agronomy

MDPI

Article

Characterization of Strubbelig-Receptor Family (SRF) Related to Drought and Heat Stress Tolerance in Upland Cotton (*Gossypium hirsutum* L.)

Furqan Ahmad [1], Shoaib Ur Rehman [1], Muhammad Habib Ur Rahman [1], Saghir Ahmad [2] and Zulqurnain Khan [1,*]

[1] Sino-Pak Joint Research Laboratory, Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan 60000, Punjab, Pakistan; furqan.ahmad@mnsuam.edu.pk (F.A.); shoaib.rehman@mnsuam.edu.pk (S.U.R.); habib.rahman@mnsuam.edu.pk (M.H.U.R.)
[2] Cotton Research Institute, Multan 60000, Punjab, Pakistan; saghirahmad_1@yahoo.com
* Correspondence: zulqurnain.khan@mnsuam.edu.pk; Tel.: +92-302-5880755

Abstract: Cotton is one of the world's leading fiber crops, but climate change, drought, heat, and salinity have significantly decreased its production, consequently affecting the textile industries globally. To acclimate to these environmental challenges, a number of gene families involved in various molecular, physiological, and hormonal mechanisms play crucial roles in improving plants response to various abiotic stresses. One such gene family is the GhSRF, a Strubbelig-Receptor family (SRF), and member of the leucine-rich repeat (LRR-V) group. This family encodes leucine-rich repeat transmembrane receptor-like kinases (LRR-RLKs) and have not yet been explored in cotton. *Arabidopsis thaliana* Strubbelig-Receptor gene sequences were used as queries to identify the homologs in cotton, with subsequent support from the literature and functional prediction through online data. In the current study, a comprehensive genome-wide analysis of cotton was conducted, identifying 22 SRF putative proteins encoded by 22 genes. We performed the detailed analysis of these proteins, including phylogeny, motif and gene structure characterization, promoter analysis, gene mapping on chromosomes, gene duplication events, and chromosomal sub-cellular localization. Expression analysis of putative genes was performed under drought and heat stress conditions using publicly available RNAseq data. The qRT-PCR results showed elevated expression of *GhSRF*2, *GhSRF*3, *GhSRF*4, *GhSRF*10, and *GhSRF*22 under drought and heat stress. So, it could be speculated that these genes may play a role in drought and heat tolerance in cotton. These findings could be helpful in cotton breeding programs for the development of climate-resilient cultivars.

Keywords: phylogenetic tree; Strubbelig-Receptor (SRF) gene family; abiotic stress; transmembrane signaling; functional divergence

check for updates

Citation: Ahmad, F.; Rehman, S.U.; Rahman, M.H.U.; Ahmad, S.; Khan, Z. Characterization of Strubbelig-Receptor Family (SRF) Related to Drought and Heat Stress Tolerance in Upland Cotton (*Gossypium hirsutum* L.). *Agronomy* **2024**, *14*, 1933. https://doi.org/10.3390/agronomy14091933

Academic Editor: Fangbin Cao

Received: 10 May 2024
Revised: 5 August 2024
Accepted: 15 August 2024
Published: 28 August 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Upland cotton belongs to the *Gossypium* genus, which is divided into two groups: "Old World cotton" and "New World cotton". The Old World cotton group includes two diploid cultivated species, *G. arboreum* and *G. herbaceum*. The New World cotton group includes tetraploid cultivated species such as *G. hirsutum* (AD1) and *G. barbadense* (AD2). Other tetraploid species, which are wild types, contain *G. tomentosum* (AD3), *G. mustelinum* (AD4), *G. darwinii* (AD5), and *G. ekmanianum* (AD6). Diploid species are further classified into eight genomes based on geographical distribution [1]. Climate change is the biggest challenge of the 21st century and significantly hampering agricultural yields [2,3]. The unprecedented and abrupt patterns of climatic changes have severely impacted the sustainability of major field crops [4]. Abiotic stresses, intensified by the changing climate, impose severe adverse effects on the natural environment of plant species [5]. The high temperature, one of the key abiotic factors, evidently reduced the production of major crops worldwide due to increases

ranging from 2.6 to 4 °C [6,7]. Heat and drought stresses have significantly influenced the seed cotton, whether these factors occurred separately, alternately, or simultaneously [8]. Heat stress accompanied by the droughts may reduce crop yields up to 60% [9].

Plant species research differs considerably in the 21st century as compared with the 20th century. Genome-based studies had been revolutionized after the whole-genome sequencing of *Arabidopsis thaliana* [10]. Utilizing genome-wide predictions has provided a comprehensive understanding of gene functions across various biological processes [11]. For sustainable cotton production, advanced fast-track breeding tools integrated with genomic selections are indispensable in changing climate [12]. The response of the cotton genome to abiotic stresses is highly complex and genome-wide analyses had assured the identification of multiple genes in cotton [13,14]. To cope with the adverse conditions and to survive in changing climate, genome-wide characterization is crucial, ultimately indicating the role of different genes [15].

The regulation of many aspects of plant life cycle is carried out through receptor-like kinases (RLKs), a distinct group of surface receptors [16]. The Strubbelig-Receptor family (SRF) belongs to the leucine-rich repeat (LRR-V) group, encoding leucine-rich repeat transmembrane receptor-like kinases (*LRR-RLKs*). These kinases play crucial roles in plant development, including the formation of different plant parts such as carpels, petals, ovules, and root hairs [17–19]. According to a study, the role of the *SRF4* gene was reported as a positive regulator of leaf size [16]. In plants, apical meristems and organ development patterns were influenced by intercellular signaling. In *Arabidopsis*, the role of leucine-rich repeat receptor-like Strubbelig proteins had been shown to regulate floral shape, ovule integument morphogenesis, cell division, and root hair patterning, indicating their presence as a typical kinase with Strubbelig members [20,21]. Variations in the *Strubbelig Receptor Kinase 3* (*SRF3*) gene had resulted incompatibilities among various accessions of *Arabidopsis thaliana* due to different immune responses [22]. Silencing of the *SCRAMBLED/STRUBBELIG (SCM/SUB)* gene resulted in decreased cell proliferation, compromised cell expansion, and clear changes in auxin distribution patterns [23]. Mutation in the sub-2 allele of the *Scrambled/Strubbelig* (*SCM/SUB*) *Receptor-like Kinase* gene resulted in slow development of leaf blades with irregular shape [24]. A study on root architecture traits resulted in *Strubbelig-Receptor Family3* (*SRF3/SUB*) as a candidate gene in canola [25]. This *SUB* gene was involved in signal transduction, ultimately regulating the synthesis of the cellulose, which is a central carbohydrate component of the cell wall. The signaling transduction of the *SUB* gene is further involved in the production of reactive oxygen species (ROS), which further influences stress-related gene activation mechanisms [26].

The SRF family has been studied in model plants and a few studies are reported in other plant species. As members of the leucine-rich repeat (LRR) class involved in membrane signal transduction, this family has been relatively underexplored for its role in conferring abiotic stress tolerance in plants. Therefore, we characterized this family for drought and heat tolerance in upland cotton.

2. Materials and Methods

2.1. Sequence Retrieval of SRF Protein Family Members

SRF genes were downloaded from cotton database CottonFGD [27] and Phytozome13 [28] using the *AtSRF* (*AT1G11130*, *AT1G53730*, *AT1G78980*, *AT2G20850*, *AT3G13065*, *AT3G14350*, *AT4G03390*, *AT4G22130*, *AT5G06820*) query sequences. A Hidden Markov Model (HMM) confirmed the mined *GhSRF* proteins in *Gossypium hirsutum* to avoid the omission of useful genes. The targeted cotton species included *G. arboreum*, *G. raimondii*, *G. tomentosum*, *G. barbadense*, *G. mustelinum*, *G. hirsutum*, and *G. darwinii*. *Arabidopsis thaliana* was selected as a model organism. *Theobroma cacao* species was selected as close relatives of upland cotton. *Oryza sativa* was selected from monocots to compare the evolutionary process of the *SRF* gene family. Organisms like *Selaginella moellendorffii*, *Chlamydomonas reinhardtii*, and *Physcomitrium patens* were selected as primitive organisms to compare the evolution of this gene family in cotton and other primitive and model organisms of diverse groups and further verified using SMART [29] and Inter-

ProScan5 [30]. The *GhSRF* genes were further examined thoroughly for biophysical properties using CottonFGD, EnsemblPlants [31], and ExPASy [32]. These properties were molecular protein length (A.A), protein molecular weight (KDa), charge, isoelectric point (pI), and grand average of hydropathy. Sub-cellular localization of the SRF gene was investigated using Cello V.2.0 [33].

2.2. Construction of Phylostratum, and Phylogeny Tree

The NCBI database (https://www.ncbi.nlm.nih.gov/ (accessed on 25 March 2024)) was used to create the phylip file and visualized in iTOL [34] to generate the phyla-stratum tree of all studied species. The ClustalW program in MEGA V.11 [35] was used for multiple sequence alignment (MSA) of the retrieved sequences from all targeted species. The phylogenetic tree using the neighbor-joining method with bootstrap values of 1000 replications was performed.

2.3. Sequence Logos and Conserved Amino Acid Analysis

Protein sequences from cotton species including *G. hirsutum*, *G. arboruem*, and *G. raimondii* were used to develop sequence logos using online WebLogo tool [36] and further visualized in TBtools [37]. The same region of all three species was used to perform this analysis.

2.4. Promoter, Protein Motifs, and Gene Structure Analyses

To investigate the *cis*-element from the promoter region of *GhSRF* genes, the 2000 bp upstream region of each gene was utilized. PlantCARE [38] was used to conduct promoter analysis, which was visualized in TBtools. Motifs present among the proteins were explored using MEME Suite [39]. All the default parameters were ensured except a few, like (i) occurrence of motif value of 1 for each sequence, (ii) residue range selected as 12–65 s in the motif width, (iii) motif number of 10, (iv) minimum sites in motif of 5. After setting default parameters, the MEME.xml or mast.xml file was retrieved. The further processing of these files to draw a motif structure was performed in TBtools. Gene structure display analysis was performed using Gene Structure Display Server 2.0 [40].

2.5. Sub-Cellular Localization, Gene Mapping on Chromosomes, Gene Duplication, Collinearity and Ratios of Ka/Ks

Sub-cellular localization was predicted using CELLO [41] and WOLFPSORT [42] and a heatmap of the studied genes was created with TBtools. MapChart v2.32 [43] was utilized to find the position of different genes on all the concerned chromosomes of the cotton. The codon alignment and duplication of genes was ensured using the CLUSTAL X 2.0 [44] PAL2NAL program [45]. The CODMEL program and PAML package [46] were the best-suited options used to evaluate the non-synonym (*Ka*) and synonym (*Ks*) values, as an indicator of duplication events. TBtools was used for the visualization of the results on the provided values of *Ka*/*Ks*. The circus-based tool "Circoletto" [47] was used to draw the sequence similarity and duplications in the *Arabidopsis* and upland cotton species. The peptide FASTA sequences of *G. hirsutum* were used as query sequences against peptide sequences of other cotton species. The similarity was provided in the form of a circle on the basis of bit-score values.

2.6. In Silico Expression Analysis of GhSRF Genes

RNA-seq data of *GhSRF* under drought and high temperature were retrieved from CottonFGD under the project available at the NCBI Geodataset (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA490626 accessed on 4 April 2024). The *G. hirsutum* line named Texas Marker 1 (TM-1) was used as experimental material. The expression of all genes under drought and heat stress conditions was presented through heat maps.

2.7. Expression Analysis and Validation of Candidate Genes

To perform the gene expression studies, the most tolerant line (VH-402) and most susceptible line (CIM-443) from previous investigations were used along with a standard line TM-1 (Texas Marker-1). The qRT-PCR was performed to investigate the relative expression patterns of selected candidate *GhSRF*s under drought and high-temperature conditions. Initially, the twenty-day-old seedlings of cotton genotypes were maintained under normal conditions with a temperature range of 30 °C during the day and 24 °C during the night, light intensity of 400–600 µmol m^{-2} s^{-1}, and 16/8 h light/dark in a growth chamber. The humidity was kept to 50–60% under normal conditions. For drought stress, (PEG-17% mass fraction) was used to induce the drought effects on twenty-day-old cotton seedlings. For heat stress induction, seedlings were placed in another growth chamber with a day temperature of 45 °C, night temperature of 35 °C, and humidity of 60 to 70%, with the same light intensity of 400–600 µmol m^{-12} s^{-1} and the same photoperiods as the normal conditions. The experiment, designed as a completely randomized design with three replications, was maintained under normal and stress conditions for 72 h, after which leaf samples were collected for RNA extraction. The complementary DNA (cDNA) was synthesized from extracted RNA and *Ghactin7* (NCBI-AY305729) was used as internal reference during the expression studies, and the experiment was independently repeated three times. The $2^{-\Delta\Delta CT}$ formula was used to determine the relative gene expression [48]. The primers used in this study are presented in (Supplementary Table S1).

3. Results

3.1. Identification and Physiochemical Properties of SRF Genes in Cotton

Thirteen (13) *SRF* family genes from *G. arboreum*, eleven from *G. raimondii*, twenty-two from *G. hirsutum*, twenty-five from *G. barbadense*, twelve from *G. tomentosum*, fifteen from *G. mustelinum*, sixteen from *G. darwinii*, eight from *A. thaliana*. Fifteen from *O. sativa*, eight from *T. cacao*, four from *S. moellendorffii*, one from *C. reinhardtii,* and six from *P. patents gene* were retrieved (Supplementary Table S2). Biophysical properties like coordinates, CDS length, strand, protein length (aa), molecular weight (kDa), charge, isoelectric point, and grand average of hydropathy of the studied *GhSRF* members from the *G. hirsutum* L. are presented in Supplementary Table S3.

3.2. Sequence Alignment, Phylostratum, and Phylogenetic Tree

The phylostratum analysis revealed the evolutionary relationships among the targeted species at the whole-genome level (Figure 1A). The phylogenetic analysis identified the origins of the SRF genes and provided knowledge of their emergence and diversification. The phylogenetic analysis further elucidated the evolutionary history of SRF proteins in cotton, highlighting their relationships with SRF gene members in other organisms (Figure 1B). A total of 156 *SRF* family genes from different organisms were used to construct the phylogenetic tree, which divided *SRF* members into five major groups viz. a, b, c, d, and e. The group (SRF-d) was the largest group, with 39 (39 proteins) members, and all belonged to the *Gossypium* genus, along with some members from *A. thaliana*, *O. sativa,* and *T. cacao,* indicating a closer evolutionary relationship and shared ancestral traits or recent common ancestry. The group (SRF-d) was followed by the group (SFR-c), exhibiting the same patterns for genes and their evolution. The groups (SRF-a and SRF-e) included 26 (26 proteins) members each, and most of the members were from the *Gossypium* species. SRF group a, c, d, and e contained most of the members from the *Gossypium* genus, including *G. arboreum*, *G. raimondii*, *G. hirsutum*, *G. barbadense*, *G. mustelinum*, *G. darwinii*, *G. tomentosum*, and only a few members from *A. thaliana*, *O. sativa*, and *T. cacao.* The group (SRF-b) represented the members from all cotton species, model plants, monocots, and most primitive organisms. The presence of *SRF* members in *Gossypium* species and primitive organisms (SRF-b) indicated the conserved behavior of SRF genes during the evolutionary process. The phylogenetic analysis indicated the evolutionary linkage of *SRF* genes in different primitive and higher organisms.

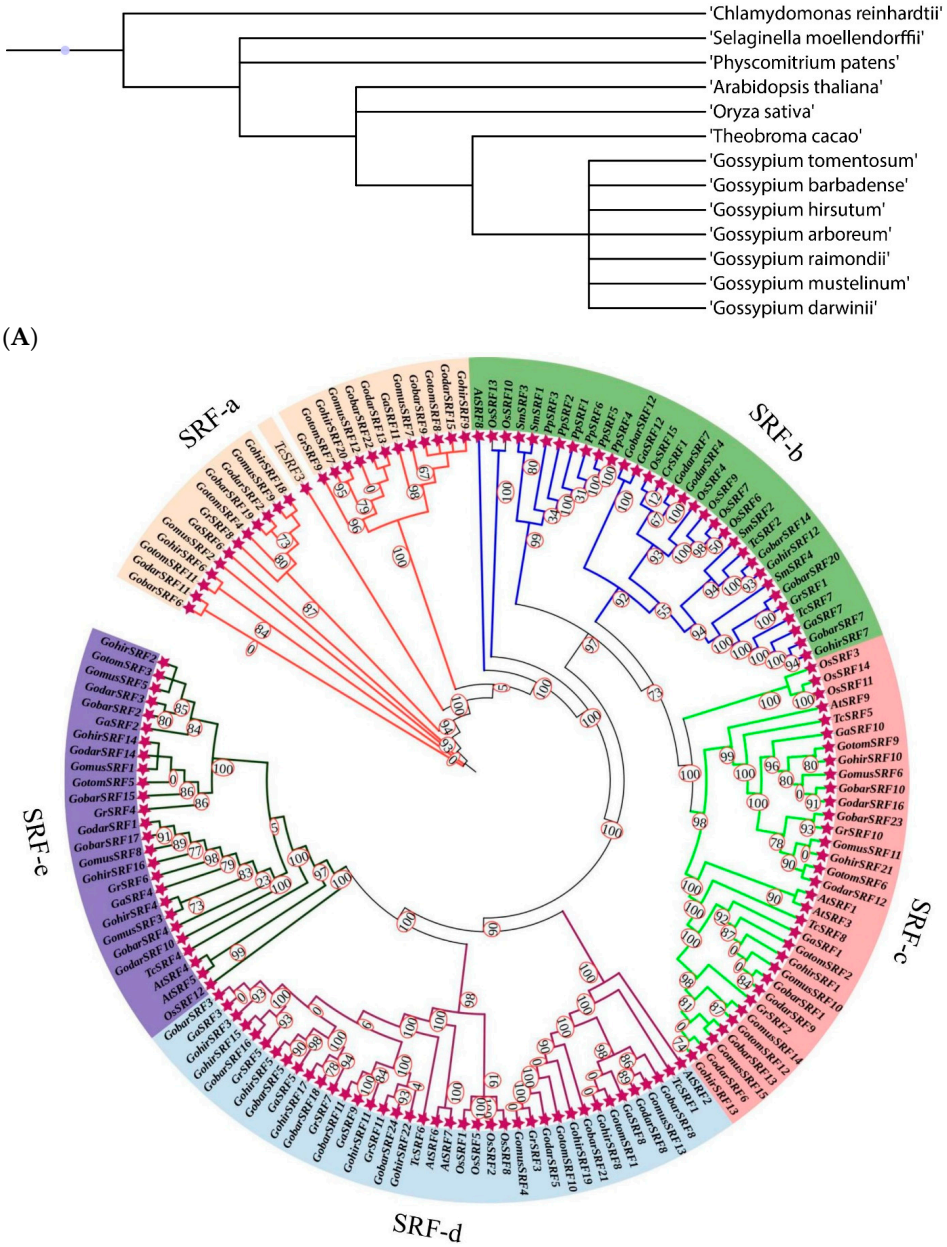


Figure 1. Phylogenetic analysis of *SRF* family genes from 13 plant species. (**A**) Phylostratum analysis of *SRF* gene family for lineage study. (**B**) A phylogenetic tree exhibiting an evolutionary relationship of *SRF* family genes in eudicots, monocots, and primitive plant species. The analysis was performed using 156 SRF family genes and five groups (SRF-a, SRF-b, SRF-c, SRF-d, and SRF-e) represented by different colors. The bootstrap values are added near each branch node.

3.3. Finding of Conserved Amino Acid Patterns

Sequence logos were generated to analyze the conserved patterns of *SRF* family proteins in *G. hirsutum (AADD genome)*, *G. arboreum* (*AA*), and *G. raimondii (DD)*. Sequence logos indicated that the SRF family is conserved among these species across the N and C termini, such as N (1), F (3), G (5), P (8), L (17), L (22), N (25), L (27), D (33), F (34), L (36), L (39), D (43), L (44), S (45), N (47), L (53), P (54), S (56), and F (57) (Figure 2). The patterns of C and N termini served as indicators to demonstrate the pattern of *SRF* protein sequence conservation in other organisms.

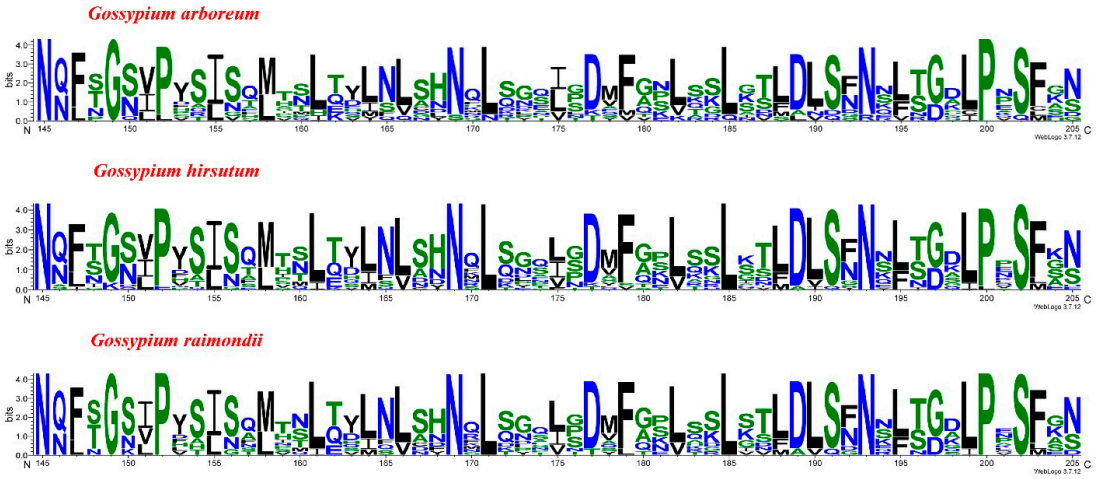


Figure 2. Sequence logos of SRF family genes in *G. hirsutum*, *G. arboreum*, and *G. raimondii*. Amino acid, in the form of alphabets in two diploid cotton species (*G. arboreum* and *G. raimondii*) of sub-genomes A and D, with one tetraploid cotton species (*G. hirsutum*) of AD genome presented. Each upper-case letter represents the amino acids being conserved at that specific location.

3.4. Analysis of Cis-Element in Promoter, Motif Finding and Gene Structure

GhSRF contained different *cis*-elements, such as MeJARE, ABRE, AUX, AUX-R, MYB-DI, GARE, SARE, WUN, BEJARE, and DSRE (Figure 3 and Supplementary Table S4). These elements are important for plant growth, development, and response to biotic and abiotic stresses. Moreover, similar patterns of motif were found in all members of this gene family among all studied genes of the *SRF* family indicating that this gene family is conserved in cotton (Figure 4 and Supplementary Table S5).

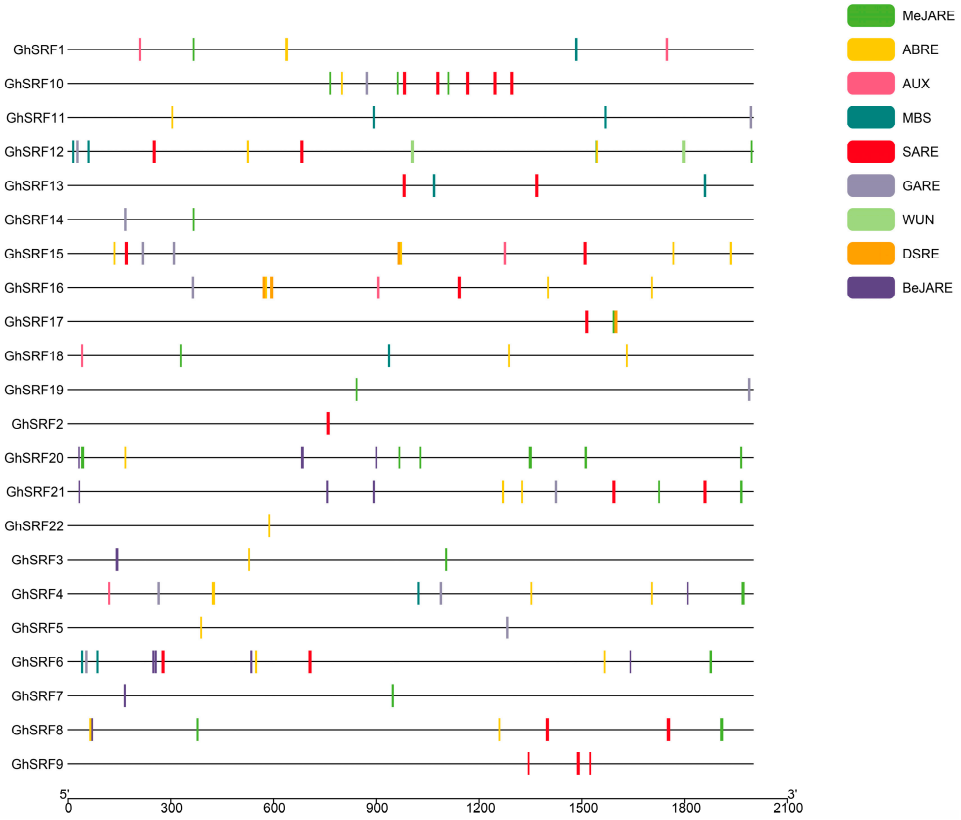


Figure 3. Cis-element analysis in the promoter region of *GhSRF* genes. The cis-elements in each gene are indicated by different colors and rectangle shapes.

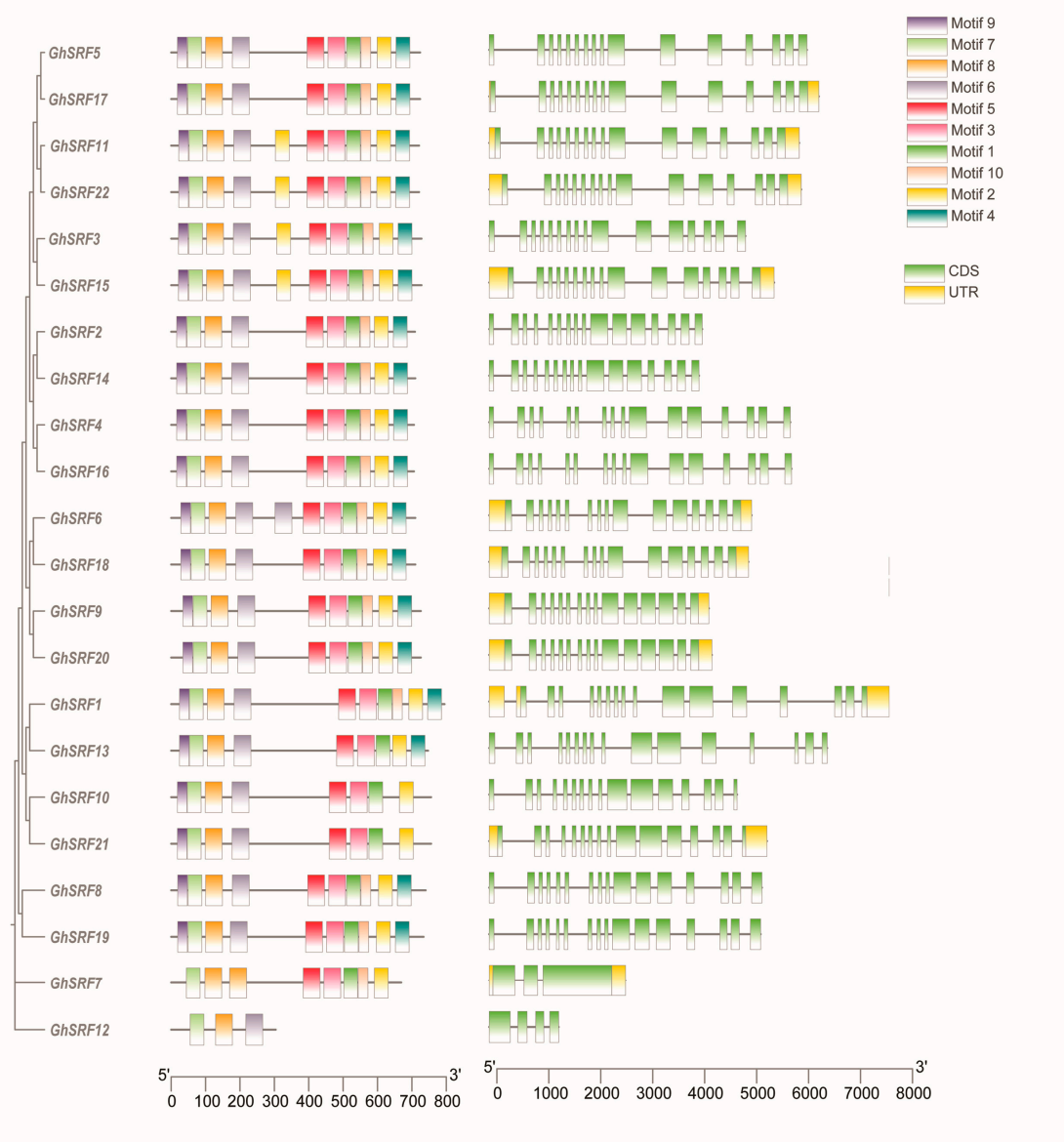


Figure 4. Phylogeny and motifs in *GhSRF* genes. A total of ten (10) motifs are presented from each *GhSRF* gene and distribution of these motifs is presented in different colors. Gene structure of SRF family genes in *G. hirsutum*. Exon/intron structure display of *GhSRF* family genes; the upstream region was indicated by yellow and CDS is presented in green.

To demonstrate the event of the evolutionary development among all *GhSRF* genes, we constructed the gene structures. Out of 22 *GhSRF* genes, 11 members contained only exons. Among all, *GhSRF12* was the smallest gene, with only four exons, and *GhSRF5* was the largest, with sixteen exons but without any intron (Figure 4).

3.5. Gene Mapping on Chromosomes, Ratio of Ka/Ks, Gene Duplication, and Sub-Cellular Localization

The gene locus linkage confirmed the paralogous gene pairs in *G. hirsutum* among *GhSRF* genes (Figure 5). The *GhSRF* genes were present in five color groups on different chromosomes. The *SRF* genes like *GhSRF1*, *GhSRF10*, *GhSRF13,* and *GhSRF19* belonged to Group-I and are present on chromosomes A-3, A-10, D-2, and D-10. Group-II enclosed the *SRF* genes, including *GhSRF6*, *GhSRF9*, *GhSRF18,* and *GhSRF20,* which occupied positions on chromosomes A-6, A-10, D-6, and D-10. Similarly, Group-III involved the *SRF* genes like *GhSRF2*, *GhSRF4*, *GhSRF14,* and *GhSRF16,* situated on chromosomes A-5, A-6, D-5, and D-6.

Group-IV was the largest group with five members, including *GhSRF3*, *GhSRF5*, *GhSRF11*, *GhSRF15,* and *GhSRF22* located at A-5, A-6, A-10, D-5, and D-6. The last group, Group-V, also had four *SRF* genes entitled *GhSRF7*, *GhSRF8*, *GhSRF12,* and *GhSRF19,* positioned at chromosomes A-9, A-9, D-2, and D-9. Among all twenty-two genes, most of these were present on the extreme terminal position and some were present near to the terminal position on both ends and only were present at the central position of the chromosomes. Most of the genes were localized on the terminals of the chromosome, with chromosome A05 and D05 having the maximum number of genes. Chromosomes A-6 and A-8 contained the largest length but the lowest number of genes on them. A set of 10 gene pairs in *G. hirsutum* were predicted through the analysis, and all experienced segmental duplication events. To further estimate the magnitude and type of selection as per Darwinian theory, we generated *Ka*/*Ks* ratios through the events of duplication in genes. It was observed that all 10 duplicated gene pairs showed *Ka*/*Ks* values < 1, indicating that the patterns of negative selection ultimately led to purifying selection (Supplementary Table S6). The peptide similarity analysis was performed in Circoletto by providing the peptide sequence of *G. hirsutum* as query; all other cotton species sequences were set as targets to see the duplications. The sequences presented in red showed more than 75 percent similarity with each other. The single blue color of *GhSRF12* in *G. hirsutum* and *G. barbadense* confirmed its singular presence with no duplication, as predicted by *Ka* and *Ks* ratios (Figure 6A). The sub-cellular location results indicated that most of the *SRF* genes are present in the plasma membrane (Figure 6B and Supplementary Table S7).

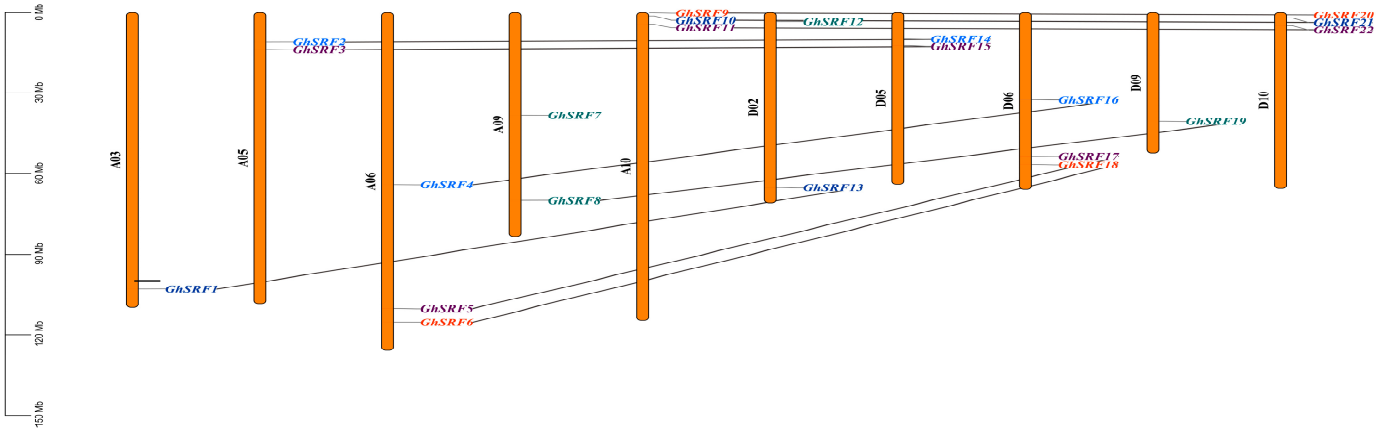


Figure 5. *GhSRF* genes distributed on different chromosomes of *G. hirsutum,* respectively.

3.6. Gene Expression Analysis of GhSRF Genes Under Drought and Heat Stress Conditions

The FPKM values of cotton accession (*TM-1)* for *GhSRF* genes were retrieved from the CottonFGD database and expression graphs of cotton *SRF* genes were developed under abiotic stress conditions of drought and high-temperature stress (Figure 7 and Supplementary Table S8). Extracted data showed that *GhSRF3*, *GhSRF4*, *GhSRF10*, and *GhSRF22* were highly expressed under drought stress conditions. The expression of *GhSRF2*, *GhSRF3*, *GhSRF4*, *GhSRF10,* and *GhSRF22* was high under high-temperature conditions. In general, *GhSRF2*, *GhSRF3*, *GhSRF4,* and *GhSRF22* were highly expressed in different conditions of drought and heat. Based on FPKM expressions, highly expressed genes under abiotic stress conditions were selected to confirm the results through qRT-PCR. Relative gene expression results showed that *GhSRF2*, *GhSRF4,* and *GhSRF10* have more transcript abundance of these genes under drought conditions, while *GhSRF3* and *GHSRF22* showed better transcript production under heat conditions (Figure 8). Further, the production of more transcripts in cotton accession VH-402 as compared to CIM-443 confirmed the higher tolerance in former accession under drought and heat conditions as compared to the latter.

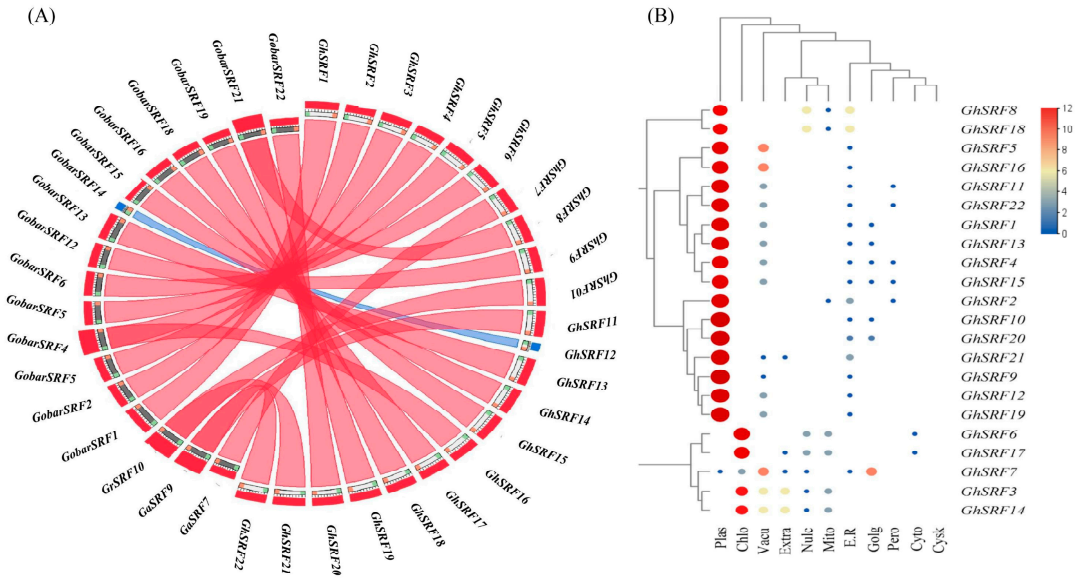


Figure 6. (**A**) Peptide similarity analysis among SRF genes of *G. hirsutum* with SRF genes of *G. arboruem*, *G. raimondii*, and *G. barbadense*. (**B**) Sub-cellular localization of *GhSRF* genes within the cell. The color and size of solid circles indicate the higher presence levels of *GhSRF* genes in different sub-cellular compartments.

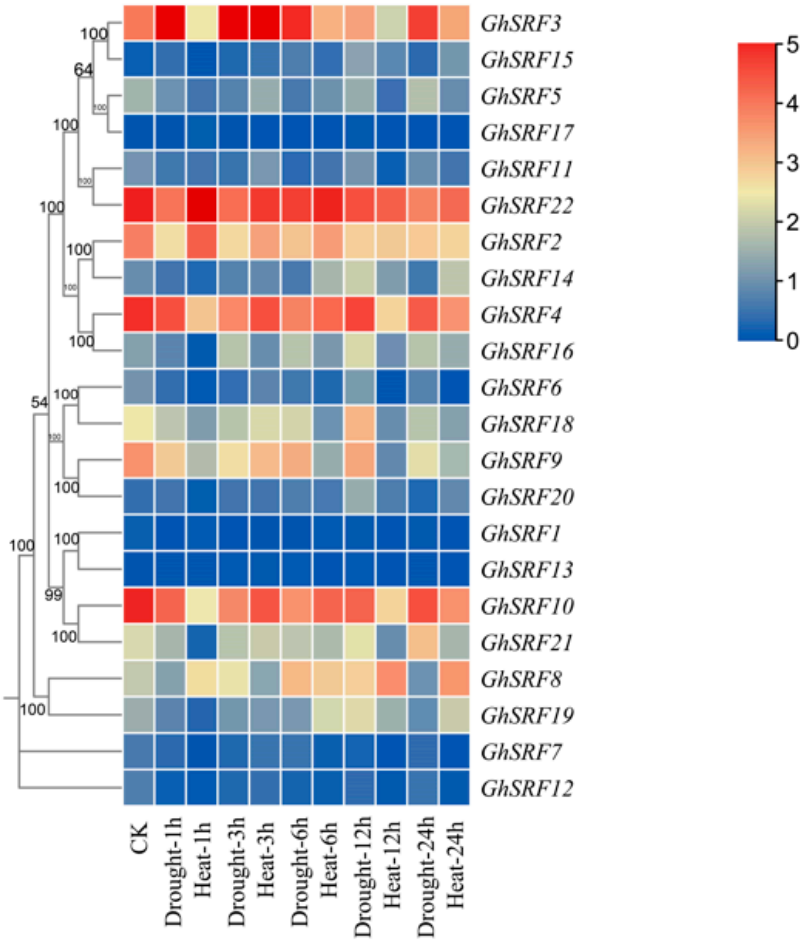


Figure 7. Expression analysis of *GhSRF* genes based on FPKM values obtained from CottonFGD for drought and heat conditions. The color scale on the right side indicates the expression level from minimum (blue) to maximum (red).

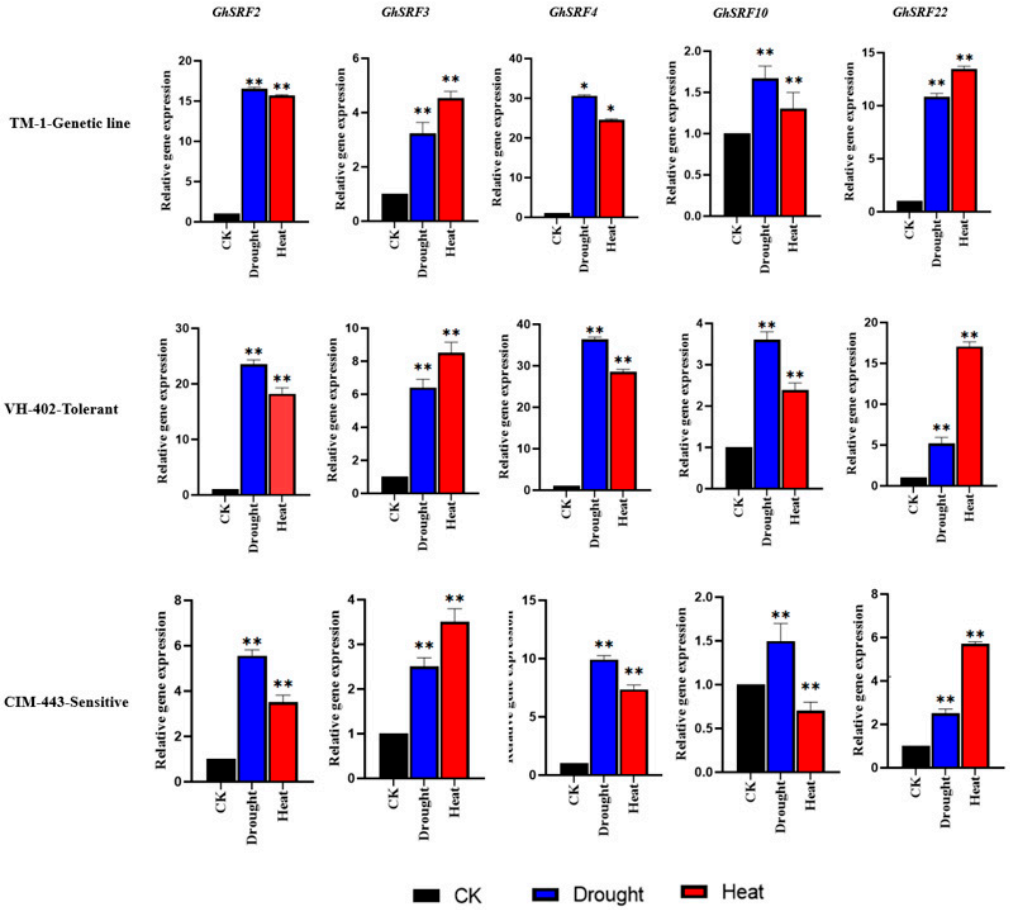


Figure 8. qRT-PCR expression of *GhSRF2*, *GhSRF3*, *GhSRF4*, *GhSRF10,* and *GhSRF22* under drought and heat stress conditions. Three cotton lines, including one Chinese line (TM-1) and two local Pakistani tolerant (VH-402) and susceptible (CIM-443) genotypes, were used in this analysis. The CK indicates the control condition and others are drought and heat in blue and red. The CK (control) was normalized and kept constant at 1 to check the folds of expression in all three lines under drought and heat conditions. Error bars indicate standard deviations among three independent replications. * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Genome-wide approaches has provided comprehensive understanding of the roles of gene families in various biological processes [11]. Genome-wide identification has facilitated the rapid identification of hundreds of novel genes in cotton research, accelerating the gene detection process [13,14]. The advancement in sequencing technologies, particularly in plant species such as "*Gossypium*" species, made the gene identification process much easier as compared to earlier times [49]. Members of the SRF protein family belong to the leucine-rich repeat (LRR-V) group, contributing in adaptation to environmental stresses. Several investigations have explored the role of *SRF* genes in various plant species [16,18–22]. In the current work, *SRF* genes in cotton (*G. hirsutum*) were explored for taxonomical classification, sequence similarities and duplication, gene mapping on chromosomes, motif, promoter, and expression analysis [50,51].

The *SRF* family is an expanded gene family across the plant kingdom due to their prints in multiple plant groups, including monocots (*O. sativa*), dicots (*A. thaliana* and *T. cacao*), tracheophytes (*S. moellendorffii*), embryophytes (*P. patens*), and chlorophytes (*C. reinhardtii*). In *G. hirsutum*, we identified 22 *GhSRF* genes, most of which present in pairs, except *GhSRF7* and *GhSRF*12, identified as singletons without any pairing. The diversity of these *SRF* genes inferred their involvement in environmental and physiological adaptation in *G. hirsutum*. Gene duplication can lead to functional diversification, where duplicated genes evolve new

functions (neofunctionalization) or subdivide the original function (subfunctionalization). Additionally, gene structure variation can result in alternative splicing, producing different protein isoforms from the same gene [52].

Phylogenetic analysis is usually performed to explore the lineage history of species [53]. We aligned the conserved peptide sequences of *SRF's* in multiples species ranging from primitive to higher organisms and identified the evolutionary connections of SRF genes among these species [54]. The phylogenetic analysis divided the *GhSRF* family members into five different groups. Group-b included the genes from all species including mosses, ferns, model organisms, monocots, and dicots, indicating the origin of SRF genes earlier than divergence of monocots and eudicots. Group b represented the most primitive members of *SRF* genes, widely diversified among other species. In contrast, Groups a, c, d, and e contained a smaller number of *GhSRF* genes from ancient plants as compared to other species. The presence of SRF genes in *C. reinhardtii* (chlorophyte) and *S. moellendorffii* (lycophyte) clearly indicated their lineage with the oldest known organisms. The phylostratum analyses suggested that *SRF* genes originated from early land plants, with potential orthologs of *SRF* genes existing throughout the kingdom plantae. Both *G. hirsutum* and *G. barbadense* relatively contained a higher number of SRF genes as compared to other studied plant species. Our results of sequence logos of cotton (*G. hirsutum*) with its progenitor species (*G. arboreum* and *G. raimondii*) displayed the conserved behavior of *SRF* genes on C and N terminals. Mutations during the evolution process of genes and gene families can lead to function-based divergence, with genes being added or deleted over time [55]. The gene length and structure varied due to duplication or deletion events, being observed across 22 *GhSRF* genes, depicting the role of variation in crop breeding [56,57]. Motif analysis provided the list of motifs present in each gene, highlighting the consistent patterns of gene functions within the same subfamily or family [58]. Chromosomal localization analysis is a fundamental approach to predict the position of genes on a chromosome. Further, it involves mapping identified genes to their respective chromosomes. It provides the crucial insight on duplication events, genome evolution, and complex regulation mechanisms [59].

The *Ka/Ks* ratios depicted the minor segmental duplication in *SRF* genes, indicating minor functional divergence. Generally, the results of the *Ka/Ks* ratio are interpreted in three ways: values of *Ka/Ks* is < 1 indicate purifying selection, *Ka/Ks* > 1 indicates positive selection, and *Ka/Ks* $= 1$ indicates neutral evolution [60]. *GhSRF* genes were found to be evenly distributed on both of the cotton genomes (A and D). Gene expression variation on different time scales provides essential understandings of gene functions and promoter diversity [61]. Cis-acting regulatory elements within the promoter are crucial for regulating the gene function under severe environments [62]. Various stress-related cis-regulatory elements were identified in *SRF* genes, including MeJARE, ABRE, MBS, AUX, SARE, GARE, WUN, BeJARE, and MYB-DI, with varying numbers across different SRF genes. Several studies have highlighted the significance of these regulatory elements in growth, development, and stress resilience [63]. The online expression was used to elucidate the role and transcriptional variation of these genes, which was further validated by qRT-PCR analysis. The candidate genes showed a clear difference in transcript abundance under different conditions. *GhSRF2*, *GhSRF3*, *GhSRF4*, *GhSRF10*, and *GhSRF22* were selected as *SRF* candidate genes with a potential role under drought and heat stress conditions. The *GhSRF2*, *GhSRF3,* and *GhSRF22* were expressed more under heat conditions, while *GhSRF4* and *GhSRF10* were expressed under drought conditions.

Future research should focus on knockout or overexpression studies of *GhSRF* genes to validate their roles in drought and heat tolerance mechanisms. Investigating interactions with other regulatory proteins and identifying downstream targets will enhance our understanding of their signaling pathways. Utilizing marker-assisted selection and gene editing technologies like CRISPR/Cas9 to develop cotton varieties with enhanced abiotic stress tolerance holds significant potential for improving yield and resilience. Field trials and breeding programs will be essential to evaluate the performance of these genetically modified plants under real-world conditions. This study lays a robust foundation for future

research on the Strubbelig gene family, paving the way for innovative strategies to enhance cotton's adaptability to environmental challenges.

5. Conclusions

In conclusion, this study has significantly advanced our understanding of the Strubbelig gene family (*GhSRF*) in cotton by elucidating their roles in drought and heat tolerance through comprehensive sequence and expression analyses. The identification of conserved motifs and regulatory elements, coupled with insights from gene duplication and evolutionary pressure analyses, highlights the genetic robustness and potential functional importance of *GhSRF* genes. Our findings suggest that *GhSRF* genes are integral to stress response mechanisms, providing a valuable resource for future genetic and biotechnological interventions. This work not only enriches the existing knowledge base but also offers practical implications for breeding programs aimed at developing more resilient cotton varieties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14091933/s1, Supplementary Table S1: Primers used in this study; primer properties; CDS and Protein Sequence, Supplementary Table S2. Gene ID's and designated names in Studied Organisms. Supplementary Table S3: Physio-chemical Properties of *GhSRF*, CDS and Protein Lengths, Protein Molecular Weight, Isoelectric point, Grand Average of Hydropathicity, Number of Intron/Exon and Sub-cellular prediction. Supplementary Table S4. Cis-elements in *GhSRF*'s. Supplementary Table S5. Protein Motif, Description and Sequences. Supplementary Table S6. Ka & Ks value, type of duplications. Supplementary Table S7. Sub-cellular localization of gene on chromosome. Supplementary Table S8. Gene expression data based on FPKM values.

Author Contributions: Conceptualization, F.A. and Z.K.; methodology, F.A., S.A. and S.U.R.; software, F.A., S.U.R. and M.H.U.R.; validation, F.A., S.U.R. and Z.K.; formal analysis, F.A., S.A. and M.H.U.R.; investigation, F.A. and Z.K.; resources, S.U.R., Z.K. and M.H.U.R.; data curation, F.A. and Z.K.; writing—original draft preparation, F.A.; writing—review and editing, F.A., S.U.R., M.H.U.R., S.A. and Z.K.; visualization, F.A. and Z.K.; supervision, Z.K.; project administration, F.A. and Z.K.; funding acquisition, F.A., Z.K. and S.U.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by grants ADP Funded Project entitled National Crop Genomics and Speed Breeding Center for Agricultural Sustainability (ADP-LO21002838 Punjab, Pak) and Pakistan Science Foundation-National Natural Science Foundation of China (PSF-NSFC-IV/Agr/P-MNSUAM (30)).

Data Availability Statement: All the relevant data are available from the corresponding authors upon request. There are no restrictions on data availability.

Acknowledgments: The authors are thankful to Institute of Crop Science, Plant Precision Breeding Academy, Zhejiang Provincial Key Laboratory of Crop Genetic Resources, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China for kind support in expression profiling experimentation.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Chattha, W.S.; Atif, R.M.; Iqbal, M.; Shafqat, W.; Farooq, M.A.; Shakeel, A. Genome-Wide Identification and Evolution of Dof Transcription Factor Family in Cultivated and Ancestral Cotton Species. *Genomics* **2020**, *112*, 4155–4170. [CrossRef] [PubMed]
2. Abdullah, M.; Ahmad, F.; Zang, Y.; Jin, S.; Ahmed, S.; Li, J.; Islam, F.; Ahmad, M.; Zhang, Y.; Hu, Y.; et al. HEAT RESPONSIVE PROTEIN Regulates Heat Stress via Fine-Tuning Ethylene/Auxin Signaling Pathways in Cotton. *Plant Physiol.* **2022**, *191*, 772–788. [CrossRef]
3. Malhi, G.S.; Kaur, M.; Kaushik, P. Impact of Climate Change on Agriculture and Its Mitigation Strategies: A Review. *Sustainability* **2021**, *13*, 1318. [CrossRef]

4. Zafar, M.M.; Chattha, W.S.; Khan, A.I.; Zafar, S.; Subhan, M.; Saleem, H.; Ali, A.; Ijaz, A.; Anwar, Z.; Qiao, F.; et al. Drought and Heat Stress on Cotton Genotypes Suggested Agro-Physiological and Biochemical Features for Climate Resilience. *Front. Plant Sci.* **2023**, *14*, 1265700. [CrossRef] [PubMed]
5. Eckstein, D.; Künzel, V.; Schäfer, L. *Global Climate Risk Index 2021*; Germanwatch: Bonn, Germany, 2021; p. 28.
6. Ali, M.M.; Ali, Z.; Ahmad, F.; Nawaz, F.; Shakil, Q.; Ahmad, S.; Khan, A.A. Transcript Abundance of Heat Shock Protein Genes Confer Heat Tolerance in Cotton (*Gossypium hirsutum* L.). *Pak. J. Bot.* **2022**, *54*, 65–71. [CrossRef]
7. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review. *Plants* **2019**, *8*, 34. [CrossRef]
8. Iqbal, M.; Ul-Allah, S.; Naeem, M.; Ijaz, M.; Sattar, A.; Sher, A. Response of Cotton Genotypes to Water and Heat Stress: From Field to Genes. *Euphytica* **2017**, *213*, 131. [CrossRef]
9. Abro, A.A.; Anwar, M.; Javwad, M.U.; Zhang, M.; Liu, F.; Jiménez-Ballesta, R.; Salama, E.A.A.; Ahmed, M.A.A. Morphological and Physio-Biochemical Responses under Heat Stress in Cotton: Overview. *Biotechnol. Rep.* **2023**, *40*, e00813. [CrossRef]
10. Provart, N.J.; Brady, S.M.; Parry, G.; Schmitz, R.J.; Queitsch, C.; Bonetta, D.; Waese, J.; Schneeberger, K.; Loraine, A.E. Anno Genominis XX: 20 Years of *Arabidopsis* Genomics. *Plant Cell* **2021**, *33*, 832–845. [CrossRef]
11. Bhat, G.R.; Sethi, I.; Rah, B.; Kumar, R.; Afroze, D. Innovative in Silico Approaches for Characterization of Genes and Proteins. *Front. Genet.* **2022**, *13*, 865182. [CrossRef]
12. Varshney, R.K.; Bohra, A.; Yu, J.; Graner, A.; Zhang, Q.; Sorrells, M.E. Designing Future Crops: Genomics-Assisted Breeding Comes of Age. *Trends Plant Sci.* **2021**, *26*, 631–649. [CrossRef] [PubMed]
13. Yang, Z.; Gao, C.; Zhang, Y.; Yan, Q.; Hu, W.; Yang, L.; Wang, Z.; Li, F. Recent Progression and Future Perspectives in Cotton Genomic Breeding. *J. Integr. Plant Biol.* **2023**, *65*, 548–569. [CrossRef]
14. Hou, S.; Zhu, G.; Li, Y.; Li, W.; Fu, J.; Niu, E.; Li, L.; Zhang, D.; Guo, W. Genome-Wide Association Studies Reveal Genetic Variation and Candidate Genes of Drought Stress Related Traits in Cotton (*Gossypium hirsutum* L.). *Front. Plant Sci.* **2018**, *9*, 1276. [CrossRef] [PubMed]
15. Bao, Y.; Zhang, X.; Xu, X. Abundant Small Genetic Alterations after Upland Cotton Domestication. *Biomed Res. Int.* **2018**, *2018*, 9254302. [CrossRef] [PubMed]
16. Eyüboglu, B.; Pfister, K.; Haberer, G.; Chevalier, D.; Fuchs, A.; Mayer, K.F.X.; Schneitz, K. Molecular Characterisation of the STRUBBELIG-RECEPTOR FAMILY of Genes Encoding Putative Leucine-Rich Repeat Receptor-like Kinases in *Arabidopsis thaliana*. *BMC Plant Biol.* **2007**, *7*, 16. [CrossRef] [PubMed]
17. Meng, J.; Yang, J.; Peng, M.; Liu, X.; He, H. Genome-Wide Characterization, Evolution, and Expression Analysis of the Leucine-Rich Repeat Receptor-like Protein Kinase (Lrr-Rlk) Gene Family in *Medicago truncatula*. *Life* **2020**, *10*, 176. [CrossRef]
18. Bai, Y.; Vaddepalli, P.; Fulton, L.; Bhasin, H.; Hülskamp, M.; Schneitz, K. *ANGUSTIFOLIA* Is a Central Component of Tissue Morphogenesis Mediated by the Atypical Receptor-like Kinase STRUBBELIG. *BMC Plant Biol.* **2013**, *13*, 16. [CrossRef]
19. Wang, H.; Chen, Y.; Wu, X.; Long, Z.; Sun, C.; Wang, H.; Wang, S.; Birch, P.R.J.; Tian, Z. A Potato STRUBBELIG-RECEPTOR FAMILY Member, *StLRPK1,* Associates with *StSERK3A/BAK1* and Activates Immunity. *J. Exp. Bot.* **2018**, *69*, 5573–5586. [CrossRef]
20. Fulton, L.; Vaddepalli, P.; Yadav, R.K.; Batoux, M.; Schneitz, K. Inter-Cell-Layer Signalling during *Arabidopsis* Ovule Development Mediated by the Receptor-like Kinase STRUBBELIG. *Biochem. Soc. Trans.* **2010**, *38*, 583–587. [CrossRef]
21. Fulton, L.; Batoux, M.; Vaddepalli, P.; Yadav, R.K.; Busch, W.; Andersen, S.U.; Jeong, S.; Lohmann, J.U.; Schneitz, K. DETORQUEO, QUIRKY, and ZERZAUST Represent Novel Components Involved in Organ Development Mediated by the Receptor-like Kinase STRUBBELIG in *Arabidopsis thaliana*. *PLoS Genet.* **2009**, *5*, e1000355. [CrossRef]
22. Alcázar, R.; García, A.V.; Kronholm, I.; De Meaux, J.; Koornneef, M.; Parker, J.E.; Reymond, M. Natural Variation at Strubbelig Receptor Kinase 3 Drives Immune-Triggered Incompatibilities between *Arabidopsis thaliana* Accessions. *Nat. Genet.* **2010**, *42*, 1135–1139. [CrossRef] [PubMed]
23. Lin, L.; Zhong, S.H.; Cui, X.F.; Li, J.; He, Z.H. Characterization of temperature-sensitive mutants reveals a role for receptor-like kinase SCRAMBLED/STRUBBELIG in coordinating cell proliferation and differentiation during *Arabidopsis* leaf development. *Plant J.* **2012**, *72*, 707–720. [CrossRef] [PubMed]
24. Zhang, T.; Yu, L.X.; Zheng, P.; Li, Y.; Rivera, M.; Main, D.; Greene, S.L. Identification of Loci Associated with Drought Resistance Traits in Heterozygous Autotetraploid Alfalfa (*Medicago sativa* L.) Using Genome-Wide Association Studies with Genotyping by Sequencing. *PLoS ONE* **2015**, *10*, e0138931. [CrossRef]
25. Arifuzzaman, M.; Oladzadabbasabadi, A.; McClean, P.; Rahman, M. Shovelomics for Phenotyping Root Architectural Traits of Rapeseed/Canola (*Brassica napus* L.) and Genome-Wide Association Mapping. *Mol. Genet. Genom.* **2019**, *294*, 985–1000. [CrossRef]
26. Chaudhary, A.; Chen, X.; Gao, J.; Leśniewska, B.; Hammerl, R.; Dawid, C.; Schneitz, K. The Arabidopsis Receptor Kinase STRUBBELIG Regulates the Response to Cellulose Deficiency. *PLoS Genet.* **2020**, *16*, e1008433. [CrossRef]
27. Zhu, T.; Liang, C.; Meng, Z.; Sun, G.; Meng, Z.; Guo, S.; Zhang, R. CottonFGD: An Integrated Functional Genomics Database for Cotton. *BMC Plant Biol.* **2017**, *17*, 101. [CrossRef] [PubMed]
28. Goodstein, D.M.; Shu, S.; Howson, R.; Neupane, R.; Hayes, R.D.; Fazo, J.; Mitros, T.; Dirks, W.; Hellsten, U.; Putnam, N.; et al. Phytozome: A Comparative Platform for Green Plant Genomics. *Nucleic Acids Res.* **2012**, *40*, 1178–1186. [CrossRef] [PubMed]
29. Letunic, I.; Khedkar, S.; Bork, P. SMART: Recent Updates, New Developments and Status in 2020. *Nucleic Acids Res.* **2021**, *49*, D458–D460. [CrossRef]

30. Jones, P.; Binns, D.; Chang, H.Y.; Fraser, M.; Li, W.; McAnulla, C.; McWilliam, H.; Maslen, J.; Mitchell, A.; Nuka, G.; et al. InterProScan 5: Genome-Scale Protein Function Classification. *Bioinformatics* **2014**, *30*, 1236–1240. [CrossRef] [PubMed]
31. Martin, F.J.; Amode, M.R.; Aneja, A.; Austine-Orimoloye, O.; Azov, A.G.; Barnes, I.; Becker, A.; Bennett, R.; Berry, A.; Bhai, J.; et al. Ensembl 2023. *Nucleic Acids Res.* **2023**, *51*, D933–D941. [CrossRef]
32. Gasteiger, E.; Gattiker, A.; Hoogland, C.; Ivanyi, I.; Appel, R.D.; Bairoch, A. ExPASy: The Proteomics Server for in-Depth Protein Knowledge and Analysis. *Nucleic Acids Res.* **2003**, *31*, 3784–3788. [CrossRef]
33. Yu, C.-S.; Lin, C.-J.; Hwang, J.-K. Predicting Subcellular Localization of Proteins for Gram-Negative Bacteria by Support Vector Machines Based on n -Peptide Compositions. *Protein Sci.* **2004**, *13*, 1402–1406. [CrossRef]
34. Letunic, I.; Bork, P. Interactive Tree of Life (ITOL) v6: Recent Updates to the Phylogenetic Tree Display and Annotation Tool. *Nucleic Acids Res.* **2024**, *52*, W78–W82. [CrossRef]
35. Sharma, S.; Vakhlu, J. Evolution and Biology of CRISPR System: A New Era Tool for Genome Editing in Plants. *Bot. Rev.* **2021**, *87*, 496–517. [CrossRef]
36. Crooks, G.E.; Hon, G.; Chandonia, J.M.; Brenner, S.E. WebLogo: A Sequence Logo Generator. *Genome Res.* **2004**, *14*, 1188–1190. [CrossRef] [PubMed]
37. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
38. Lescot, M.; Déhais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van De Peer, Y.; Rouzé, P.; Rombauts, S. PlantCARE, a Database of Plant Cis-Acting Regulatory Elements and a Portal to Tools for in Silico Analysis of Promoter Sequences. *Nucleic Acids Res.* **2002**, *30*, 325–327. [CrossRef]
39. Nystrom, S.L.; McKay, D.J. Memes: A Motif Analysis Environment in R Using Tools from the MEME Suite. *PLoS Comput. Biol.* **2021**, *17*, e1008991. [CrossRef]
40. Hu, B.; Jin, J.; Guo, A.Y.; Zhang, H.; Luo, J.; Gao, G. GSDS 2.0: An Upgraded Gene Feature Visualization Server. *Bioinformatics* **2015**, *31*, 1296–1297. [CrossRef]
41. Yu, C.S.; Cheng, C.W.; Su, W.C.; Chang, K.C.; Huang, S.W.; Hwang, J.K.; Lu, C.H. CELLO2GO: A Web Server for Protein SubCELlular LOcalization Prediction with Functional Gene Ontology Annotation. *PLoS ONE* **2014**, *9*, e99368. [CrossRef] [PubMed]
42. Horton, P.; Park, K.J.; Obayashi, T.; Fujita, N.; Harada, H.; Adams-Collier, C.J.; Nakai, K. WoLF PSORT: Protein Localization Predictor. *Nucleic Acids Res.* **2007**, *35*, 585–587. [CrossRef] [PubMed]
43. Voorrips, R.E. Mapchart: Software for the Graphical Presentation of Linkage Maps and QTLs. *J. Hered.* **2002**, *93*, 77–78. [CrossRef] [PubMed]
44. Larkin, M.A.; Blackshields, G.; Brown, N.P.; Chenna, R.; Mcgettigan, P.A.; McWilliam, H.; Valentin, F.; Wallace, I.M.; Wilm, A.; Lopez, R.; et al. Clustal W and Clustal X Version 2.0. *Bioinformatics* **2007**, *23*, 2947–2948. [CrossRef]
45. Suyama, M.; Torrents, D.; Bork, P. PAL2NAL: Robust Conversion of Protein Sequence Alignments into the Corresponding Codon Alignments. *Nucleic Acids Res.* **2006**, *34*, 609–612. [CrossRef] [PubMed]
46. Yang, Z. PAML 4: Phylogenetic Analysis by Maximum Likelihood. *Mol. Biol. Evol.* **2007**, *24*, 1586–1591. [CrossRef] [PubMed]
47. Darzentas, N. Circoletto: Visualizing Sequence Similarity with Circos. *Bioinformatics* **2010**, *26*, 2620–2621. [CrossRef]
48. Schmittgen, T.D.; Livak, K.J. Analyzing Real-Time PCR Data by the Comparative CT Method. *Nat. Protoc.* **2008**, *3*, 1101–1108. [CrossRef]
49. Hong, K.; Radian, Y.; Manda, T.; Xu, H.; Luo, Y. The Development of Plant Genome Sequencing Technology and Its Conservation and Application in Endangered Gymnosperms. *Plants* **2023**, *12*, 4006. [CrossRef]
50. Akram, U.; Song, Y.; Liang, C.; Abid, M.A.; Askari, M.; Myat, A.A.; Abbas, M.; Malik, W.; Ali, Z.; Guo, S.; et al. Genome-Wide Characterization and Expression Analysis of NHX Gene Family under Salinity Stress in *Gossypium barbadense* and Its Comparison with *Gossypium hirsutum*. *Genes* **2020**, *11*, 803. [CrossRef]
51. Shahid, S.; Sher, M.A.; Ahmad, F.; ur Rehman, S.; Farid, B.; Raza, H.; Ali, Z.; Maqbool, A.; Alfarraj, S.; Ansari, M.J. Prediction of RNA Editing Sites and Genome-Wide Characterization of PERK Gene Family in Maize (*Zea mays* L.) in Response to Drought Stress. *J. King Saud Univ.—Sci.* **2022**, *34*, 102293. [CrossRef]
52. Su, J.; Song, S.; Wang, Y.; Zeng, Y.; Dong, T.; Ge, X.; Duan, H. Genome-Wide Identification and Expression Analysis of DREB Family Genes in Cotton. *BMC Plant Biol.* **2023**, *23*, 169. [CrossRef] [PubMed]
53. Yang, X.; Xu, Y.; Yang, F.; Magwanga, R.O.; Cai, X.; Wang, X.; Wang, Y.; Hou, Y.; Wang, K.; Liu, F.; et al. Genome-Wide Identification of OSCA Gene Family and Their Potential Function in the Regulation of Dehydration and Salt Stress in *Gossypium hirsutum*. *J. Cott. Res.* **2019**, *2*, 11. [CrossRef]
54. Liu, Z.; Ge, X.; Yang, Z.; Zhang, C.; Zhao, G.; Chen, E.; Liu, J.; Zhang, X.; Li, F. Genome-Wide Identification and Characterization of SnRK2 Gene Family in Cotton (*Gossypium hirsutum* L.). *BMC Genet.* **2017**, *18*, 54. [CrossRef] [PubMed]
55. Li, Z.; Liu, Z.; Wei, Y.; Liu, Y.; Xing, L.; Liu, M.; Li, P.; Lu, Q.; Peng, R. Genome-Wide Identification of the MIOX Gene Family and Their Expression Profile in Cotton Development and Response to Abiotic Stress. *PLoS ONE* **2021**, *16*, e0254111. [CrossRef]
56. Hu, Y.; Chen, J.; Fang, L.; Zhang, Z.; Ma, W.; Niu, Y.; Ju, L.; Deng, J.; Zhao, T.; Lian, J.; et al. *Gossypium barbadense* and *Gossypium hirsutum* Genomes Provide Insights into the Origin and Evolution of Allotetraploid Cotton. *Nat. Genet.* **2019**, *51*, 739–748. [CrossRef]

57. Peng, R.; Xu, Y.; Tian, S.; Unver, T.; Liu, Z.; Zhou, Z.; Cai, X.; Wang, K.; Wei, Y.; Liu, Y.; et al. Evolutionary Divergence of Duplicated Genomes in Newly Described Allotetraploid Cottons. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2208496119. [CrossRef] [PubMed]
58. Yadav, M.; Saxena, G.; Verma, R.K.; Asif, M.H.; Singh, V.P.; Sawant, S.V.; Singh, S.P. Genome-Wide Identification and Expression Analysis of Autophagy-Related Genes (ATG) in *Gossypium* spp. Reveals Their Crucial Role in Stress Tolerance. *S. Afr. J. Bot.* **2024**, *167*, 82–93. [CrossRef]
59. Kilwake, J.W.; Umer, M.J.; Wei, Y.; Mehari, T.G.; Magwanga, R.O.; Xu, Y.; Hou, Y.; Wang, Y.; Shiraku, M.L.; Kirungu, J.N.; et al. Genome-Wide Characterization of the SAMS Gene Family in Cotton Unveils the Putative Role of *GhSAMS2* in Enhancing Abiotic Stress Tolerance. *Agronomy* **2023**, *13*, 612. [CrossRef]
60. Wei, W.; Ju, J.; Zhang, X.; Ling, P.; Luo, J.; Li, Y.; Xu, W.; Su, J.; Zhang, X.; Wang, C. GhBRX.1, GhBRX.2, and GhBRX4.3 Improve Resistance to Salt and Cold Stress in Upland Cotton. *Front. Plant Sci.* **2024**, *15*, 1353365. [CrossRef]
61. Zhang, H.; Xiao, X.; Li, Z.; Chen, Y.; Li, P.; Peng, R.; Lu, Q.; Wang, Y. Exploring the Plasmodesmata Callose- Binding Protein Gene Family in Upland Cotton: Unraveling Insights for Enhancing Fiber Length. *PeerJ* **2024**, *12*, e17625. [CrossRef]
62. Li, Y.; Zheng, A.; Li, Z.; Wang, H.; Wang, J.; Dong, Z.; Yao, L.; Han, X.; Wei, F. Characterization and Gene Expression Analysis Reveal Universal Stress Proteins Respond to Abiotic Stress in *Gossypium hirsutum*. *BMC Genom.* **2024**, *25*, 98. [CrossRef] [PubMed]
63. Ali, F.; Qanmber, G.; Wei, Z.; Yu, D.; Li, Y.H.; Gan, L.; Li, F.; Wang, Z. Genome-Wide Characterization and Expression Analysis of Geranylgeranyl Diphosphate Synthase Genes in Cotton (*Gossypium* spp.) in Plant Development and Abiotic Stresses. *BMC Genom.* **2020**, *21*, 561. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.