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Genome-Wide Identification and Expression Profiling of the *SRS* Gene Family in *Arabidopsis thaliana*

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ABSTRACT

Development & formation of several plant organs and regulating processes, including morphogenesis, signal transduction, and floral meristem determinacy, are regulated by the *SRS* (*SHI*-related sequence) transcription factor family. However, the functional contributions of *SRS* family genes in *Arabidopsis* related to phenology, hormone-mediated regulation, and abiotic stress adaptation have not been comprehensively elucidated. The objective of this study is to comprehensively investigate the *SRS* gene family in the *Arabidopsis* genome and to clarify their prospective functional roles in mediating responses to diverse hormonal signals and abiotic stress conditions. Comprehensive analyses were performed using multiple databases and bioinformatics platforms to assess chromosomal positioning, gene organization, conserved motifs & domains, structural homology, *cis*-acting regulatory elements, evolutionary relationships, duplication events, transcriptional expression patterns, and potential protein interaction networks. Through phylogenetic analysis, along with conserved domain (DUF702 and IGGH) and motif characterization, ten identified *SRS* genes in *Arabidopsis* were classified into two distinct groups: Group I (eight members) and Group II (two members). The results demonstrated that all the *Arabidopsis* chromosomes contain *AtSRS* genes and comprise 74 distinct *cis* acting regulatory elements in their distinct promoter regions, which facilitates a wide range of tissue expression patterns in response to various hormonal activities and abiotic stressors. Based on phylogenetic analysis, some *AtSRS* genes appear to have undergone functional diversification, while others may function similarly to their orthologs in other plant species. This study reveals the door to the determination of *AtSRS* gene-specific functions and the clarification of the mechanisms via which *SRS* genes regulate hormones, stress responses, floral formation and development, and lateral root formation.

Keywords: *Arabidopsis thaliana*, *In-silico*, Genome-wide, *Shi*-related sequence (*SRS*), Expression pattern, Stress response

1. INTRODUCTION

Plants continuously balance growth and survival by modifying morphological characters, such as elongating stems, forming roots, and producing flowers, while responding to different abiotic and biotic stresses by producing various metabolites as well as undergoing morphological and biochemical adaptations, and expressing specific stress-related genes. These modifications include changes in shoot and root growth, leaf number and flower formation under drought, salinity, cold, as well as other abiotic and biotic stresses¹. This coordinated regulation is obtained through transcription factor interrelationship and connectivity that control gene expression in response to both endogenous and exogenous stimuli. Among these regulators, the *Shi*-related sequences (*SRS*) gene family has emerged as a considerable influential group of plant-specific transcription factors². Typically, *SRS* proteins are characterized by two conserved domains, including a cysteine-rich RING-like zinc finger domain at the N-terminus and a transcriptionally active IGGH domain at the C-terminus. The IGGH domain, enriched with acidic amino acids residues, is associated with homologous isomerization activity. On the other hand, the RING-like zinc finger is a C3HC3H type domain with a C-X2-C-X(7)-C-X4-C-X2-C-X(7)-C structure, where X denotes any amino acid²⁻⁴. These features facilitate them to act as multifaceted regulators across developmental and physiological processes⁵. The *SHI* (SHORT INTERNODES) gene, representing the first characterized member of this family, was identified in the *Arabidopsis shi* mutant. Since then, ten *AtSRS* genes have been documented in *Arabidopsis thaliana*, including *SHI*, *STY1*, *STY2*, *LATERAL ROOT PRIMORDIUM1 (LRP1)*, *SRS4*, *SRS5*, *SRS6*, *SRS7*, and *SRS8*⁵⁻⁷. Functional studies have shown that *AtSRS* proteins are pleiotropic regulators, influencing root primordium initiation, pistil morphology, floral induction, photomorphogenesis, and defense responses⁸. Their roles are closely tied to phytohormone biosynthesis and signaling pathways⁹. For example, *SHI* negatively regulates GA signaling^{2,4}. The NF-Y family protein LiNF-YA1 in *Lotus japonicus* directly interacts with *STY1*, *STY2*, and *STY3*, which are known to play important roles in developmental regulation¹⁰. *STY1*, together with other members of the *SHI/STY* family, may influence the development and maintenance of the shoot apical meristem by regulating auxin levels during embryogenesis³. *AtSRS5* demonstrated multiple effects, including participation in defense responses¹¹, promoted photomorphogenesis¹², and negatively regulated lateral root formation and inhibited by auxin¹³. Jasmonic acid (JA) signalling causes *SRS7* to be overexpressed, and its mutation results in plant dwarfism and disruption of anther dehiscence¹⁴. Apart from their established roles in plant development, *SRS*

family genes are now being increasingly associated with regulatory functions in abiotic stress responses. The significance of *SRS* gene functions has also been verified by studies conducted in other organisms. The *MsSRS* genes of alfalfa responded to cold and salt stress, and their expression levels increased significantly¹⁵. The *GmSRS18* gene of the model plant soybean suppressed drought and salt tolerance¹⁶. Evidence indicates that *GhSRS21* in cotton may negatively regulate salt tolerance, likely by modulating the balance of reactive oxygen species (ROS)¹⁷. The expression pattern of *MaSRS* genes under a series of stresses and treatments, including cold stress, salicylic acid (SA), and methyl jasmonate (MeJA), was significantly upregulated, highlighting their role in plant growth regulation¹⁸. These results confirm that the *SRS* genes play a vital role as mediators that connect the regulation of plant growth with hormone and stress responses. An important part of *SRS* activity involves its interaction with *cis*-acting elements. The *AtSTY1* protein has been demonstrated to specifically interact with the ACTCTAC element, which explains the mechanism of regulation of auxin biosynthesis and related development by the *SRS* protein⁸. However, understanding the roles of these transcription factors in regulating gene expression associated with development and stress responses remains challenging, largely due to the limited characterization of *SRS* protein interactions with *cis*-regulatory elements. Despite progress, the molecular mechanisms of *AtSRS* genes that integrate hormone regulation, abiotic stress responses, and *cis*-element binding remain incompletely understood in *Arabidopsis thaliana*. It facilitates a full functional study of the *AtSRS* gene family because it is a well-established model organism. To resolve this, a thorough genome-wide assessment of the *AtSRS* gene family was undertaken. This study included the analyses of genomic characteristics, phylogenetic analysis, chromosomal distribution, conserved amino acids in RING-like zinc finger and IGGH domain, collinearity, and *cis*-regulatory elements. Additionally, our approach involved the analysis of gene architecture, expression activity and pattern in plant tissues. Moreover, it involves evaluating the expression triggered by six plant hormones and several abiotic stresses. Collectively, these findings provide valuable insights into expression profiles and indicate potential gene-specific functions of *AtSRS* family proteins.

2. MATERIALS AND METHODS

2.1. Genome-Wide Identification, Domain Characterization, and Sequence Retrieval of *AtSRS* Genes in *Arabidopsis*

Genomic information pertaining to *AtSRS* genes was accessed via the NCBI database (<https://www.ncbi.nlm.nih.gov/>), TAIR10 (<http://www.Arabidopsis.org/>)¹⁹, Phytozome

(<https://phytozome-next.jgi.doe.gov/>)²⁰, Plant transcription factor database v5.0 (<https://planttfdb.gao-lab.org/>) databases^{21–24} and iTAK (<http://itak.feilab.net/>). Incorporating the collected gene IDs from these five databases and a previously published article⁵, a total of 11 unique gene IDs were retained. Next, the NCBI CDD search was used to conduct the domain analysis (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>)²⁵. Analysis revealed that all ten AtSRS proteins possess the conserved DUF702 (RING-like zinc finger) and IGGH domains, leading to their selection for subsequent analyses. The domain structures were further analyzed and graphically represented using TBtools (v1.089).²⁶ Additional SRS sequences were collected from 22 species, namely, *Solanum lycopersicum*⁸, *Oryza sativa*²⁷, *Triticum aestivum*²⁸, *Zea mays*²⁹, *Brassica napus*³⁰, *Benincasa hispida*, *Citrullus lanatus*, *Cucurbita maxima*, *Cucurbita moschata*, *Cucumis melo*, *Cucumis sativus*, *Lagenaria siceraria*³¹, *Chenopodium quinoa*, *Nicotiana glauca*, *Psycotriton patens*, *Selaginella moellendorffii*³², *Glycine max*³³, *Melilotus albus*, *Spinacia oleracea*¹⁸, *Medicago sativa*¹⁵, *Phaseolus vulgaris*³⁴, *Sesamum indicum*³⁵.

2.2. Characterization of AtSRS Genes

For each gene, information regarding chromosome number, gene location, gene length, coding sequence, and protein length was obtained from the genome annotation data server TAIR10 and Uniprot. These data were further validated by the NCBI database, and also rechecked through the online webserver Interpro (<https://www.ebi.ac.uk/interpro/search/sequence/>)³⁶. Molecular weight, isoelectric point (pI) and Grand Average of Hydropathicity (GRAVY) data retrieved from ExPASy ProtParam (<https://web.expasy.org/protparam/>)³⁷. Further, the exon-intron numbers were obtained from UniProt (<https://www.uniprot.org/>)³⁸. Predictions of subcellular localization for the ten AtSRS proteins were performed using UniProt and Plant-mPLoc web resources (<http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/>)³⁹.

2.3. Mapping of Chromosomal Locations and Analysis of Gene Duplications

To illustrate their chromosomal distribution, gene positions were plotted using the MG2C online tool (http://mg2c.iask.in/mg2c_v2.1/)⁴⁰. The reciprocal BLAST searches of the TBtool software were used to analyze AtSRS genes. The TBtool software's sophisticated circos feature was used to map the duplicated gene pairs throughout the genome (<https://bio.tools/tbtools>)²⁶.

2.4. Genomic Structure, Motif Distribution, *Cis*-Acting Elements

Gene architecture was investigated by analyzing and graphically representing the exon–intron arrangements of *AtSRS* genes using GSDS 2.0 (<https://gsds.gao-lab.org/>)⁴¹. Detection of conserved motifs among *AtSRS* genes was performed using the MEME Suite online platform (<https://meme-suite.org>)⁴², with the analysis restricted to a maximum of 10 motifs. Promoter regions were investigated by extracting 2,000 bp upstream sequences from the transcription start site (TSS) of each *SRS* gene from NCBI, followed by examined with PlantCARE database (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>)⁴³ to detect and annotate cis-regulatory elements and certain functions of each *cis* element were obtained mainly from the previously published literatures^{44,45}.

2.5. Sequence Alignment and Phylogenetic and Evolutionary Analysis

BioEdit v7.7.1.0 (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>)⁴⁶ was used to perform multiple sequence alignment of SRS protein sequences, where the conserved RING-like zinc finger and IGGH domains were highlighted using a 50% similarity threshold. Maximum likelihood-based phylogenetic analysis was performed in MEGA11 to examine the evolutionary relationships among SRS proteins, with pairwise deletion and 1,000 bootstrap repetitions under the Poisson model, and the tree was graphically represented using iTOL (Interactive Tree of Life) (<https://itol.embl.de/>)⁴⁸.

2.6. Synteny Relationship and Selective Pressure Analysis

The syntenic relationships of *Arabidopsis* were conducted with rice (*O. sativa*), maize (*Z. mays*), melon (*C. melo*), soybean (*G. max*), mustard (*B. rapa*), and tomato (*S. lycopersicum*). Genomic sequences and annotation files retrieved from the Phytozome²⁰ database were analyzed, and synteny results were visualized using TBtools. To evaluate evolutionary pressures, synonymous (Ks) and non-synonymous (Ka) substitution ratios between collinear *AtSRS* gene pairs were determined using TBtools' Ka/Ks calculator.

2.7. Protein Structure Prediction

Secondary structure predictions of the ten *AtSRS* proteins were performed using Jpred 4.0 (<https://www.compbio.dundee.ac.uk/jpred/>)⁴⁹ and AlphaFold (<https://www.alphafoldserver.com>)^{50,52}, the three-dimensional structures and corresponding Ramachandran plots of the proteins were predicted using Swiss-Model (<https://swissmodel.expasy.org/>)⁵¹ web server.

2.8. KEGG Pathway, Gene Ontology and Similarity Analysis

The ATTED-II database (<https://atted.jp/>)⁵³ was used to retrieve the KEGG pathway data for the *AtSRS* genes, and the functional annotation was performed using the TAIR10 database, including gene ontology (GO) classification to assign the genes to cellular components, molecular functions, and biological processes. The resulting GO data were further visualized using the SRplot (<https://bioinformatics.com.cn/en>) tool to generate the GO structure and enrichment diagrams⁵⁴. Additionally, similarity analysis among the genes was performed through the SIA webserver (<http://imed.med.ucm.es/Tools/sias.html>)⁵⁵.

2.9. Protein Network, Biological Network, Molecular Network Analysis

To explore interactions among *Arabidopsis*'s SRS proteins, STRING (<https://string-db.org/>)⁵⁶ was employed to construct the protein–protein interaction (PPI) network, and subsequent analysis of biological and molecular relationships was performed with SRplot.

2.10. Computational Analysis of *AtSRS* Gene Expression Profiles

Gene expression information for *AtSRS* family members was collected through the Arabidopsis eFP Browser (<https://bar.utoronto.ca/efp/cgi-bin/efpweb.cgi>)⁵⁷. Expression data under various abiotic stresses, including drought, heat, cold, and osmotic stress, were available for five of the identified *AtSRS* genes. These genes were therefore selected for *in-silico* expression prediction via log₂-fold changes analysis under stress. Additionally, the available expression data in response to hormonal treatment for seven genes and *in silico* expression for both tissue-specific and developmental stage-specific data of all the identified genes obtained from travadb (<https://travadb.org/>).

2.11. Statistical Analysis

The collected expression data were normalized with z-scores⁵⁸ following the z-score was calculated using the formula $z = \frac{x-\mu}{\sigma}$, in this equation, x corresponds to the observed value, μ to the mean, σ to the standard deviation, and z indicates how far the value deviates from the mean. Finally, clustered heatmaps (z-score scaled) were illustrated to clearly visualize expression patterns across stresses and in response to hormonal treatments.

3. RESULTS

3.1. Comprehensive Identification and Functional Characterization of *AtSRS* Genes in the *Arabidopsis* Genome

Performing extensive search from PlantTFDB and iTAK, 16 *AtSRS* transcription factors (TF) genes were found. Additionally, ten *AtSRS* genes were obtained from earlier research⁵. These steps yielded 11 unique gene IDs, which underwent conserved domain and motif analyses. Subsequently, ten sequences harboring the conserved *AtSRS* domains (RING-like zinc finger and IXGH) were selected for downstream analyses (Supplementary File 1). These sequences were named following the nomenclature proposed by Kuusk et al., (2006). The lengths of *AtSRS* proteins ranged from 173bp (*AtSRS8*) to 370bp (*AtSTY1*) amino acids, with molecular weights of 19.54268 (*AtSRS8*) to 38.55396 (*AtSRS5*) kDa. Analysis of physicochemical properties revealed that the isoelectric point spanned from 6.19 in *AtSRS7* to 9.77 in *AtSRS6*, where six *AtSRS* proteins out of 10 were basic in nature (pI > 7) and three genes, namely, *AtSTY2*, *AtSRS7*, and *AtSRS5*, were found to be acidic, having pI values 6.48, 6.19 and 6.67, respectively. Only *AtSTY1* was found neutral, with a pI of 7.13. *AtSRS* proteins' GRAVY values ranged from -0.981 (*AtSRS4*) to -0.426 (*AtSRS8*), suggesting that they are hydrophobic and non-polar. All members of the *AtSRS* family were predicted to be nuclear-localized proteins (Table 1).

3.2. Chromosomal Localization

The *AtSRS* family genes' chromosomal location showed that they are dispersed throughout five chromosomes in *Arabidopsis*: chromosome 1 (*AtSRS7* and *AtSRS5*), chromosome 2 (*AtSRS3* and *AtSRS4*), chromosome 3 (*AtSRS6* and *AtSTY1*), chromosome 4 (*AtSTY2*), and chromosome 5 (*AtSRS8*, *AtLRP1*, and *AtSHI*). The distribution pattern showed that most genes were situated far from the chromosomal midpoint, approximately halfway along the chromosome, reflecting a bias toward telomeric regions. Three *SRS* family genes (*AtSRS3*, *AtSRS4*, and *AtSRS8*) were located approximately in the middle of the chromosomes (Fig. 1).

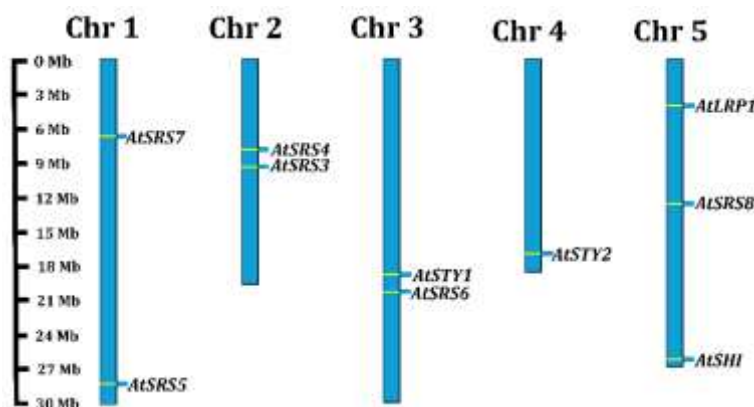


Fig. 1. Chromosomal distribution of 10 *AtSRS* genes in *Arabidopsis*. The scale on the left shows the chromosomal length

217 **Table 1.** Features of the 10 *SRS* family genes and their encoding proteins of *Arabidopsis*

Gene name	Gene ID	Chr ID	Chromosomal Location		Strand	Gene (bp)	CDS (bp)	Protein (aa)	Molecular Weight (kDa)	pI	GRAVY	Exon	Intron	Subcellular localization
			Start	End										
<i>AtSHI</i>	AT5G66350	5	26503941	26506809	reverse	3695	996	331	35.37683	8.52	-0.733	4	2	Nucleus
<i>AtSTY1</i>	AT3G51060	3	18964129	18966405	forward	2277	1113	370	38.35213	7.13	-0.441	2	1	Nucleus
<i>AtSTY2</i>	AT4G36260	4	17155556	17157645	reverse	2090	969	322	34.14416	6.48	-0.776	2	1	Nucleus
<i>AtLRP1</i>	AT5G12330	5	3987375	3989833	reverse	2502	963	320	33.61781	8.77	-0.621	2	0	Nucleus
<i>AtSRS3</i>	AT2G21400	2	9158310	9159732	forward	1423	525	174	19.81372	9.39	-0.56	2	1	Nucleus
<i>AtSRS4</i>	AT2G18120	2	7876544	7878072	reverse	1529	669	222	24.87936	8.08	-0.981	2	1	Nucleus
<i>AtSRS5</i>	AT1G75520	1	28351522	28353549	reverse	2028	1041	346	38.55396	6.67	-0.968	2	1	Nucleus
<i>AtSRS6</i>	AT3G54430	3	20146626	20148221	reverse	1596	552	183	20.32632	9.77	-0.538	2	1	Nucleus
<i>AtSRS7</i>	AT1G19790	1	6838120	6840293	reverse	2194	1038	345	37.92837	6.19	-0.809	2	1	Nucleus
<i>AtSRS8</i>	AT5G33210	5	12465057	12465713	reverse	1019	522	173	19.54268	9.42	-0.426	3	2	Nucleus

Note: Chr: Chromosome, bp: base pair, CDS: Coding Sequence, aa: No. of Amino Acid, pI: Isoelectric point, GRAVY: Grand Average of Hydropathicity

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3.3. The Alignment of AtSRS Proteins

To investigate protein structural diversity and conserved regions, multiple sequence alignment of SRS proteins in *Arabidopsis thaliana* was conducted (Fig. 2). This revealed that the identified AtSRS proteins contained the RING-like zinc finger domain, which is highly conserved, and notably, the α -helix structure common to every RING-like zinc finger domain at the N-terminus of the protein sequences. This demonstrated that the RING-like zinc finger domain, which is highly conserved, and the α -helix structure shared by all RING-like zinc finger domains at the protein sequences' N-terminus were present in the analyzed *AtSRS* proteins. The other conserved domain of AtSRS family proteins, the IGGH domain, is located at the C-terminus and contains acidic amino acids that function as homologous isomerization^{4,5,9}.

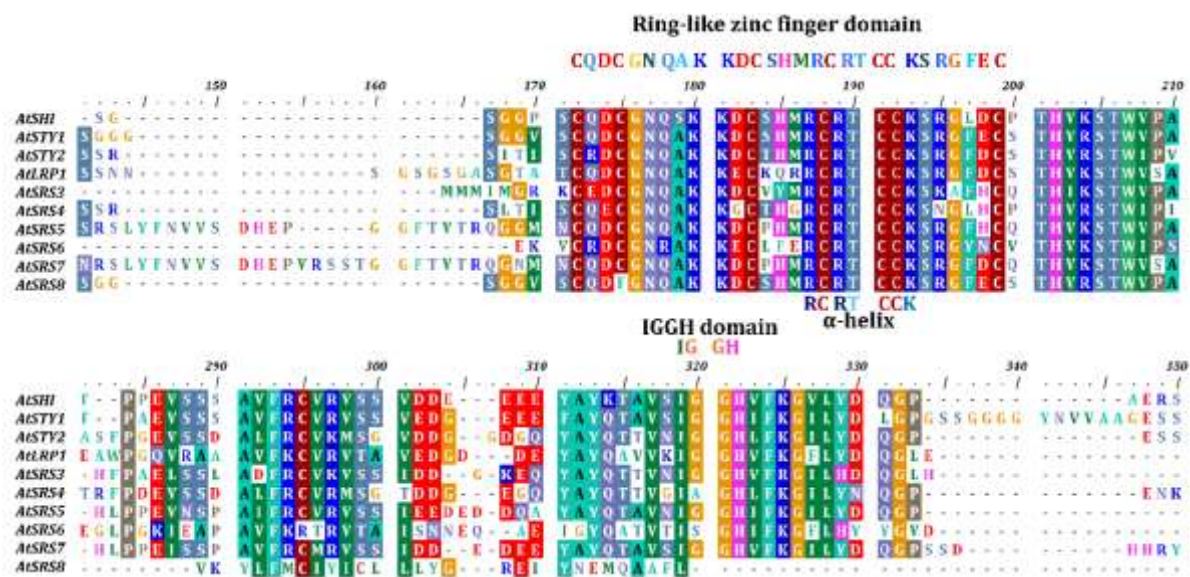


Fig. 2. The multiple sequence alignment of 10 AtSRS protein sequences, where the threshold value for shading similar amino acids was set at 50%. AtSRS protein sequence alignment of domain-related regions is displayed here, while a complete alignment of the sequences is presented in Supplementary File 2.

3.4. Phylogenetic Analysis, Gene Structure, and Conserved Motif Distribution

A rectangular phylogeny was constructed using 10 AtSRS protein sequences. A total of four paralogous protein pairs were found among the two groups, three pairs in Group01 (*AtSTY1/AtSRS8*, *AtSTY2/AtSRS4*, and *AtSRS5/AtSRS7*) and one in Group02 (*AtLRP1/AtSRS6*) (Fig. 3). AtSRS proteins were found to have ten motifs in total (Fig. 3). Every AtSRS protein has motifs 1, 2, and 3 that are extremely conserved, where no other motifs were found in

Group02. Along with those motifs, motif 6 is fairly conserved across most members (except for *AtSRS3* and *AtSRS8*), while motifs 4, 7 and 10 are sparsely distributed in Group01. Delving deeper into motif distribution patterns further revealed a closer relationship among *AtSRS* Group01 family members: Motif 5 (*AtSHI/AtSRS8/AtSTY1*), Motif 8 [*AtSTY2/AtSRS4; AtSHI/AtSTY1/AtSRS8*], and Motif 9 (*AtSRS5/AtSRS7*). The genomic structure exhibited a strong similarity of *AtSRS* genes, each of which contains equal exons (2) and an intron (Fig. 3). However, the *AtSRS* genes differ in genomic length, ranging from 1019 for *AtSRS8* (shortest) to 3695 for *AtSHI* (longest) (Table 01).

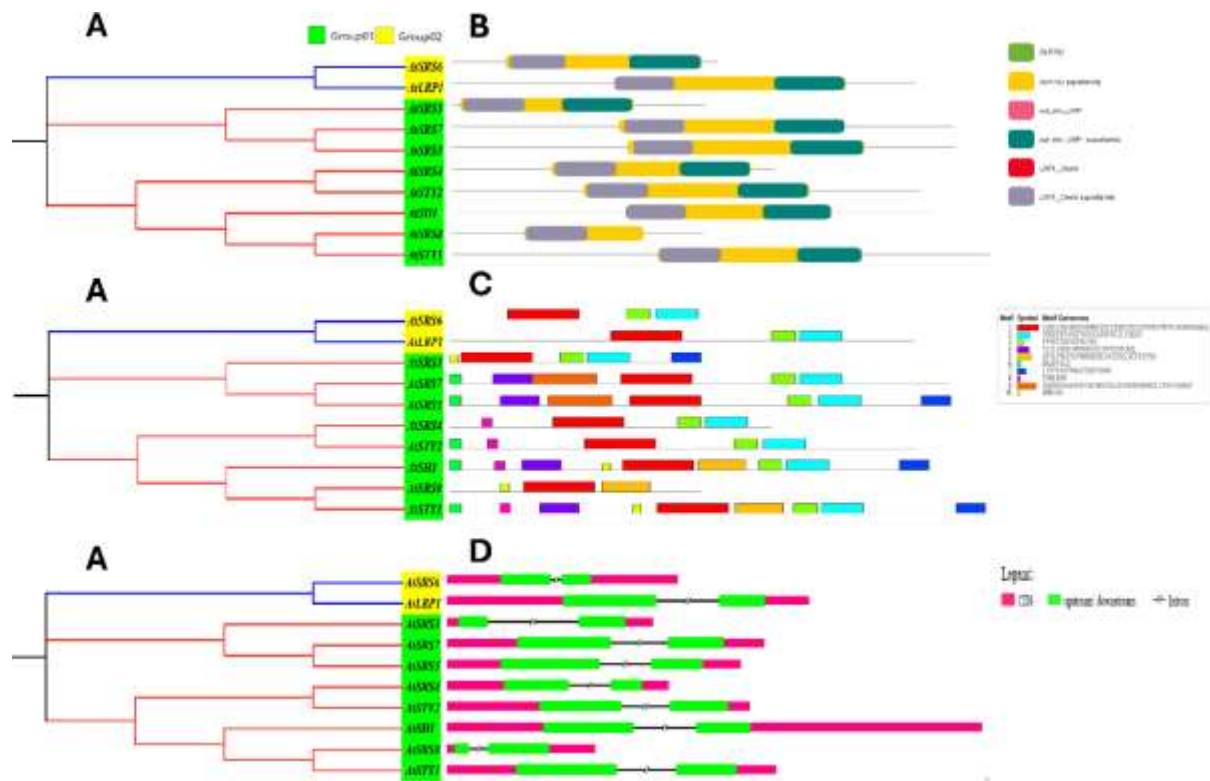


Fig. 3. *AtSRS* sequence analysis- **A** Phylogenetic tree; **B** Domain analysis; **C** Motif distribution. According to the legends displayed on the right, motif numbers are denoted by colored boxes; **D** Analysis of gene structure. Exons, UTRs, and introns are represented by pink, light green, and the shrinking line, respectively. The chromosomal, motif, and exon/intron lengths are represented by scales beneath the plots.

3.5. Identification of *Cis*-Acting Elements in Promoter Regions

Cis-regulating elements found in the promoter region play significant role in determining patterns of gene expression⁵⁹. A total of 74 *cis*-acting elements were discovered to be engaged in growth and developmental processes (14), light responsiveness (23), phytohormone

responsiveness (12), reaction to biotic (04) and abiotic stressors (17), and promoter-related functions (04). Promoter analysis revealed a wide variety of cis-acting elements implicated in responses to abiotic stress, including CCAAT-box, ARE, AT-rich element, DRE core, GC-motif, MYB, Myb, MYB recognition site, Myb-binding site, MYB-like sequence, LTR, MBSI, MBS, , MYC, STRE, TCA and TC-rich repeats. Additionally, four elements, Box S, W box, WRE3, and WUN-motif were found to be associated with responses to biotic stress. The analysis further revealed fourteen *cis*-acting elements linked to growth and developmental regulation, including AAGAA-motif, A-box, AC-I, as-1, CARE, CAT-box, CCGTCC motif, CCGTCC-box, circadian element, F-box, HD-Zip 3, O2-site, RY-element, and telo-box. Light-responsive *cis*-regulatory elements constituted the largest group identified in this study. This included AE-box, ATC-motif, ATCT-motif, Box 4, Box II, chs-CMA1a, chs-CMA2a, GA-motif, L-box, MRE, Sp1, GATA-motif, G-Box, G-box, Gap-box, GT1-motif, , TCCC-motif, TCT-motif, GTGGC-motif, I-box, LAMP-element, 3-AF1 binding site, and 3-AF3 binding site. Twelve cis-regulatory elements (ABRE, ABRE3a, ABRE4, AuxRR-core, CGTCA-motif, ERE, JERE, P-box, TATC-box, TCA-element, TGACG-motif, and TGA-element) were discovered to be actively sensitive to hormones. AT~TATA-box, CAAT-box, TATA, and TATA-box were shown to be responsive to promoter functioning and had the highest availability among all types of *cis*-acting elements. Considering the individual abundance of *cis*-regulatory elements, the TATA-box exhibited the highest frequency (67 occurrences). (Fig. 4, Supplementary File 3, SP1: *Cis*-acting elements).

3.6. Evolutionary Relationships and Phylogenetic Analysis of the *AtSRS* Gene Family

A total of 259 *AtSRS* proteins from 23 different plants namely, *Arabidopsis* (10 proteins), Tomato (08), Rice (05), Wheat (15), Maize (09), Canola (34), Wax gourd (07), Watermelon (08), Pumpkin (13), Moschata pumpkin (12), Melon (05), Quinoa (10), Cucumber (09), Soybean (21), Bottle gourd (07), Sweet clover (09), Alfalfa (27), Tobacco (20), Earthmoss (05), Common bean (10), Sesame (06), Spikemoss (04), Spinach (05) were retrieved from their databases. As illustrated in Fig. 5, all 259 genes are distinctly grouped into three subfamilies (Groups I, II, and III), consistent with the findings of Lu et al. (2023). Nearly 56% (145 genes) belong to Group I, followed by 85 genes in Group II and 29 in Group III. In *Arabidopsis*, Group I contains eight members and Group II contains two members, while Group III contains no *AtSRS* members. Phylogenetic research found that just one member of the *AtSRS* genes showed orthologous relationships with *BnSRS* (*AtSTY1/BnSRS30*), demonstrating their functional

291 similarities. Moreover, the majority of *AtSRS* genes are closely connected to the *BnSRS* genes³⁰
 292 implying a common origin (Fig. 5, Supplementary File 3, SP2: Sequence of 259 Genes).

Function	Specific function	Cis elements	AtSH1	AtSTY1	AtSTY2	AtLRP1	AtSRS3	AtSRS4	AtSRS5	AtSRS6	AtSRS7	AtSRS8
Abiotic stress responsive	Anaerobic Induction	ARE	1	1	2	3	2	2	3	1		4
	Transcriptional Activators	AT-rich element				1		1			1	
	Cold/ Dehydration	DRE core				1	1				1	1
	Anoxic Specific Inducibility	GC-motif				1			1			
	Low Temperature	LTR		2			1			2	2	
	Flavonoid biosynthesis	MBSI							1			
	Drought, Salinity, Low temp.	MBS	1				1			1		
	Abiotic stress response	MYB	3		4	3	3	4		1	4	3
	Heat stress	Myb	1				2			2	1	
	ABA expression	MYB recognition site	1				2					
	Abiotic stress response	Myb-binding site	1		1	2					2	1
	Abiotic stress response	MYB-like sequence	1		2	1	3				1	1
	ABA expression	MYC	2	2	4	2	1	1		8	5	1
Biotic stress responsive	Stress	STRE	2	2		2	2	1		1		3
	Salicylic Acid	TCA	2			1	1			1		
	Defence/Stress	TC-rich repeats		1			1		1	1	1	
Growth & development related	Biotic Stress	box S										1
	Biotic Stress	W box	1					1		1		2
	Wound/Pathogen	WRE3			2		1	1		4		1
	Wound	WUN-motif						1	3			
	Seed Specific Expression	AAGAA-motif	3	1		1		2	1		3	1
	Specific Day Length	A-box					1		1			1
	Vascular Specific Expression	AC-1				1	1					
	Drought	as-1	1		1	2			3		1	
	Hormone	CARE								1		
	Meristematic development	CAT-box			2	1		1				
	Meristem Specific Gene Activ.	CCGTCC motif					1		1			1
	Meristem Specific Gene Activ.	CCGTCC-box					1		1			1
	Growth & Development	circadian				1				1		
	Growth & Development	F-box				1			1			
Light responsive	Cell metabolism	HD-Zip 3							1			
	Zeln Metabolism	OZ-site					1			1	2	
	Seed-specific gene regulation	RY-element						2		1		
	Placenta-specific activity	CCAAT-box	1				2					
	Expression in root meristem	telo-box	1									
	Regulation of GapA	AE-box			1			1	2	1		
	Light Stress	ATC-motif			1					1		
	Light Stress	ATCT-motif										1
	Light responsive	Box 4	1	5	2	1		1	3		2	2
	Light responsive	Box II			1							
	Light responsive	chs-CMA1a			1	1			1		2	
	Light responsive	chs-CMA2a					1					
	Light responsive	GA-motif		1					1			
	Light responsive	GATA-motif						1			1	
	Light responsive	G-Box	8	1	3	6		1	7	1		3
	Light responsive	G-box		2				2		1		
	Light responsive	Gap-box								1		
	Light responsive	GT1-motif		1	4	3	1		1		2	2
	Light responsive	GTGGC-motif				1						
	Light responsive	I-box						1			2	
	Light responsive	LAMP-element				1				1	1	
	Light responsive	L-box			1							
	Metal response element	MRE		1	1			1		1		2
	Light responsive	Sp1		1							1	
	Light responsive	TCCC-motif										1
	Light responsive	TCT-motif	2	1		2			1		1	
	Light responsive	3-AF1 binding site	1									
	Light responsive	3-AF3 binding site	1							1		
Phyto-hormone responsive	ABA	ABRE	7	2	3	4		5	6	2	1	3
	ABA	ABRE3a		1	2	1			2			1
	ABA	ABRE4		1	2	1			2			1
	Auxin	AuxRR-core		1	1					1		
	MeJA	CGTCA-motif	1		1	2			3		1	
	Ethylene	ERE	1	1			1	2	2			
	Jasmonate and ethylene	JERE									1	
	Gibberellin	P-box						1			1	
	Gibberellin	TATC-box					1	1				
	Salicylic Acid	TCA-element	1	1						1		1
Promoter related cis elements	MeJA	TGACG-motif	1		1	2			3		1	
	Auxin	TGA-element	1		1				1	1		
	Promoter binding site	AT-TATA-box		9	6	4	2	11	5	1	13	1
	NOS promoter activity	CAAT-box	10	17	25	18	26	20	12	24	28	33
	Promoter binding site	TATA		1	1		2		2		1	
	Promoter binding site	TATA-box	9	58	44	30	45	58	41	21	67	17

293
 294 **Fig. 4.** Mapping of cis-regulatory elements within the promoter regions of *AtSRS* genes.

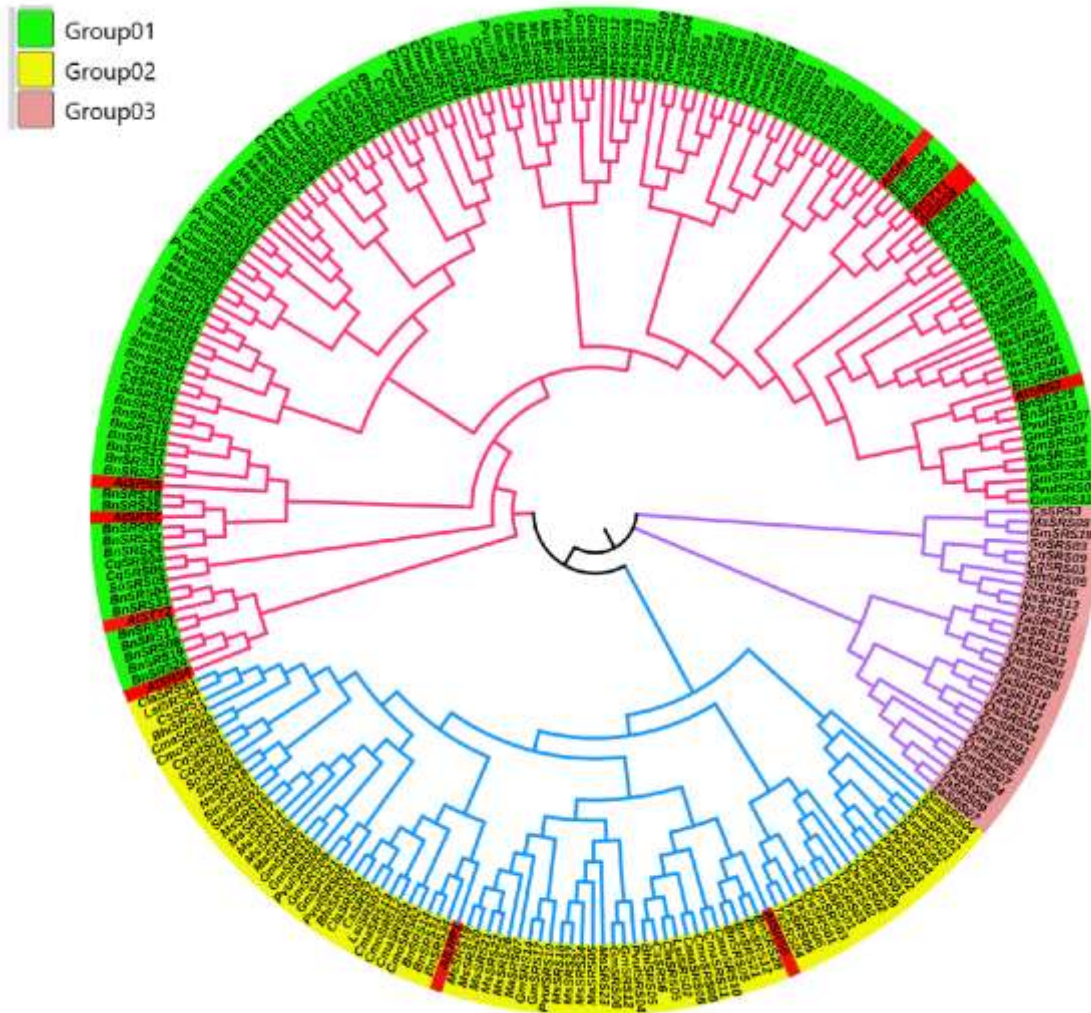
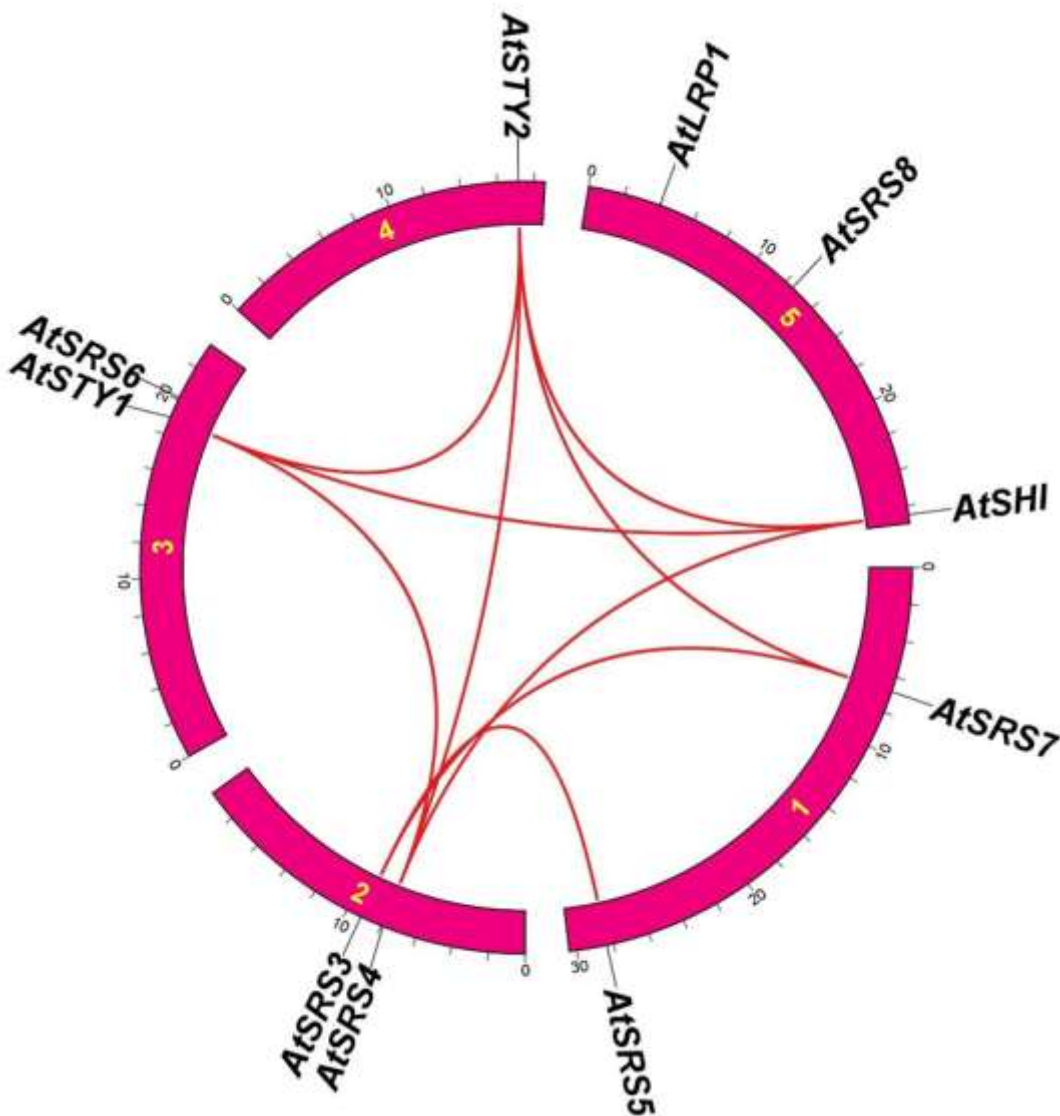


Fig. 5. The phylogenetic analysis of SRS proteins from Arabidopsis (*AtSRS*), Tomato (*SlSRS*), Rice (*OsSRS*), Wheat (*TaSRS*), Maize (*ZmSRS*), Canola (*BnSRS*), Wax gourd (*BhiSRS*), Watermelon (*ClaSRS*), Pumpkin (*CmaSRS*), Moschata pumpkin (*CmoSRS*), Melon (*CmSRS*), Quinoa (*CqSRS*), Cucumber (*CsSRS*), Soybean (*GmSRS*), Bottle gourd (*LsiSRS*), Sweet clover (*MaSRS*), Alfalfa (*MsSRS*), Tobacco (*NsSRS*), Earthmoss (*PpSRS*), Common bean (*PvulSRS*), Sesame (*SinSRS*), Spikemoss (*SmSRS*), Spinach (*SoSRS*). This phylogenetic tree was built employing the maximum likelihood method with 1,000 bootstrap replicates, with *AtSRS* proteins highlighted in red for clarity.

3.7. Gene Duplication Events and Syntenic Relationships in *AtSRS* Genes

Analysis revealed nine duplication events, including whole-genome and segmental duplications, representing syntenic pairings among *AtSRS* genes (Fig. 6, Supplementary File 3, SP3: Gene Duplication Data). Out of four homologous gene pairs (Fig. 3), only one showed gene duplication events (*AtSRS4/AtSTY2*) and the rest of the eight duplication events,

309 *AtSRS5/AtSRS3*, *AtSTY1/AtSTY2*, *AtSTY1/AtSHI*, *AtSRS7/AtSTY2*, *AtSRS7/AtSRS3*,
 310 *AtSTY2/AtSHI*, *AtSRS4/AtSTY1*, *AtSRS4/AtSHI*, were not among the homologous gene pairs.
 311 The syntenic links among *AtSRS* genes and other crops were investigated to better understand
 312 their evolution and abundance. These five species shared 103 syntenic pairs of *AtSRS* genes.
 313 *G. max* had the maximum number of pairwise syntenic blocks (40), followed by *Brassica rapa*
 314 (33), *S. lycopersicum* (17), *C. melo* (12), and *Z. mays* (1) (Fig. 7, Supplementary File 3, SP3:
 315 Gene Duplication Data).



316
 317 **Fig. 6.** Analysis of gene duplication events among 10 *AtSRS* genes. Duplicated genes within
 318 the analysis are highlighted using red lines.

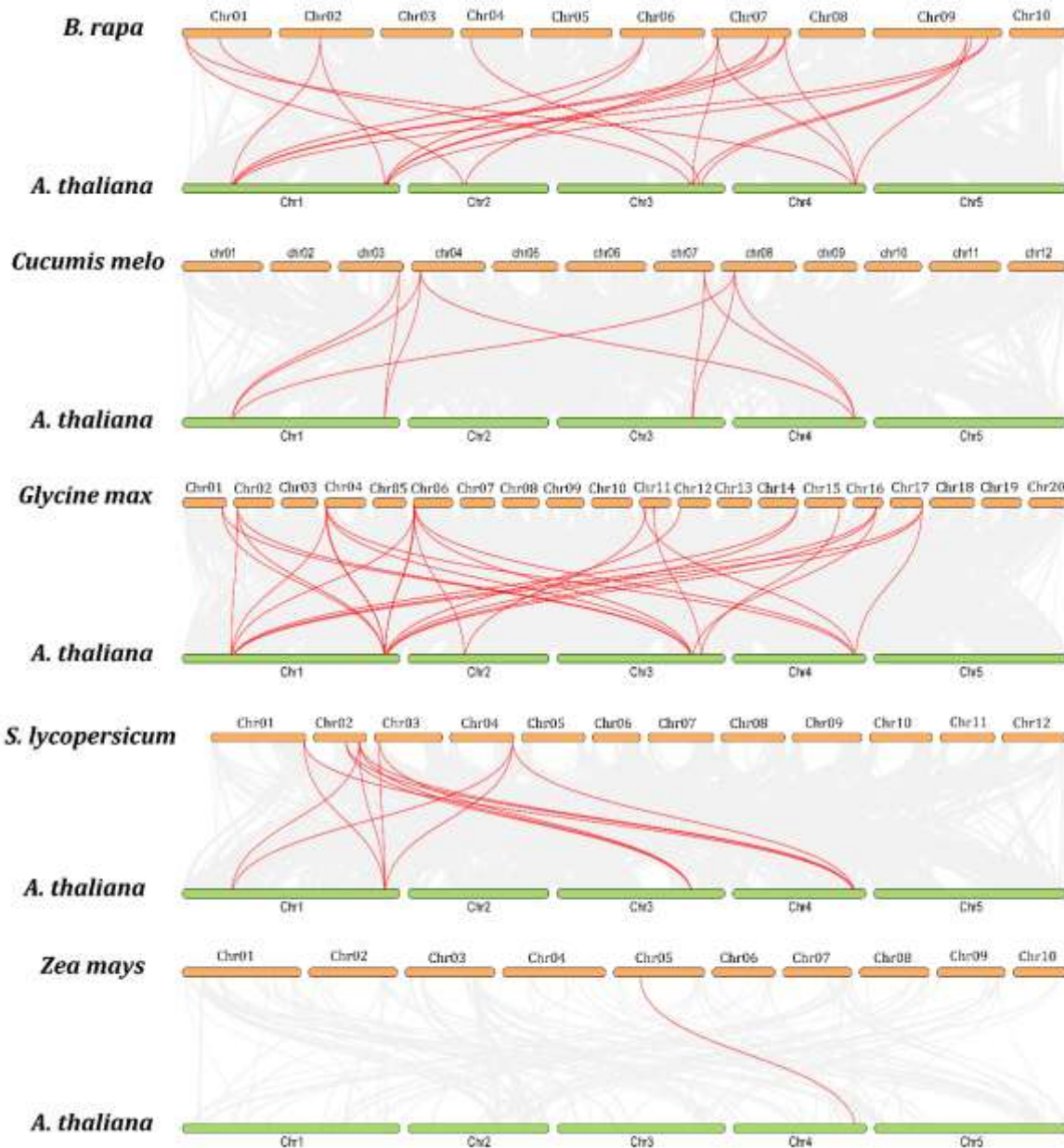


Fig. 7. Syntenic links of 10 *Arabidopsis* SRS genes with *B. rapa*, *C. melo*, *G. max*, *S. lycopersicum*, and *Z. mays*. Grey lines in the background depict collinear genomic blocks across *Arabidopsis* and other plant genomes, whereas red lines emphasize syntenic *AtSRS* gene pairs across *Arabidopsis* and related plant genomes.

3.8. Prediction of Protein Structures and Analysis of Sequence Similarity in *AtSRS* Proteins

Protein three-dimensional structures can be predicted using homology modeling, which infers structural information by aligning the target amino acid sequence with homologous proteins of known structures⁵⁰. Predicted structures of *AtSRS* proteins, Ramachandran plots for each

protein, and template information were reported (Fig. 8, Supplementary File 4). GMQE (Global Model Quality Estimation) provides a measure of the confidence and reliability of models generated through protein homology modeling. AtSRS6 had the greatest GMQE value (0.65), followed by AtSRS3 (0.62), while AtSTY1 and AtSRS7 had the lowest values (0.54). Similarity analysis among the AtSRS proteins also illustrates significant differences, ranging from 11.25% (AtSRS6 vs AtSRS8) to 61.50% (AtSRS5 vs AtSRS7), with an average similarity percentage of 33.06% (Supplementary File 3, SP4: Similarity Analysis).

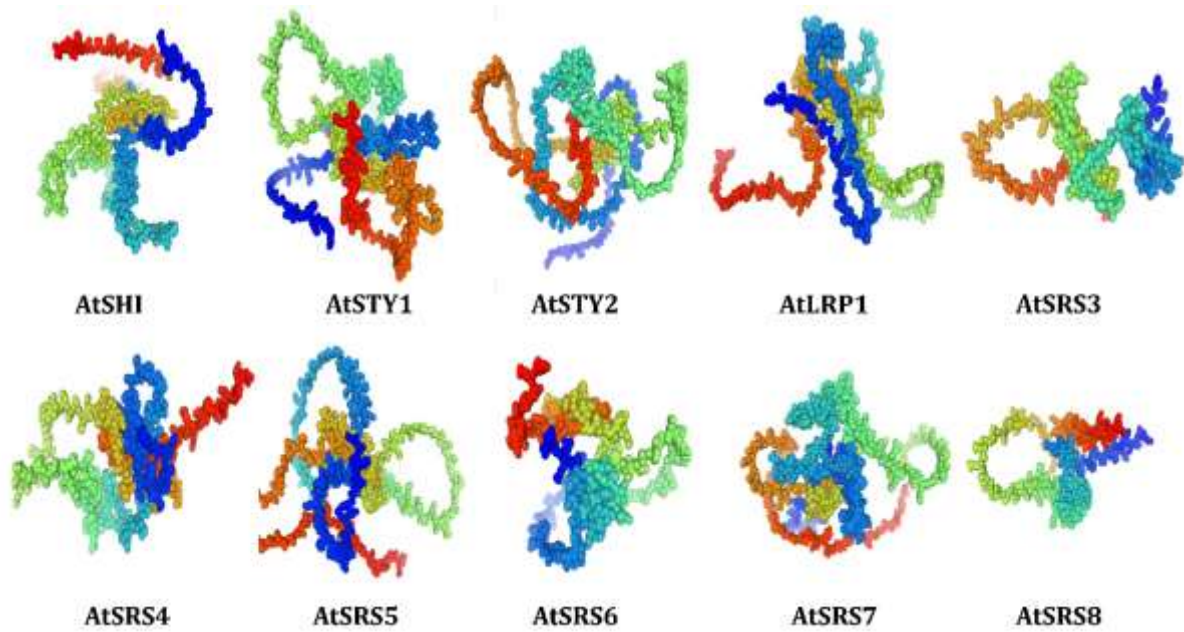


Fig. 8. Predicted 3D structures of AtSRS proteins generated through homology modeling in *Arabidopsis thaliana*

3.9. Gene Ontology

To better understand the functional roles of *AtSRS* genes in *Arabidopsis thaliana*, Gene Ontology (GO) enrichment analysis was carried out, categorizing differentially expressed genes (DEGs) according to biological process, molecular function, and cellular component. By mapping the DEGs to enriched GO terms, key cellular features and functional categories associated with *AtSRS* activity were identified. The analysis revealed distinct patterns between upregulated and downregulated genes, highlighting their potential involvement in diverse cellular and metabolic processes. Visualization of the enriched terms through bar plots provided a clear representation of the most significant categories (Fig. 9, Supplementary File 3, SP5: Gene Ontology data). Overall, these results establish a functional framework for *AtSRS* genes

in *Arabidopsis thaliana*, highlighting their participation in diverse biological processes, molecular activities, cellular organization, and regulatory networks.

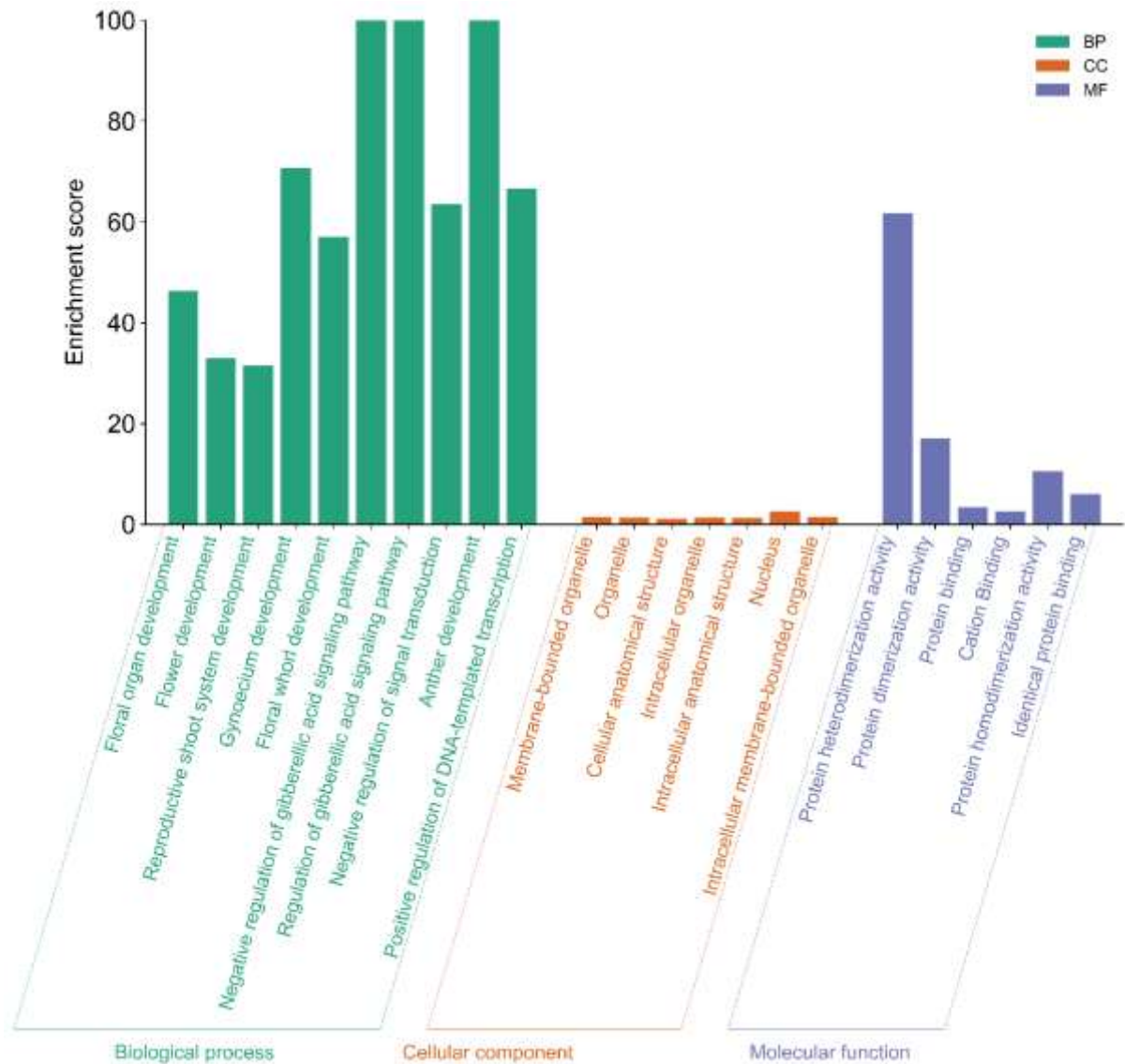


Fig. 9. The functional enrichment of *AtSRS* genes was analyzed using gene ontology (GO). The horizontal axis reflects functional classification, and the vertical axis indicates the degree of gene enrichment. Here, **BP**: Biological process, **CC**: Cellular component, **MF**: Molecular function

3.10. Predicted Network of AtSRS Proteins

Investigation of Protein–Protein interactions among *AtSRS* Proteins revealed a high level of network interconnectivity and density, with no isolated nodes, demonstrating that all *AtSRS* proteins are implicated in an overall functional or overlapping framework. Therefore, members of the *AtSRS* family collaborate to facilitate coordinated biological processes rather than acting

independently. The interaction network further revealed that *AtSRS6*, *AtSRS4*, *AtLRP1*, and *AtSTY2* have significantly more connections than the other AtSRS family proteins, positioning them as hubs of the AtSRS interaction network. As such, they may act as transcriptional regulators and/or integrators of multiple signaling pathways, thereby regulating the various cellular responses coordinated by the AtSRS family. Conversely, the other six AtSRS family proteins (*AtSRS8*, *AtSRS3*, *AtSHI*, *AtSRS7*, *AtSTY1*, and *AtSRS5*) exhibited a moderate number of interactions but remained closely associated with the network's central core (Fig. 10).

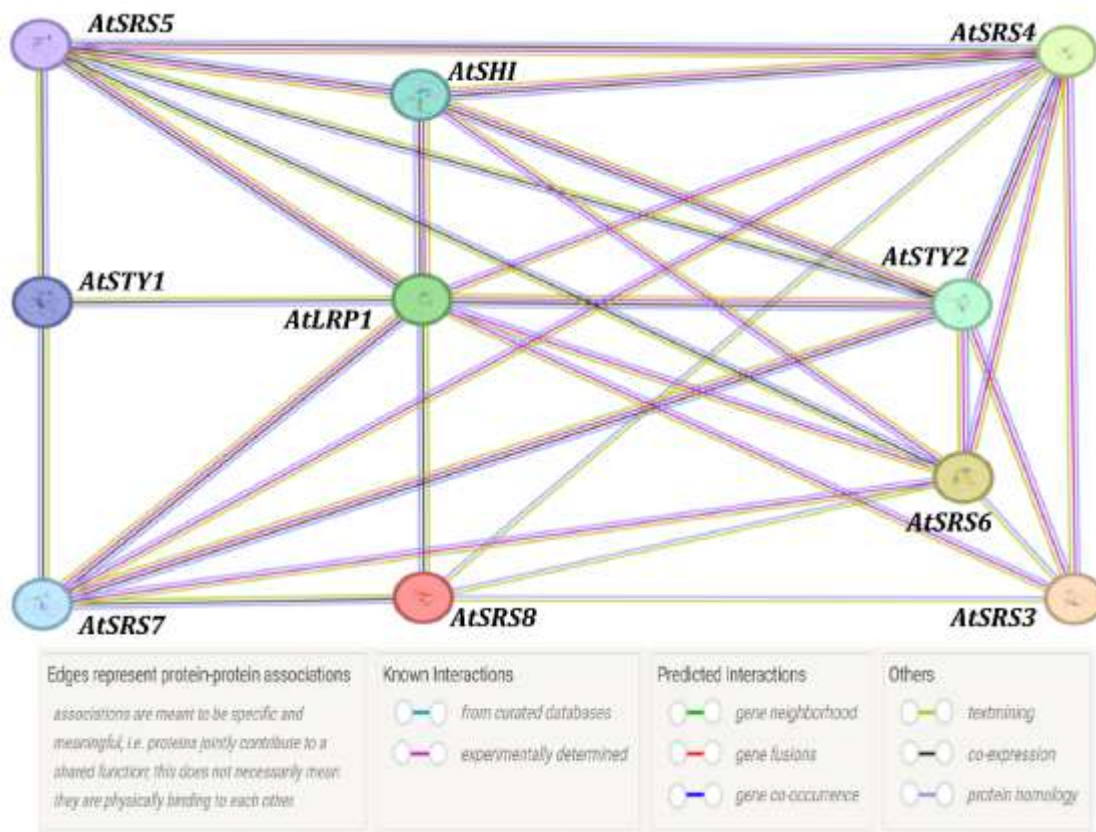


Fig. 10. Predicted interaction network among AtSRS proteins in *Arabidopsis thaliana*. Nodes represent proteins, while connecting lines highlight their interactions.

3.11. Biological and Molecular Interaction Network

The biological and molecular interaction analysis revealed that the biological process category is highly associated with pathways integral to several developmental processes. Based on interactions and enrichment analysis (Fig. 11A, Supplementary File 5, SP1: Biological Enrichment), Processes including auxin biosynthesis and metabolism, hormone biosynthetic pathways, and the positive regulation of both transcription (DNA-templated) and RNA synthesis displayed the greatest enrichment and were highly interconnected. The enrichment

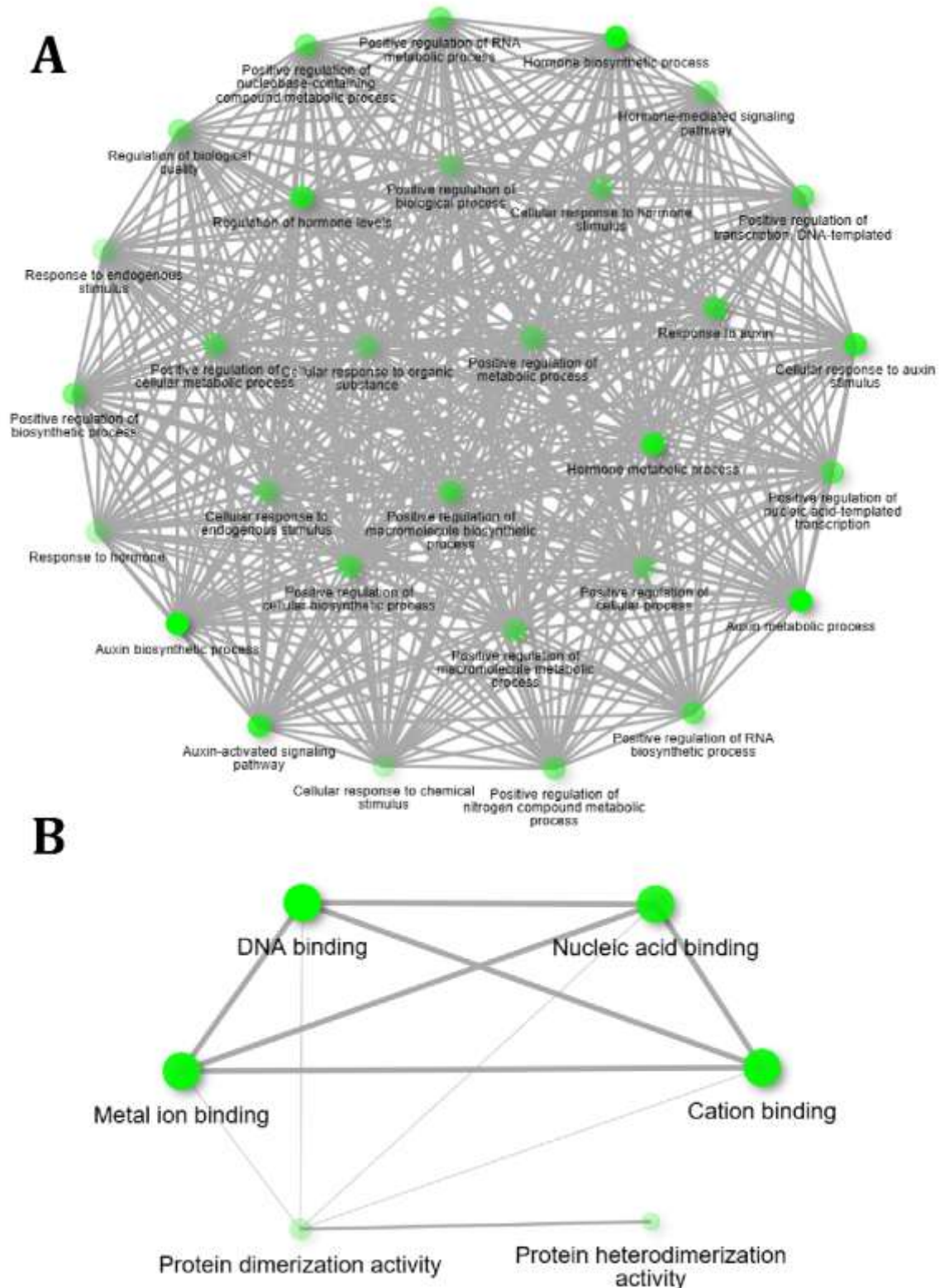
and interconnectivity were substantially lower for processes including regulation of biological quality, cellular responses to hormones and endogenous signals, cellular reactions to organic compounds, and the positive regulation of biological processes. Within the molecular interaction network, DNA binding, cation binding, metal ion binding, and nucleic acid binding exhibited the highest enrichment and strongest interactions, and protein heterodimerization and dimerization activity exhibited comparatively lower levels (Fig. 11B, Supplementary File 5, SP2: Molecular Enrichment).

3.12. KEGG Pathway Enrichment and Functional Analysis

To further investigate the functional relevance of the *AtSRS* gene family in *Arabidopsis thaliana*, KEGG pathway enrichment analysis was performed. This analysis identified 37 pathways with significantly enriched pathways, providing insight into the broader metabolic and signaling networks influenced by the differentially expressed genes (DEGs). Among these, several pathways stood out due to their strong statistical significance and enrichment levels, including plant hormone signal transduction, ABC transporters, DNA replication, nucleocytoplasmic transport, the MAPK signaling pathway in plants, and phenylpropanoid biosynthesis (Supplementary File 3, SP6: KEGG Pathway Enrichment). The prominence of these pathways highlights the diverse functional roles of *AtSRS* genes, ranging from hormone regulation and signal transduction to transport mechanisms, DNA metabolism, and secondary metabolite biosynthesis. These findings suggest that *AtSRS* genes are integrated into both regulatory and metabolic paths that are essential for cellular activity and plant adaptability. By linking DEGs to multiple interconnected pathways, the results from KEGG enrichment analysis establish a foundation for elucidating the contribution of *AtSRS* genes to growth, development, and stress adaptation in *Arabidopsis thaliana*.

3.13. Analysis of Selective Pressure (Ka/Ks)

Ka and Ks analyses were performed on gene pairs to quantify the evolutionary selection pressure following gene duplication events (Supplementary File 6). The Ka and Ks for all duplicated gene pairs are substantially lower than 1.0 (0.13 to 0.17). Thus, indicating there has been substantial and consistent purifying selection of duplicated genes. For *AtSRS4/AtSHI* and *AtSRS4/AtSTY1* pairs, Ks was zero in both cases, making Ka/Ks zero and undefined. This result indicated the absence of detectable synonymous substitutions, which is characteristic of a very recent gene duplication event, and this gene pair was excluded from Ka/Ks-based evolutionary inference (Supplementary File 6).



410

411 **Fig. 11.** These interactive maps depict the networks of (A) Biological and (B) Molecular
 412 function enriched network. Nodes are connected if they share 20% or more genes (default
 413 setting). Darker nodes represent higher enrichment, larger nodes correspond to bigger gene
 414 sets, and thicker edges indicate greater gene overlap.

3.14. Comparative Analysis of *AtSRS* Gene Expression in Various *Arabidopsis thaliana* Tissues

Gene expression profiling across *Arabidopsis* tissues and developmental stages (seed to flower; Fig. 12A, B) revealed significant stage- and tissue-specific transcription patterns of *AtSRS* genes, providing crucial information about their proper developmental roles and regulatory domains. In (Fig. 12A), broad expression trends across tissues become apparent. *AtSRS8* showed its strongest transcriptional expression in young seeds, while *AtSRS7* in stigmatic tissue, with relatively lower expression in the other stages for both genes.

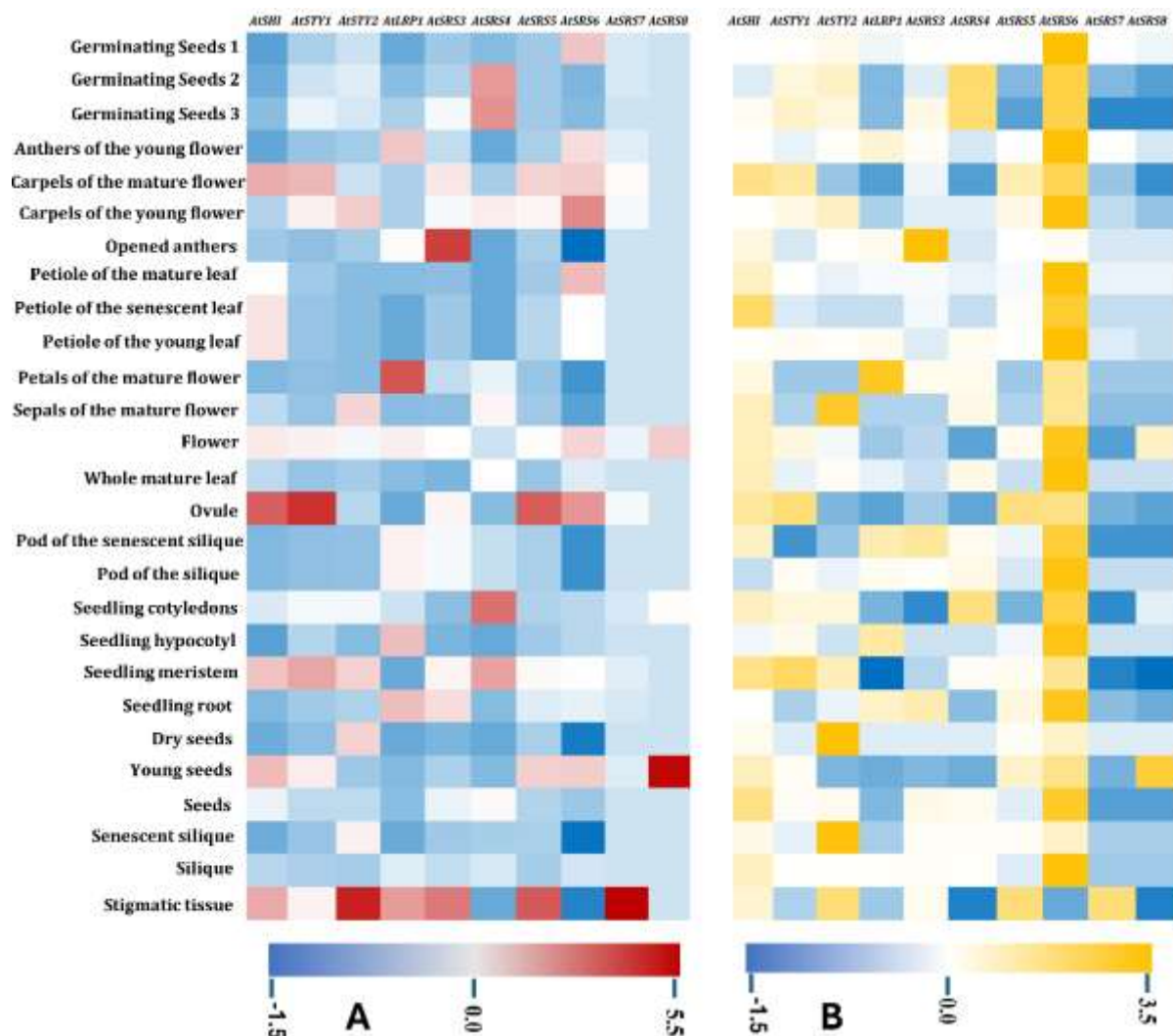


Fig. 12. Expression profile of *Arabidopsis AtSRS* genes in diverse tissues. (A) In silico analysis of relative *AtSRS* gene expression in different tissue types, represented by columns. (B) Comparative expression of selected *AtSRS* genes across tissues. Values are normalized and displayed as Z-scores.

AtSRS6 showed high expression in carpels of young flowers and ovules, moderately high expression in petioles of mature leaves, followed by germinating seeds, carpels of mature flowers, young flower anthers, and young seeds. *AtSRS5* exhibited greater expressions in the stigmatic tissue and ovule, while moderate to lower expressions in others. *AtSRS4* displayed high response levels in seedling cotyledons, germinated seeds, and seedling meristems. *AtSRS3* showed high expression in open anthers and stigmatic tissues, and moderate expression in carpels of mature flowers and seedling roots. *AtLRP1* is most strongly expressed in petals of mature flowers, stigmatic tissues, and moderately expressed in seedling hypocotyl and root. *AtSTY1* showed its highest response in stigmatic tissue, while moderate to low expressions in others. More or less similar expressions were observed for *AtSHI* and *AtSTY2*: high in Ovule, moderate in carpels of mature flowers, seedling meristem, young seeds and stigmatic tissue. Relative expression enrichment scores in Fig. 12B further revealed almost identical expression patterns with transcriptomic expression for *AtSRS* genes except for *AtSRS6*, which consistently showed the highest expression levels in nearly all examined tissues.

3.15. Analysis of *AtSRS* Gene Expression under Plant Hormone Treatments

Analysis of seven *AtSRS* genes' expression responses to six plant hormones revealed their roles in regulating growth, development, and environmental adaptation. *AtSRS* genes displayed both rapid and delayed transcriptional changes, demonstrating their dynamic nature in hormone-mediated signaling pathways. Under abscisic acid (ABA) treatment, *AtSTY2* showed the strongest positive responses, followed by *AtLRP1*. *AtSRS8* and *AtSRS4* showed moderate response, although their expression levels changed over time. On the other hand, *AtSHI* expression was downregulated and *AtSRS5* exhibited an early negative response followed by partial recovery at later time points. In response to methyl jasmonate (MeJA), the expressions of *AtSRS4* and *AtLRP1* were upregulated, while expressions of *AtSRS5* and *AtSRS8* were downregulated. GA (Gibberellic Acid) treatment strongly upregulated *AtSRS4* and moderately upregulated *AtSRS8*, while most other *AtSRS* genes showed little change except *AtSTY2*, which was initially upregulated but downregulated at subsequent period. Zeatin induced strong upregulation of *AtSRS4* and moderate upregulation of *AtLRP1*, but strong downregulation of *AtSRS5* and slight downregulation of *AtSHI*. The other three genes exhibited transient changes in expression that returned to baseline levels by the end of treatment. Additionally, during ACC treatment (1-aminocyclopropane-1-carboxylic acid), which acts as an ethylene precursor, the expression level was higher at subsequent period, where *AtSRS4* and *AtSTY2* experienced significant upregulation. On the contrary, *AtSHI*, *AtSRS6* and *AtSRS8* showed slight

downregulation while the expression of the remaining two genes showed no change. Upon exposure to IAA, *AtLRP1* and *AtSTY2* showed strong and moderate upregulation, respectively. *AtSRS5* showed significant downregulation with no major changes of the remaining four genes (Fig. 13).

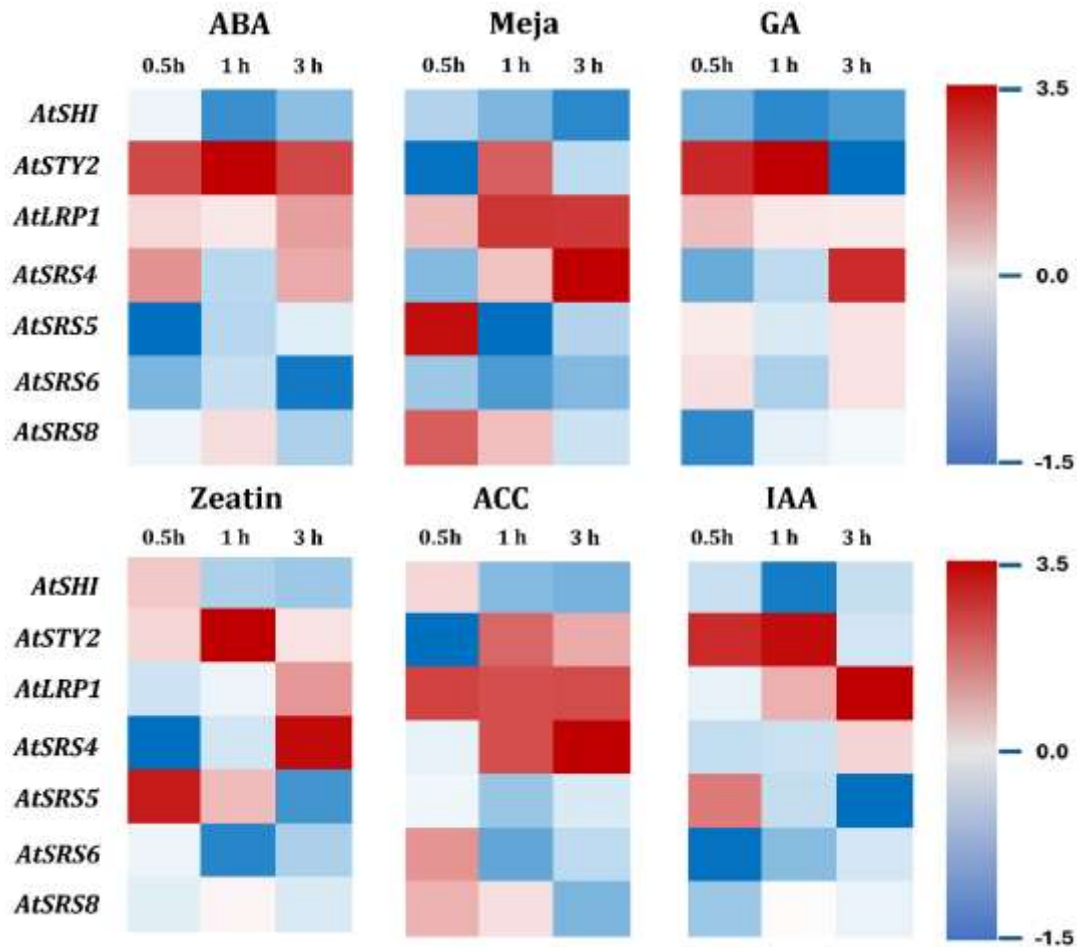


Fig. 13. Relative expression of *AtSRS* genes in response to distinct plant hormone treatments. *AtSRS* gene expression was measured after treated with ABA, Meja, GA, Zeatin, ACC, and IAA at time intervals of 0.5 h, 1 h, and 3 hours. Expression data were converted to log₂ fold change (log₂FC), and heatmaps were generated to illustrate transcriptomic patterns of *AtSRS* genes. Blue and red indicate down-regulated and up-regulated genes, respectively, under the various stress treatments.

3.16. Analysis of *AtSRS* Gene Expression in Response to Abiotic Stresses

To study the potential response of the *AtSRS* genes to diverse abiotic stressors, the transcriptional changes of the *AtSRS* genes under cold, osmotic, salt, drought, genotoxic, oxidative, UV-B, wound, and heat stresses were assessed. Under cold stress, *AtSHI* exhibited

the highest expression in both roots and shoots compared to *AtSTY2* and *AtSRS5*. In contrast, *AtSRS8* and *AtSRS6* showed consistently low expression throughout the treatment period. Osmotic stress promotes more variations in shoots than in roots. In shoots, *AtSRS5* showed the greatest induction, followed by *AtSTY2*. Furthermore, *AtSRS8* and *AtSHI* showed moderate expressions, whereas *AtSRS6* had extremely low expressions. At another time, *AtSRS8* and *AtSTY2* were the most highly expressed in roots, while *AtSRS6* had the lowest expression in comparison with *AtSHI* and *AtSRS8*. Oxidative stress contributed to significant activation of *AtSRS8* and *AtSRS6* in shoot tissues. In contrast, *AtSTY2* responded considerably less than *AtSHI* and *AtSRS5*. On the other hand, *AtSRS8* and *AtLRP1* showed strong responsiveness in roots. But *AtSRS5* remained moderately expressed among all focused *AtSRS* genes, indicating a significant role in root-specific oxidative responses. Under salt stress, *AtSRS6* again revealed as a significantly expressed gene in shoots, followed by *AtSRS8*, *AtSTY2*, *AtSHI* and *AtSRS5*. Additionally, *AtSRS8*, *AtSRS6*, and *AtSHI* showed significant activity in roots at later stages, indicating their role in salt adaptation specific to roots. During drought stress, *AtSRS8* and *AtSRS6* showed the highest expression in shoots, while *AtSTY2* had the lowest response, with intermediate expression of *AtSHI* and *AtSRS5*. By contrast, *AtSRS5* had the highest expression, followed by *AtSRS8* and *AtSRS6*. There was also displayed an average response in *AtSTY2*, indicating a significant contribution to drought adaptation. Shoots exposed to genotoxic stress showed the highest expression in *AtSRS8*, followed by *AtSRS5*, *AtSRS6*, *AtSHI* and *AtSTY2*. In roots, the highest transcriptomic expression exhibited by *AtSRS5*, followed by *AtSRS8* and *AtSRS6*, where *AtSHI* demonstrated the lowest expression. UV-B exposure strongly activated *AtSRS8*, *AtSRS6*, *AtSHI*, and *AtSRS5* in shoots, whereas *AtSTY2* showed less induction. In roots, *AtSRS6* had the highest expression, followed by *AtSRS8* and *AtSTY2*, with *AtSRS5* having the lowest reactivity. Wounding stress in shoots resulted in the rapid induction of *AtSHI* and moderate expression of *AtSRS5*. But *AtSRS5* had the highest expression in roots after injury, followed by *AtSRS6* and *AtSRS8*. Furthermore, *AtSRS6* showed root-specific induction in later stages, indicating a role in wound-induced regeneration. During heat stress in shoots, *AtSRS5* had high induction, followed by *AtSRS6* and *AtSTY2*. In roots, *AtSRS8*, *AtSRS6*, and *AtSTY2* had high expression levels, indicating tissue-specific regulatory diversity among *AtSRS* family members (Fig. 14).

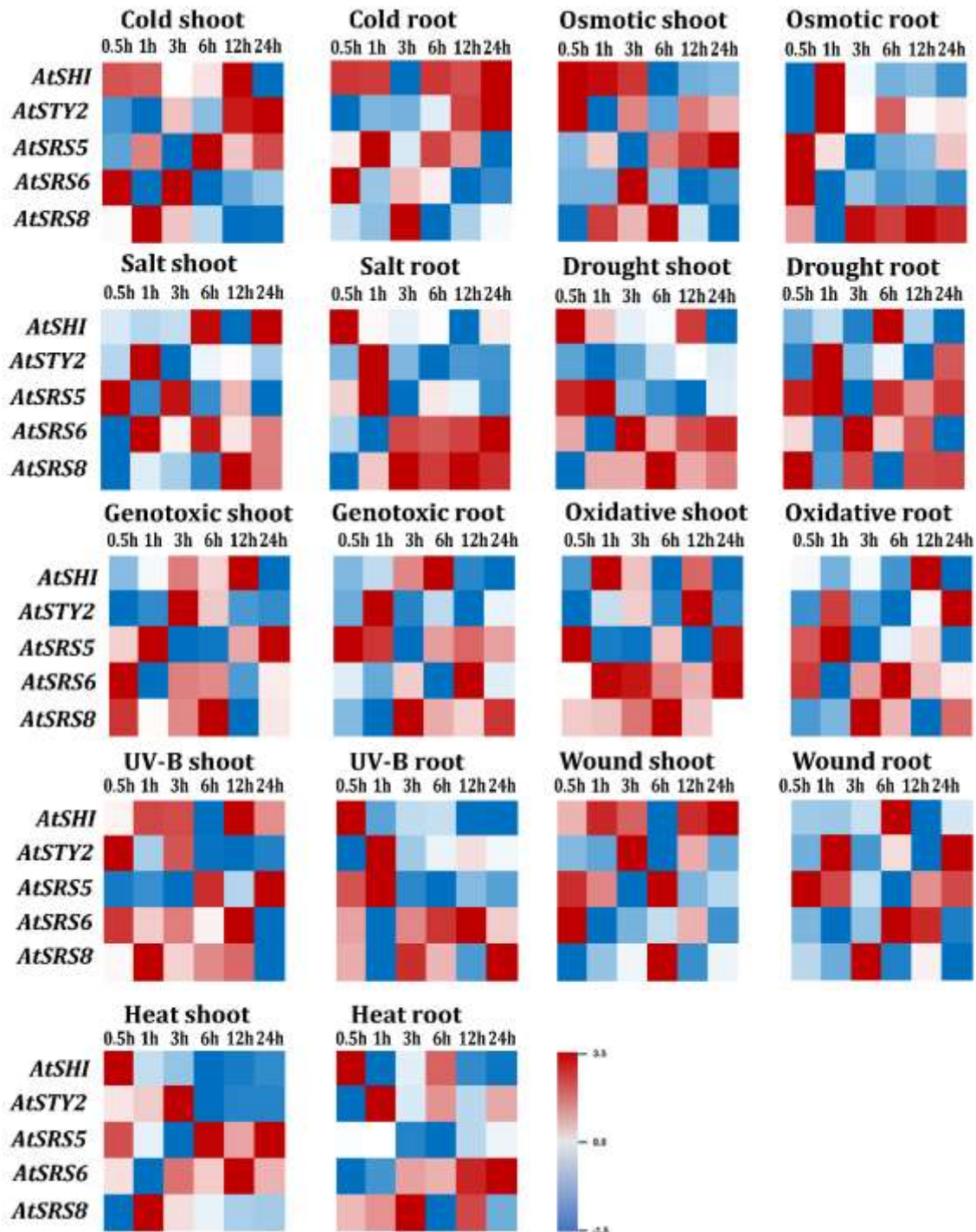


Fig. 14. Expression of *AtSRS* genes in shoots and roots under various abiotic and biotic stresses, including cold, osmotic, salt, drought, genotoxic, oxidative, UV-B, wound, and heat. Gene expression levels were measured at 0.5, 1, 3, 6, 12, and 24 hours, normalized using $\log_2$ fold change ($\log_2FC$), and displayed in heatmaps to visualize differences in *AtSRS* gene expression. Blue and red indicate down-regulated and up-regulated genes, respectively, under the various stress treatments.

4. DISCUSSION

The *SRS* gene family is involved in a range of biological processes^{2,4,5,9} including light response⁸, reproduction⁵, growth and development^{5,9} hormonal signaling^{2,4}, response to biotic and abiotic stress conditions^{8,18,28,31,34}. We conducted genome-wide *in-silico* bioinformatic analyses to identify and characterize the *SRS* family genes in *Arabidopsis*, to understand their genomic features, evolutionary processes, and functional characteristics.

4.1. *SRS* Family Genes: Numbers and Chromosomal Distribution

Significant interspecific variation exists in the number of *SRS* genes among plant species, ranging from 01 (*Oryza longistaminata*)^{21–24} to 34 (*Brassica napus*)³⁰, with intermediate counts in Rice (05)²⁷, *Solanum lycopersicum* (08)⁸, *Arabidopsis* (10)⁵, *Triticum aestivum* (15)²⁸, and *Glycine max* (21)^{16,33} etc. However, we identified 10 *SRS* genes in the *Arabidopsis* genome (Table 1). The observed variation in gene number across species is likely driven by factors such as genome size, chromosome number, duplication events, and evolutionary diversification^{8,29}. All the selected genes are distributed across the five *Arabidopsis* chromosomes, predominantly toward the telomeric regions (Fig. 1), except for *AtSRS3*, *AtSRS4*, and *AtSRS8*. Similar telomeric distributions of the *SRS* gene family were observed in *T. aestivum*²⁸, *Chenopodium quinoa*³², *S. lycopersicum*⁸, *Sesamum indicum*³⁵, *Melilotus albus*¹⁸, *G. max*^{16,33}. The predicted nuclear localization of all *AtSRS* genes (Table 1) indicates that they may function as transcription factors by interacting with specific DNA target sequences to regulate gene expression⁸.

4.2. Gene Structure, Motif and Protein Domains

Each of the ten *AtSRS* genes has one intron (Fig. 3). Furthermore, as illustrated in Fig. 3, *AtSRS* genes from the same group had similar alignment, conserved domains, motifs, and exon-intron architectures, showing a significant evolutionary link among these genes. Analysis of sequence alignment demonstrated strong conservation of the RING-like zinc finger and IGGH domains among *AtSRS* proteins, as well as motif analysis, reported in earlier works^{5,8,9,16–18,28,29,31,34}. So, it can be assumed that both domains were conserved during the evolution of the *AtSRS* gene family.

4.3. Evolutionary Relationship

Variations in *SRS* gene sequences among species are likely driven by evolutionary forces such as natural selection, mutation, and diversification. To investigate gene relationships, synteny

analysis using whole-genome datasets from multiple species was conducted to identify orthologous and paralogous gene pairs.^{60,61} Gene duplication serves as a source of extra genetic material, which can be modified through selection, drift, and mutation to generate specialized or unique functions⁶². The diversity observed among *SRS* genes implies an evolutionary origin from common ancestors (Fig. 5), with gene duplication playing a key role in increasing gene abundance and promoting functional differentiation among *AtSRS* members. Comparative phylogenetic analysis of homologous genes across diverse genomes enables a comprehensive understanding of the evolutionary patterns and functional divergence within a gene family⁶³.

Comparative phylogenetic analysis indicated a significant evolutionary relationship between *Arabidopsis thaliana* and *Brassica napus* (Canola), highlighting the close relatedness of *AtSRS* and *BnSRS* genes. *AtSTY1* was discovered to be completely grouped with canola homologs (*BnSRS30*), indicating that these proteins may have comparable functions in canola and *Arabidopsis*⁶⁴.

4.4. Analysis of *Cis*-Regulating Elements

A variety of functionally relevant *cis*-acting elements were detected in the promoter regions. These components included those pertaining to meristem and endosperm expression, light responses, hormonal response (including auxin, gibberellin, salicylic acid, MeJA etc.), and stress (low temperature, heat, cold, drought, salinity etc.). Overall, the data imply that the *AtSRS* gene family contributes to the regulation of hormone levels, thereby affecting the growth and developmental processes of *Arabidopsis thaliana*^{2,4} apart from contributing to plant responses under various abiotic stress conditions^{8,16–18,28,31,34,44}.

4.5. Expression Profiles of *AtSRS* Genes in *Arabidopsis* Organs and Developmental Stages

The expression patterns indicate that nearly all *AtSRS* genes exhibit low to high expression levels across the various tissues analyzed (Fig. 10, Supplementary File 3). These findings indicate that specific *AtSRS* genes may contribute to the development and functioning of diverse organs, including leaves, flowers, and fruits^{2,4,5,9}. *SRS* genes have been reported to function predominantly in gynoecium development, seedling growth, and the morphogenesis of floral organs and siliques^{5,9}. In our study, we found that *AtSTY2* and *AtSTY1* become highly expressed in carpels of young flowers, flowers, ovules, and stigmatic tissue, consistent with previous reports^{5,65}. Almost similar functions have also been reported for *AtLRP1* and *AtSH1*⁵. These genes further contribute to carpel fusion and the differentiation of all marginal structures in the apical gynoecium, such as the style, stigma, internal septum, placenta, and ovules.

According to phylogenetic analysis, *AtLRP1* forms homologues with *AtSRS6*, suggesting that they may play a similar role in *Arabidopsis*⁵. Previous studies showed that *AtSRS5* is involved in leaf and style development, with *AtSRS5*:GUS expression detected in young rosette leaves, hydathodes, and cells near lateral root initiation sites, supporting a functional role similar to what we observed. *AtSRS4* forms homologues with *AtSTY2*, suggesting close functioning and phenotypic functioning in gynoecium development^{5,65}. *AtSRS3* had been reported as inactive in leaves and style development; it did not form a homologue with any other *SRS* genes, implying a different action. In our study, we found *AtSRS3* was highly expressed in petals of mature flowers, anthers of young flowers, and stigmatic tissue. Based on phylogenetic relationships, *AtSRS7* forms homologues with *AtSRS5*, suggesting similar activities. Finally, *AtSRS8* was reported as a pseudogene that forms homologs with *AtSTY1*, indicating comparable functioning in *Arabidopsis* tissue^{5,9,65}.

4.6. *AtSRS* Gene Expression under Phytohormone Treatments

In this study, we could examine the responses of seven *AtSRS* genes against six plant hormones. Considering abscisic acid (ABA) treatment, *AtSTY2* showed the strongest positive responses followed by *AtLRP1* which indicates their involvement in ABA-mediated stress signaling. From promoter analysis we found they have *cis* elements related to ABA expression. In case of methyl jasmonate *AtSRS5* showed prompt response likewise *AtLRP1* and *AtSRS4* showed greater expression for subsequent period, suggesting their involvement in the jasmonate-mediated defense response. Considering gibberellic acid *AtSTY2* expressed fast and greater response than others while *AtSHI* constantly suppressed the expression consistent with previous research^{2,4}. Regarding zeatin, *AtSRS5* showed a prompt response and *AtSRS4* expressed later while *AtSTY2* maintained a balance of expression indicates their potential role in cytokinin response. Assessing ACC (1-Aminocyclopropane-1-carboxylic acid) response, *AtSRS4*, *AtLRP1*, and *AtSTY2* demonstrated higher expression, implying their potential role in ethylene response. Finally, evaluating IAA response, *AtLRP1* and *AtSTY2* displayed subsequent increase over time except at 3h for *AtSTY2*. *AtSTY2* experienced a duplication event with *AtSTY1*, promoting normal style development and influencing apical–basal patterning of the gynoecium by regulating auxin homeostasis, highlighting their role in auxin-mediated responses^{9,65–67}.

4.7. Expression Profiles of *AtSRS* Genes in Response to Stresses

Considering cold root and cold shoot, *AtSHI* exhibited the highest expression may be for consisting of several types of *cis* elements for abiotic stresses indicates their potential role

against these stresses. In case of osmotic shoot *AtSRS5* showed greater expression and *AtSRS8* in osmotic root implying their significance towards these stresses. Under salt, drought, genotoxic, oxidative and UV-B stress, *AtSRS6* demonstrated as a significant expressive gene in shoots and roots along with *AtSRS8* denoting their possible action against these stresses as they both have more *cis*-regulatory elements against abiotic stresses. Regarding wound shoot *AtSHI* mostly expressed following *AtSRS5* and *AtSRS6* displayed greater expression than others possibly implying their active consistence of WUN motif and WRE3, respectively. Considering heat shoot *AtSRS5* and *AtSRS6* expressed higher and *AtSRS6* and *ATSR8* were highly expressed in heat root, implying their potential functioning against heat stress.

5. CONCLUSIONS

Ten *SRS* genes are found in the *Arabidopsis* genome, spread across all chromosomes. The genes in this species are highly conserved, according to genomic structure, domain, motif distribution, and sequence alignment. Gene duplication and synteny research showed that genes are formed by genome duplication and during evolution. Certain *AtSRS* genes may be involved in floral development, lateral root initiation, hormone modulation, and stress response, according to expression data and earlier research. The results open the door to any further functional genomic research that aims to clarify the precise roles of the *SRS* genes in *Arabidopsis*, which may subsequently be utilized to improve this model plant using biotechnological and breeding methods. Further wet lab validation will be necessary to confirm the functional roles of specific genes in *Arabidopsis*.

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Author Contributions

MMHM: Conceptualization, Methodology, Formal analysis, Visualization, Writing - Original Draft; **TJ**: Formal analysis, Visualization, Writing - Original Draft; **YI**: Writing - Original Draft, Writing - Review & Editing; **MH**: Writing - Review & Editing; **MJHJ**: Conceptualization, Methodology, Supervision, Writing - Review & Editing

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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