genetic evolution, and gene duplications can occur through various mechanisms, including tandem duplications, segmental duplications and retroposition [45,46]. In the present study, the results of chromosomal localization showed the tandem duplication events of StSRO3 and StSRO4. In addition to tandem duplication, we also obtained one gene pair, StSRO5 and StSRO6, through genome-wide synteny analysis of the potato genome, which was a segmental duplication. From these results, it was concluded that tandem duplications and segmental duplications were involved in the potential functional diversity of StSRO genes and the expansion of the StSRO gene family.

Promoter activity plays a key role in regulating gene function [46,47]. We obtained seven phytohormone responsiveness elements, nine tissue-specific expression elements, two stress responsiveness elements and fifteen light responsiveness elements by analyzing the cis-acting elements of StSRO genes. Thus, StSRO genes can respond to plant hormones, tissue-specific expression and light responsiveness. Additionally, these results indicated that StSRO genes were involved in the growth and development of potatoes by responding to different cis-acting elements. The complex interactions between StSRO genes were detected and obtained from the protein–protein interaction network [47]. The results of the protein–protein interaction network obtained in this study suggested a possible interaction between these StSRO proteins in potato growth and development. This conclusion provides new insight into studying the regulation of potato growth and development by SROs. This study also revealed the possible role of StSRO genes through GO analysis. StSRO genes were mainly enriched in BP, CC and MF groups and played corresponding functions. This indicated that StSRO genes played an important role in regulating gene expression.

Generally, the expression patterns of genes were closely related to their potential functions. In Arabidopsis, AtRCD1 and AtSRO1 were highly expressed in young developing tissues [11]. In sesame, group I SiSROs were generally expressed in all tissues, while group II SiSROs were highly expressed in root [9]. In rice, OsSRO1a and OsSRO1c exhibited
A vast number of studies have shown that SRO genes can not only respond to abiotic stress but also to stress-related hormones. In this study, to verify the stress-specific expression of SISRO genes in the downloaded and our RNA-seq data, SISRO genes were confirmed by qRT-PCR in potato leaves treated with NaCl, PEG and ABA treatments. Under salt stress, qRT-PCR, our transcriptome data and online transcriptome data were basically consistent; the reason may be that the SISRO gene has different tolerance to salt stress caused in different potato varieties and different NaCl concentrations. Under the ABA treatment, the results of qRT-PCR were basically consistent with the online transcriptome data, but no drought stress related data were obtained from the online RNA-seq database.

The SRO gene family has been shown to respond to ABA treatment in many plants. The former results showed that the upregulated genes in response to ABA in tea plants were CsRCD3 and CsRCD4 [32]. In rice, OsSRO1c was obviously upregulated under ABA treatment [12]. In apples, MdSRO4 expression was upregulated and was 14-fold that of the control under ABA treatment [13]. In bananas, MaSRO5 and MaSRO6 were more sensitive to ABA treatment [48]. The results of exogenous ABA treatment in this study showed that the expression of SISRO2, SISRO3 and SISRO6 was dramatically upregulated at different treatment times. It can be concluded that the three members were more sensitive to ABA treatment than the other members in potatoes. SISRO2, SISRO3, SISRO5 and SISRO6 could respond to severe ABA treatment, while SISRO1 and SISRO4 were not expressed under severe ABA treatment. It can be concluded that the three members were more sensitive to ABA treatment than the other members in potatoes. In Arabidopsis, AtRCD1 participated in the signal transduction process of plant hormone ABA [11]. In this study, AtRCD1, SISRO2 and SISRO6 were distributed in group III with high homology, and SISRO2 and SISRO6 could actively respond to ABA stress. Therefore, this result fits well with previous studies.

The SRO gene family also responded to drought conditions under PEG stress treatment. In apples, MdSRO2, MdSRO3 and MdRCD1 were upregulated 18-fold, 17-fold and 14-fold, respectively. MdRCD1 was supposed to be an essential regulator of abiotic stress, which regulated stomata through the ABA signal pathway and enhanced its tolerance to oxidative stress, drought and salt [13]. In Chinese cabbage, the genes responding to drought stress were BrSRO1, BrSRO5 and BrSRO9 [40]. In maize, the expression levels of ZmSRO1c and ZmSRO1f were upregulated considerably under drought stress [41]. In this study, the expression levels of SISRO genes in leaves were analyzed under PEG stress. The expression peak of SISRO genes was reached at the same time (12 h), and under severe drought stress (48 h), SISRO2, SISRO3, SISRO5 and SISRO6 were downregulated, while SISRO1 and SISRO4 did not respond to severe drought stress.

Studies have suggested that the SRO gene family also plays a vital role in response to salt stress. In tomatoes, SISRO1 was significantly upregulated in roots under salt stress [38]. In wheat, TaSRO2a.1–1D, TaSRO2a.2–4A and TaSRO2b.3–4A were significantly upregulated in the root system under NaCl treatment. The above results indicated that these genes could quickly respond to salt stress [1]. In Chinese cabbage, the genes of BrSRO5 and BrSRO8 responded to NaCl stress [40]. In this study, the expression levels of SISRO1 at different treatment times were significantly higher than the control. When the expression reached a peak, it was 18.9-fold that of the control, and SISRO1, SISRO3 and SISRO5 had the same expression pattern. This indicated that SISROs could respond positively to salt stress. In this study, it was found that SISRO1, BrSRO5 and BrSRO8 were distributed in group II by evolutionary analysis and showed high homology. At the same time, they could respond positively to salt stress. Therefore, the results of this study and previous studies are highly consistent.
Studies have shown that different levels of salt stress will lead to varying degrees of molecular damage, growth arrest and even death of many salt-sensitive crops [49,50]. In upland cotton, two SRO genes, GHSCR04 and GHSCR08, were cloned from upland cotton and expressed in cotton leaves under high salt stress (400 mM) and reached a peak at 24 h and 6 h, respectively, indicating that the SRO gene plays an important role in the salt stress of cotton [51]. In maize, the expression of ZmSRO1a/ZmSRO1b/ZmSRO1c/ZmSRO1d/ZmSRO1e was significantly upregulated in roots after 1 h of high salinity treatment. By comparison, the expression of ZmSRO1f in the shoots was upregulated considerably after high salinity treatment for 6 h [41]. In this research, the expression levels of StSRO genes were analyzed by RNA-seq data from the online database and qRT-PCR data under mild and moderate salt stress, and our transcriptome data under moderate and severe salt stress. The results showed that StSRO3, StSRO5 and StSRO6 were highly expressed under mild salt stress (6 h); the expression levels of StSRO1 and StSRO3–5 were higher under moderate salt stress (24 h); and StSRO1 and StSRO4–6 were highly expressed under severe salt stress for 48 h. Therefore, this study demonstrated that StSRO genes and SRO genes of other species played similar functions under adverse circumstances, and StSRO genes could respond to different salt conditions based on our transcriptome data. StSRO genes may be more inclined to respond to severe salt stress. StSRO5 obviously alleviated mild, moderate and severe salt stress in potatoes and improved salt tolerance to a certain extent.

4. Materials and Methods

4.1. Identification and Sequence Analysis of SRO Gene Family Members in Potato

To obtain the StSRO genes, the whole protein sequences of potatoes were retrieved and downloaded from the database of Spud DB Potato Genomics Resource (http://solanaceae.plantbiology.msu.edu/, accessed on 16 September 2022) [25]. The hidden Markov model files (HMM file) of the PARP, RST and WWE domains of SRO genes were downloaded from the online database Pfam (http://pfam.xfam.org/, accessed on 16 September 2022). Then, the Hmmsearch tool implemented in HMMER 3.0 was used to screen the ID of StSRO gene family members with the expected value (E-value) of 1e-5 [52,53]. Finally, the protein sequence of StSRO gene family members was extracted by TBtools 1.09876 and screened using the target domain by uploading the obtained protein sequence file to the online database pfamscan (https://www.ebi.ac.uk/Tools/pfa/pfamscan/, accessed on 16 September 2022) [54]. To ensure that the domains of the candidate genes contained at least one PARP and RST domain, the online website SMART (http://smart.embl-heidelberg.de/, accessed on 16 September 2022) was used for comparison with Blast-P of NCBI and verified the identified StSRO genes [55]. The physicochemical properties of StSRO proteins, including GRAVY, II, AI, pI, MW and the number of AA compositions, were obtained from ExPASY (https://web.expasy.org/compute_pi/, accessed on 16 September 2022) [56]. Subcellular localization of the StSRO genes was predicted by Wolf PSORT (https://wolfpsort.hgc.jp/, accessed on 16 September 2022) [57,58].

4.2. Gene Structure and Conserved Motifs of SRO Proteins in Potato

The conserved regions of StSROs protein were identified using the MEME program (https://meme-suite.org/meme/, accessed on 16 September 2022) [59]. The corresponding motif number parameter was set to 10, and the other parameters were set as default. The mast XML file was downloaded; then, the motif and gene structure of StSRO genes were visualized by TBtools.

4.3. Chromosomal Mapping and Secondary Structure Analysis of SRO Genes in Potato

To determine the distribution of StSRO genes on the chromosome, the chromosome location information of the identified StSRO genes was downloaded from the Spud DB Potato Genomics Resource database, and TBtools was used to draw the chromosome location map of StSRO genes. The secondary structure of StSRO proteins was predicted
by the online SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa
SOPMA.html, accessed on 16 September 2022) [60].

4.4. Synteny Analysis of SRO Gene Family

MCScanX was used for gene duplication events in the potato genome and synteny
relationship between potato and Arabidopsis, tomato. The collinearity relationship was
visualized by TBtools, and default parameters were selected for all parameters [61].

4.5. Phylogenetic Tree Analysis of SRO Genes in Potato

The evolutionary relationships of SRO genes from four plant species, including potato,
Arabidopsis thaliana, Brassica pekinensis and Zea mays, were investigated. The protein se-
quencies of 6 SRO genes from Arabidopsis thaliana, 12 SRO genes from Brassica pekinensis
and 6 SRO genes from Zea mays were downloaded from the database of Ensembl plants
(https://plants.ensembl.org/index.html, accessed on 16 September 2022). Six SISRO gene
protein sequence alignments were performed using ClustalW of MEGA-X, and the phylo-
genetic tree of the above four species was constructed by the neighbor-joining method. The
repetition bootstrap was set to 1000, and all other parameters were set as default [62,63].

4.6. Analysis of Cis-Acting Elements of SRO Gene Family in Potato

The 2000 bp upstream sequence of the start codon of StSRO genes was extracted
from the whole genome of potato by TBtools, and the cis-regulatory elements in the
promoter region of StSRO genes were searched and analyzed by online plantcare (http://
//bioinformatics.psb.ugent.be/webtools/PlantCARE/html/, accessed on 16 September
2022) [64].

4.7. Construction of SRO Protein Interaction Network in Potato

The protein–protein interaction network of the StSRO gene family was predicted in
potatoes based on the model plant Arabidopsis. The SRO genes in Arabidopsis were orthol-
ogous to those used in potatoes. The protein network interaction map was constructed us-
ing STRING (STRING: functional protein association networks (https://cn.string-db.org/,
accessed on 16 September 2022) [65].

4.8. Gene Ontology (GO) Analysis of SRO Genes in Potato

Potato protein sequences were submitted to the website eggNOG-mapper (http://
eggnog-mapper.embl.de/, accessed on 16 September 2022) for gene function classification
and annotation, and the results of gene function annotation were further collated by
TBtools [66].

4.9. Analysis of Potato SRO Gene Expression in Different Tissues and Different Treatments in DM

The publicly available DM potato RNA sequencing data were downloaded from the
database of Spud DB Potato Genomics resource. Then, the cluster heatmap was drawn
using the plug-in heatmap of TBtools software.

4.10. RNA Isolation and qRT-PCR

The CDS sequence of StSRO genes was downloaded from the Spud DB Potato Ge-
nomics Resource database, and the primers were designed through NCBI (National Center
for Biotechnology Information (https://www.ncbi.nlm.nih.gov/tools/primer-blast/, ac-
cessed on 16 September 2022). The sequences of all primers used for qRT-PCR analysis
are listed in Supplementary Materials in Table S2. The elongation factor (ef1a) gene (Gen-
Bank ID: AB061263) was used as the housekeeping gene to quantify cDNA abundance.
Total RNA was extracted with Tiangen’s RNA easy fast plant tissue kit (DP452). Reverse
transcription was performed using the cDNA Synthesis Kit (KR118) and determined by
qRT-PCR. Three technical replicates were performed for each sample. The qRT-PCR data
were used to calculate and analyze the relative expression of *SISRO* genes using the $2^{-\Delta\Delta Ct}$ quantitative method to determine differences between the treatments. The histogram was generated using the originPro 2018c software [67].

### 4.11. Plant Materials, Growth Conditions and Treatments

Potato cultivar “Zihuabai” was used as the material. Potato seedlings were propagated in vitro on liquid Murashige–Skoog (MS) medium and under a 16 h light/8 h dark cycle in an illuminated incubator maintained at 25 °C and 80% relative humidity (RH) and cultured for 30 d; then, plants with a similar growth trend were selected for treatment with the following stress treatments [68,69]. For the drought stress treatment, the original liquid MS medium was replaced with the new liquid MS medium containing 20% PEG-6000 and continued to be cultured under the same conditions as above. For the salt stress treatment, the original liquid medium was replaced with the new MS liquid medium containing 200 mM NaCl and then continued to be cultured under the same conditions as above. For the hormone ABA treatment, 100 µM ABA solution (freshly prepared) was evenly sprayed on the surface of plant leaves. The leaves were collected from plants at 0 h, 2 h, 6 h, 12 h, 24 h and 48 h after treatment. Samples immediately frozen at −80 °C were used for subsequent real-time fluorescence quantitative tests and transcriptome analysis. Transcriptome analysis was carried out on potato leaves treated with 200 mM NaCl for 24 h, 48 h and control (0 h). Leaf samples under each treatment were obtained with three independent biological replicates.

### 4.12. RNA-Seq Data Analysis

Total RNA of the previous samples was extracted, and the RNA-seq library was constructed. Each sample had three biological replicates. Second-generation sequencing was executed by Gene Read Biotechnology (Wuhan, China). The raw RNA-seq data were not published. After RNA sequencing, the clean reads were obtained by removing the linker information, low-quality bases and undetected bases from the raw reads through fine filtering. The cleaned data were aligned to potato database DM v6.1 gene models downloaded from the potato database.

### 4.13. Differential Expression Genes Analysis

Differential genes were identified using fragments per kilobase of exon per million fragments mapped (FPKM). Genes with the absolute value of $|FC| \geq 1.5$ and $p$-value $\leq 0.05$ were considered significantly differential expression genes (DEGs). The DEGs were annotated against the non-redundant database (Nr), SwissProt/UniProt Plant Proteins, Kyoto Encyclopedia of Genes and Genomes (KEGG) and eggNOG. Then, the DEGs were subjected to enrichment analysis of the KEGG pathways and GO functions.

### 5. Conclusions

In this study, six *SISRO* genes were identified from the potato genome, and their corresponding structures and functions were analyzed. Analysis of the synteny relationships showed that only one pair of duplication genes was identified in the potato genome, which played a pivotal role in the expansion of the *SISRO* gene family. The results also indicated that two and seven pairs of *SISRO* genes were orthologous to Arabidopsis and tomato, respectively. The GO data showed that *SISRO* genes played an influential role in regulating gene expression, including genes in response to stress. *SISRO* genes were highly expressed in petiole and immature fruit. In addition, the leaves of potato seedlings wilted slightly under mild and moderate salt or drought stress, while they wilted and showed chlorosis under severe salt or drought stress, but they were more sensitive and intolerant of the ABA treatment. This study further analyzed the expression pattern of the *SISRO* gene under mild, moderate and severe drought stress, salt stress and ABA treatment by qRT-PCR. Furthermore, the expression profiling of *SISRO* genes in the “Zihuabai” potato cultivar under salt stress was analyzed by our transcriptome data under moderate and severe salt.
stress. The six SISRO genes displayed different expression patterns under different degrees of salt stress, which was consistent with the expression profiling of DM potato and qRT-PCR results. This study lays an important basis for further research on the function of the potato SRO gene family, especially the function of SISRO genes with potential functions under hormone induction and abiotic stress. It also provides an essential potential application value for stress-resistance breeding in potatoes.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms232113518/s1.

**Author Contributions:** X.Z. and B.W. conceived, designed the study and reviewed the manuscript. Y.M. performed bioinformatics analysis, wrote the manuscript and conducted the experiments. Z.L. took care of the plant samples and contributed to the bioinformatics analysis. All authors have read and agreed to the published version of the manuscript.

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