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Genome-wide identification and characterization of *HSP90* family gene in cotton and their potential role in salt stress tolerance

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Heat shock proteins 90 (HSP90s) are conserved molecular chaperones essential for cellular homeostasis under abiotic stress. While several studies have been reported to elucidate the role of HSP90s in heat stress responses in cotton, their involvement in salt stress remains underexplored. *Gossypium hirsutum* L. is highly susceptible to salt stress. The Current study investigated the evolutionary aspects, expression patterns, and functional validation of *HSP90* family members in cotton under salt stress. A comprehensive genomic analysis of *G. hirsutum*, *G. raimondii*, and *G. arboreum*, identified 56 *HSP90* genes which were classified into three distinct phylogenetic groups. Gene structure and motifs analysis revealed a conserved nature of *HSP90s* within each group. Additionally, *cis*-acting elements suggested the potential roles of *HSP90s* in biotic and abiotic stresses. The Ka/Ks ratio of all genes was < 1 suggesting negative and purifying selection pressure during molecular evolution. Expression analysis demonstrated the potential role of *HSP90* genes in salt tolerance. Notably, out of ten *HSP90* genes five genes exhibited highly differential expression under salt stress, as confirmed by qRT-PCR analysis. Moreover, virus-induced silencing of the two salt stress-responsive genes, *Ghir_D03G016230* and *Ghir_D02G013530*, which were upregulated under salt stress, resulted in a significant decrease in SOD, POD, and CAT, accompanied by a marked increase in MDA content in the salt-tolerant cotton cultivar. These findings provide novel insights into the functional roles of *HSP90s* in *G. hirsutum* under salt stress.

KEYWORDS

Gossypium hirsutum, *Hsp90*, salt stress, VIGS, cotton

Introduction

Plants have evolved intricate mechanisms to survive abiotic stresses, including heat and salinity. These include stress avoidance, osmotic adjustment, antioxidant defense, stress signaling, symbiotic association, and epigenetic regulation. Rather than working independently, these mechanisms function in a coordinated way to improve plant resilience. For example, stress avoidance strategies like stomata closure and dormancy alongside stress tolerance mechanisms such as osmolyte accumulation and antioxidant production synergistically mitigate the effects of abiotic stress (Bandurska, 2022). Symbiotic association with endophytic fungi improves stress tolerance by inducing physiological and molecular changes in host plants (Rodriguez et al., 2008). Stress signaling, involving hormones like salicylic acid and abscisic acid modulates stress-responsive genes leading to the activation of various tolerance mechanisms simultaneously (Khan et al., 2015; Sha Valli Khan et al., 2014; Yasir et al., 2019). Epigenetic regulation through DNA methylation, histone modifications, and small RNAs further fine-tune gene expressions. These coordinated mechanisms suggest shared regulatory pathways and cross-talk between stress responses.

Among various molecular players mediating stress responses, heat shock proteins (HSP90) are critical. The HSP90 supports stress signaling pathways and assists in the proper functioning of stress-induced proteins. Recent studies have unraveled the potential of HSP90 family genes as targets for improving stress tolerance in crops, including cotton, which is susceptible to salinity. While heat shock protein (HSP) families are primarily involved in heat stress response, growing evidence suggests their involvement in salt tolerance as well. For instance, overexpression of *ZmHsf01* and *OsHsp20* in *Arabidopsis thaliana* enhanced tolerance to both heat and salt stress (Huang et al., 2022). Similarly, the maize gene *ZmHsf11* negatively regulates heat stress tolerance by modulating oxidative stress-related genes, which are also implicated in salt stress response (Qin et al., 2022). Moreover, genes like *OsRCI2-10* and *PGR5*, upregulated in salt-tolerant rice genotypes, are annotated for abiotic stress response, indicating their potential roles in both salt and heat tolerance (Razzaque et al., 2019). The Na⁺/H⁺ antiporter NHX1, known for salt tolerance by sequestering Na⁺ ions in vacuoles, also improves thermotolerance (Trono and Pecchioni, 2022). These findings highlight the existence of shared genetic networks and cross-tolerance mechanisms. When stimulated by various factors such as heat, plants produce highly conserved proteins known as heat shock proteins (HSPs) (Hsu et al., 2003; Queitsch et al., 2002; Robert, 2003). HSPs are well-characterized proteins that function as molecular chaperones, ensuring the proper folding and protection of cellular proteins (King & MacRae, 2015). HSPs have been identified across plants and animals (Xiong and Ishitani, 2006). These proteins have been categorized into several families based on their molecular weights, including the HSP100/ClpB family, HSP90 family, HSP70/DnaK family, chaperonin (HSP60/GroEL) family, and small heat-shock proteins (sHSP) family (Al-Wahaibi, 2011; Caplan et al., 2003; Pratt and Toft, 2003; Wang et al., 2004).

The HSP90 gene family was initially discovered in *Drosophila* with a single gene, *Hsp82*. HSP90 chaperones are expressed constitutively in most organisms under normal conditions, however their expression increases significantly under stress. HSP90, as a class of chaperones, is involved in DNA repair, substrate activation, and initial stress signaling (Lachowicz et al., 2014; Shinozaki et al., 2006; Zuehlke and Johnson, 2010). It functions to prevent protein aggregation and facilitates the refolding of inactive proteins in both normal and stress conditions (Picard, 2002). When plants are exposed to stress, the upregulated HSP90 interacts with non-proteinaceous substances, repairing deformed proteins (Mukhopadhyay et al., 2003). HSP90 is highly expressed in plant cells, constituting about 1-2% of total protein in the cytoplasm, and is characterized by conserved amino acid sequences (Prasinos et al., 2004). HSP90 consists of three domains: an N-terminal ATP-binding motif, a middle domain, and a C-terminal domain (CTD) responsible for HSP90 dimerization. This protein family acts as a mediator in plant abiotic stress signal pathways however, the underlying mechanisms remain unclear (Liu and Ekramoddoullah, 2006).

Although the HSP90 protein is well studied in heat tolerance in, however, due to the integrated mechanism of stress tolerance in plants we hypothesized that this protein family has a potential role in salt tolerance in upland cotton. Previous studies identified 9, 7, and 21 HSP90 genes in *Oryza sativa*, *Arabidopsis thaliana*, and *Nicotiana tabacum*, respectively (Hu et al., 2009; Krishna and Gloor, 2001). To the best of our knowledge, no genome-wide study has yet explored the role of the HSP90 protein family in cotton under salt stress. Upland cotton is an important commercial crop that provides feed and food for animals and humans, and lint for the textile industry (Yasir et al., 2022). However, salt stress is one of the major challenges for sustainable cotton production. Therefore, breeding for salt tolerance is an important objective of cotton breeding. In this study, we conducted a genome-wide analysis of HSP90 family members, analyzing gene structures, evolutionary relationships, chromosomal locations, and conserved domains in detail using bioinformatics tools. Additionally, we investigated their expression patterns under salt stress through qRT-PCR and validated results through virus-induced gene silencing (VIGS), antioxidants scavenging enzymes (SOD, POD, CAT), and oxidative stress marker i.e., malondialdehyde (MDA). These results provide a foundation for understanding the genomic organization and functional role of the HSP90 gene family in *G. hirsutum*.

Materials and methods

Plant materials and stress treatments

Salt-tolerant cotton lines HNZ2019–2520 and salt-sensitive line HNZ2019–2521 were used for gene expression experiments. The seeds were surface sterilized with 10% sodium hypochlorite and three times rinsed with distilled water followed by planting in mixed soil

(vermiculite: humus = 1:1) saturated with water, in trays in a greenhouse at 22°C with a 16 h/8 h (light/dark) photoperiod until complete unfolding of cotyledonary leaves. Seedlings were then shifted to hydroponic boxes and divided into two groups for control and salt treatment, each having three replications. Salt treatment of 300 mM NaCl (validated to cause significant stress in a pilot experiment) at two true leaf stages. Untreated control plants were grown normally. Samples were taken at 0, 3, 12, 24, and 48, hours post-treatment with three biological and three technical repeats. Leaf, stem, and root tissues were sampled to determine tissue-specific analyses. After collection, all samples were quickly frozen in liquid nitrogen and stored at −80°C for RNA isolation and downstream analysis like qRT-PCR, VIGS, and biochemical analysis. RNA-seq expression data of *G. hirsutum* accession TM-1 was retrieved from NCBI-Bioproject PRJNA490626 (Hu et al., 2019).

Identification of HSP90 genes

The *Arabidopsis thaliana* HSP90 protein sequences were retrieved from the TAIR databases (<http://www.arabidopsis.org/>) (Krishna and Gloor, 2001). The *Oryza sativa* and *Triticum aestivum* protein sequences were downloaded from NCBI (<https://www.ncbi.nlm.nih.gov/>) and *G. hirsutum* _HAU-AD1_v1.1 (TM-1), *G. arboreum* (A2) 'SXY1' genome CRI-updated_v1, and *G. raimondii* (D5) genome JGI_v2_a2.1 from cotton functional genomics database website (<https://cottonfgd.net/>). The Pfam (<http://pfam.sanger.ac.uk/search>) and SMART (<http://smart.embl-heidelberg.de/>) databases were employed to confirm each predicted HSP90 protein (Finn et al., 2015). Additional characteristics of HSP90 proteins, including amino acid (aa) or protein length and molecular weight (kDa), isoelectric points (pIs), grand average of hydropathy, and charge were analyzed using the Cotton Functional Genomic Database (<http://www.cottonfgd.org/>).

Phylogenetic analysis

Multiple sequence alignment of HSP90 was performed using ClustalW (Larkin et al., 2007). The MEGA 7.0 software was employed to construct an unrooted phylogenetic tree by the neighbor-joining (NJ) method (Kumar et al., 2016) with 1000 bootstraps for statistical reliability.

Chromosomal mapping of HSP90 family genes

The genomic locations of all HSP90 genes were obtained from the gff3 files of three cotton species available on the Cotton Functional Genomic Database (<http://www.cottonfgd.org/>). The TBtools software was employed to assess the chromosomal distribution and positions of HSP90 genes (Chen et al., 2020).

Gene structure motif analysis and sub-cellular localization of HSP90 family members

A diagrammatic sketch of the HSP90 gene structure was constructed using the Gene Structure Display Server (GSDS) (<http://gsds.cbi.pku.edu.cn>) (Hu et al., 2015) based on the alignment of the CDS with their corresponding genomic DNA sequences. The conserved motifs of the full length of HSP90 family proteins were analyzed using the online MEME tool (Multiple Expectation Maximization for Motif Elicitation, <http://memesuite.org/tools/meme>) (Bailey et al., 2006). The maximum motif search value was set at 10. Sub-cellular localization of proteins was determined using online bioinformatics tools Cell-Ploc (Chou and Shen, 2008), WoLF PSORT (Horton et al., 2007), and LocTree3 (Goldberg et al., 2014) with their protein sequences.

Quantitative RT-PCR expression analysis of HSP90 genes

Plant total RNA was extracted by the Total RNA Extraction Kit (R1200) (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). PrimeScript™ II 1st Strand cDNA Synthesis Kit (TAKARA, Dalian, China) was used for reverse transcription to obtain the first-strand cDNA for transcriptomic and RT qPCR analysis. The expression heatmap was visualized by TBtools. The fluorescent quantitative Taq Pro Universal SYBR qPCR Master Mix (Q712- 02) (Vazyme Biotech Co., Ltd, Nanjing China) kit was employed for the qRT-PCR reactions. The data were calculated according to the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). According to the candidate gene sequence, a relatively specific primer for real-time fluorescence quantitative PCR was designed by Oligo 7 software Supplementary Table S1, and the amplification product was 150–300 bp (Rychlik 2007; Livak and Schmittgen, 2001).

Virus-induced gene silencing and stress treatment

The plasmid TRV2 (pYL156) and TRV1 (pYL192) were employed for VIGS (Supplementary Figure 1). The VIGS sequence was obtained using the SGN VIGS Tool (<http://vigs.solgenomics.net/>) and cloned into the TRV2 vector using the T4 DNA ligation cloning method. The lengths of the silencing fragments for *Ghir_D03G016230* and *Ghir_D02G013530* were 212 and 243 bp, respectively. The TRV-VIGS constructs were also transformed into *A. tumefaciens* strain GV3101. Then, strain GV3101, containing the different constructs of gene of interest, was infiltrated into 10-day-old cotton seedlings with fully expanded cotyledonary leaves according to the method described by Gao et al. (2011) TRV2:CLA1 (CLOROPLASTOS ALTERADOS 1) was

utilized as the positive control and the empty vector TRV2:00 was the negative control (Gao et al., 2011). When the plants grew up to 2~3 true leaves stage they were shifted to hydroponic boxes. The primers list has been given in [Supplementary Table S2](#). Two weeks after infection, the albino leaf phenotype appeared in plants transformed with the positive control gene CLA1. Subsequently, cotton plants containing TRV2: *Ghir_D03G016230*, TRV2: *Ghir_D02G013530*, and the negative control TRV2:00 were exposed to salt treatments at two true leaf stage with the same volume of water or salt solution (300 mM/L NaCl) ([Supplementary Figure 2](#)). The plants were observed and photographed during the treatment. Furthermore, the effect of gene silencing on SOD, POD, CAT, and MDA was observed.

POD activities were detected according to the previous reports (Alici and Arabaci, 2016; Ullah et al., 2018). The MDA concentration was determined using thiobarbituric acid method (Schmedes and Holmer, 1989). The absorbance was measured using a UV-2550 UV-vis spectrophotometer (SHIMADZU). Leaf relative water content and chlorophyll contents were measured as described in previous studies (Magwanga et al., 2019). All the samples were replicated three times.

Results

The assessment of salt tolerant and salt sensitive genotypes

The salt-tolerant and salt-sensitive genotypes were subjected to salt stress. The biochemical outcomes of SOD, POD, CAT, and MDA from different tissues such as leaf, root, and stem revealed that activities of SOD, POD, and CAT were significantly high in salt tolerant genotype however, the MDA expression was low in salt-tolerant and vice versa as shown in [Figure 1](#). These results

Measurement of physiological indices

To determine the physiological indices variations, the candidate genes-silenced cotton leaves of the salt-tolerant cultivar were used to observe the activities of SOD, POD, CAT, and concentration of MDA after salt treatments. The SOD activity, CAT activity, and

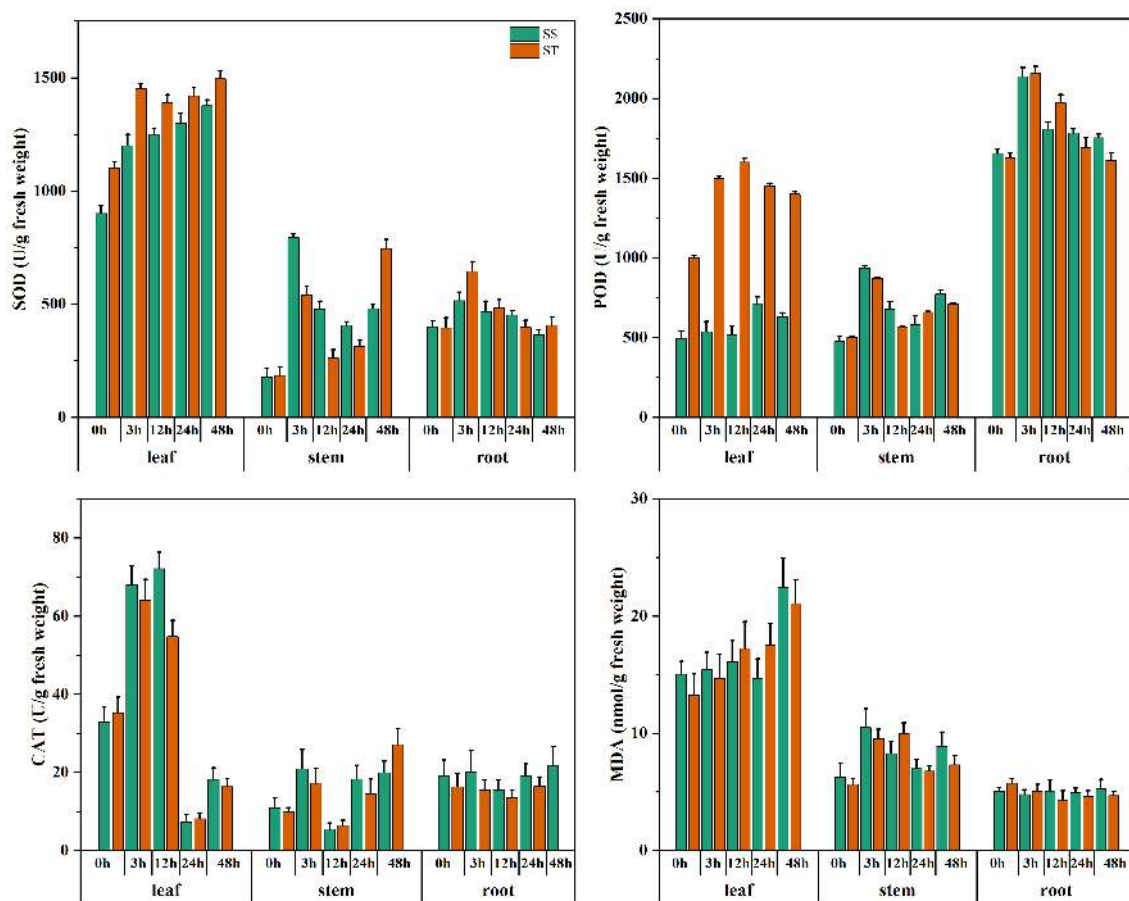


FIGURE 1

The assessment of salt tolerant and sensitive genotypes. The expression of SOD, POD, CAT and MDA of salt tolerant (ST) HN2019-2520, and salt sensitive (SS) HN2019-2521 genotypes. The red bar graph represents salt tolerant genotype whereas, green salt sensitive. Error bars represent the standard deviation of the triplicated data.

TABLE 1 Physicochemical properties of three cotton species.

Gene ID	Protein Length (aa)	Molecular Weight (kDa)	Charge	Isoelectric Point	Grand Average of Hydropathy
<i>Ghir_A01G008350</i>	699	80.066	-27.5	4.726	-0.572
<i>Ghir_A01G009870</i>	794	90.742	-30.5	4.684	-0.689
<i>Ghir_A03G002790</i>	329	37.867	-8	5.042	-0.513
<i>Ghir_A03G002800</i>	137	15.92	-1	5.838	-0.573
<i>Ghir_A03G012060</i>	779	88.764	-27.5	4.682	-0.557
<i>Ghir_A05G018960</i>	134	15.239	-7	4.423	-0.457
<i>Ghir_A06G000260</i>	805	91.916	-37	4.605	-0.713
<i>Ghir_A07G020520</i>	622	71.284	-16.5	4.848	-0.633
<i>Ghir_A08G003090</i>	699	80.054	-27.5	4.726	-0.579
<i>Ghir_A08G003100</i>	700	80.139	-28.5	4.704	-0.571
<i>Ghir_A08G026410</i>	258	29.383	-15.5	4.466	-0.531
<i>Ghir_A12G026820</i>	703	80.643	-26.5	4.718	-0.605
<i>Ghir_A13G009660</i>	766	86.834	-35.5	4.519	-0.518
<i>Ghir_A13G013590</i>	699	80.143	-26.5	4.748	-0.591
<i>Ghir_D01G008810</i>	699	80.022	-27.5	4.726	-0.572
<i>Ghir_D01G010630</i>	809	92.49	-31	4.693	-0.688
<i>Ghir_D02G013530</i>	790	90.159	-33.5	4.589	-0.569
<i>Ghir_D03G016230</i>	707	81.116	-23.5	4.815	-0.617
<i>Ghir_D06G000070</i>	804	92.04	-34	4.654	-0.718
<i>Ghir_D06G016230</i>	240	27.947	-1	5.979	-0.587
<i>Ghir_D07G020620</i>	844	96.022	-8	5.443	-0.527
<i>Ghir_D08G003190</i>	699	80.022	-27.5	4.728	-0.584
<i>Ghir_D08G003210</i>	699	80.048	-27.5	4.729	-0.576
<i>Ghir_D08G012920</i>	704	81.051	-25	4.772	-0.595
<i>Ghir_D11G023100</i>	287	33.097	11.5	9.324	-0.122
<i>Ghir_D12G026910</i>	730	84.354	-2	6.219	-0.525
<i>Ghir_D13G009090</i>	791	90.098	-32	4.605	-0.428
<i>Ghir_D13G014300</i>	699	80.125	-27.5	4.725	-0.589
<i>Ga01G0999</i>	699	80.066	-27.5	4.726	-0.572
<i>Ga01G1189</i>	812	92.816	-33	4.66	-0.695
<i>Ga01G2599</i>	707	81.145	-27.5	4.728	-0.609
<i>Ga02G1769</i>	699	80.001	-28.5	4.704	-0.57
<i>Ga02G1770</i>	699	80.068	-27.5	4.726	-0.58
<i>Ga03G1468</i>	790	90.034	-32.5	4.602	-0.56
<i>Ga05G1968</i>	128	14.527	-9	4.428	-0.663
<i>Ga06G0030</i>	789	90.293	-40	4.549	-0.729
<i>Ga06G0031</i>	804	91.746	-36.5	4.599	-0.702
<i>Ga07G2203</i>	797	90.453	-15.5	4.995	-0.577

(Continued)

TABLE 1 Continued

Gene ID	Protein Length (aa)	Molecular Weight (kDa)	Charge	Isoelectric Point	Grand Average of Hydropathy
<i>Ga08G1356</i>	689	79.615	-23	4.815	-0.628
<i>Ga12G0240</i>	703	80.742	-27.5	4.701	-0.613
<i>Ga13G1166</i>	792	90.016	-33.5	4.577	-0.564
<i>Ga13G1641</i>	699	80.139	-26.5	4.748	-0.587
<i>Gorai.001G220600</i>	797	90.448	-15.5	4.995	-0.569
<i>Gorai.002G103000</i>	699	80.007	-28.5	4.707	-0.566
<i>Gorai.002G122800</i>	809	92.407	-32	4.674	-0.684
<i>Gorai.003G155600</i>	707	81.117	-24.5	4.792	-0.617
<i>Gorai.004G033900</i>	699	80.02	-27.5	4.728	-0.576
<i>Gorai.004G034000</i>	666	76.096	-24.5	4.765	-0.561
<i>Gorai.004G138600</i>	704	81.025	-24	4.794	-0.591
<i>Gorai.005G148100</i>	832	94.919	-34.5	4.578	-0.47
<i>Gorai.007G238900</i>	274	31.959	4.5	8.557	-0.398
<i>Gorai.008G274600</i>	703	80.744	-27	4.724	-0.613
<i>Gorai.010G003000</i>	1,084	123.601	-34	4.816	-0.666
<i>Gorai.010G173700</i>	226	26.195	3	8.415	-0.522
<i>Gorai.013G098300</i>	777	89.036	-32	4.639	-0.506
<i>Gorai.013G150300</i>	699	80.125	-27.5	4.725	-0.589

corroborated the findings of HNZZ2019–2520 as salt tolerant genotype, which was further used for downstream experiments and validation results.

Genome-wide identification of HSP90 gene family members

A total of 28 *G. hirsutum*, 14 *G. arboreum*, and 14 *G. raimondii* genes were identified. The genomic, CDS, and protein features of the *HSP90* genes of *G. hirsutum* were then identified, such as genomic length (bp), protein length (aa), CDS length (bp), locus ID with corresponding chromosome number, strand polarity, start and end points, predicted isoelectric points (PI), predicted masses, and protein molecular weights (MW), and charges as shown in [Tables 1, 2](#). A comparative visualization of the protein length and molecular weight of *HSP90* proteins has been shown in [Figure 2](#). Retrieving the information of *HSP90* genes revealed that *Ga05G1968*, which was detected on chromosome chr05 in *G. arboreum*, coded the smallest protein of 128 amino acids (aa), with a molecular weight of 14.527 kDa. Meanwhile, *Gorai.010G003000*, identified on chromosome chr10 in *G. raimondii*, coded the largest protein among all *HSP90* members in three *Gossypium* species, having 1084 aa, with a molecular weight of 123.601 kDa as shown in [Figure 2](#) and [Table 2](#). The isoelectric point (PI) of the *HSP90* genes ranged from 4.423 (*Ghir_A05G018960*) to 9.324 (*Ghir_D11G023100*). The theoretical isoelectric point of all the

proteins except three proteins was > 7. Whereas, the grand average of hydropathy of all *HSP90* proteins was negative. Moreover, in-silico analysis using Cell-Ploc, WoLF PSORT, and LocTree3 revealed that the highest number of genes were localized in the cytoplasm 26, 29, and 43 respectively, with the LocTree3 predicting the highest as given in [Supplementary Table S4](#). Cell-Ploc and WoLF PSORT were significantly in agreement in terms of gene localization in the cytoplasm. The endoplasmic reticulum contained 14, 7, and 13 genes respectively, with WoLF PSORT predicting the most. The results of Cell-Ploc and LocTree3 were in compliance in terms of *HSP90*s localization to the endoplasmic reticulum.

Evolutionary relationship and classification of HSP90 family genes

To analyze the evolutionary relationships of the *HSP90* genes, we constructed a neighbor-joining (NJ) phylogenetic tree based on multiple sequence alignments of 56 *HSP90* proteins of *Gossypium* species (*G. hirsutum* (28), *G. arboreum* (14), and *G. raimondii* (14), 7 proteins of *A. thaliana*, 9 of *Oryza sativa*, and 18 of *Triticum aestivum* as shown in [Figure 3A](#). To get further insights into the evolutionary relationship among the three cotton species under study, another phylogenetic tree was constructed as shown in [Figure 3B](#). Both the phylogenetic trees were divided into three subgroups ([Figures 3A, B](#)). The *Arabidopsis thaliana*, *Oryza sativa*,

TABLE 2 Genomic features of three cotton species.

Gene ID	Chromosome	Start	End	Strand	Length (bp)
<i>Ghir_A01G008350</i>	A01	14,553,402	14,556,889	–	3,488
<i>Ghir_A01G009870</i>	A01	21,024,434	21,028,720	+	4,287
<i>Ghir_A03G002790</i>	A03	4,241,366	4,243,296	+	1,931
<i>Ghir_A03G002800</i>	A03	4,243,323	4,243,736	+	414
<i>Ghir_A03G012060</i>	A03	70,117,279	70,123,061	+	5,783
<i>Ghir_A05G018960</i>	A05	17,997,552	17,998,809	+	1,258
<i>Ghir_A06G000260</i>	A06	168,499	179,965	+	11,467
<i>Ghir_A07G020520</i>	A07	89,766,421	89,770,702	–	4,282
<i>Ghir_A08G003090</i>	A08	3,069,702	3,072,964	–	3,263
<i>Ghir_A08G003100</i>	A08	3,101,817	3,105,495	–	3,679
<i>Ghir_A08G026410</i>	Scaffold1354	10,888	12,251	–	1,364
<i>Ghir_A12G026820</i>	A12	105,567,937	105,571,751	–	3,815
<i>Ghir_A13G009660</i>	A13	50,901,086	50,906,257	+	5,172
<i>Ghir_A13G013590</i>	A13	88,404,614	88,408,124	+	3,511
<i>Ghir_D01G008810</i>	D01	12,233,303	12,236,902	–	3,600
<i>Ghir_D01G010630</i>	D01	16,985,080	16,989,632	+	4,553
<i>Ghir_D02G013530</i>	D02	45,437,332	45,444,255	+	6,924
<i>Ghir_D03G016230</i>	D03	48,953,760	48,957,307	–	3,548
<i>Ghir_D06G000070</i>	D06	42,078	53,776	+	11,699
<i>Ghir_D06G016230</i>	D06	54,670,770	54,673,493	–	2,724
<i>Ghir_D07G020620</i>	D07	51,989,192	51,994,307	–	5,116
<i>Ghir_D08G003190</i>	D08	2,955,211	2,962,627	–	7,417
<i>Ghir_D08G003210</i>	D08	2,974,607	2,978,282	–	3,676
<i>Ghir_D08G012920</i>	D08	44,530,614	44,533,841	–	3,228
<i>Ghir_D11G023100</i>	D11	34,873,214	34,875,498	–	2,285
<i>Ghir_D12G026910</i>	D12	60,716,055	60,719,148	–	3,094
<i>Ghir_D13G009090</i>	D13	18,346,522	18,351,714	+	5,193
<i>Ghir_D13G014300</i>	D13	46,081,670	46,084,965	+	3,296
<i>Ga01G0999</i>	Chr01	15,324,156	15,327,045	–	2,890
<i>Ga01G1189</i>	Chr01	22,951,441	22,955,605	+	4,165
<i>Ga01G2599</i>	Chr01	110,018,507	110,021,550	+	3,044
<i>Ga02G1769</i>	Chr02	98,686,251	98,689,179	+	2,929
<i>Ga02G1770</i>	Chr02	98,720,919	98,723,567	+	2,649
<i>Ga03G1468</i>	Chr03	94,539,619	94,545,399	+	5,781
<i>Ga05G1968</i>	Chr05	17,861,689	17,862,394	+	706
<i>Ga06G0030</i>	Chr06	171,955	176,076	+	4,122
<i>Ga06G0031</i>	Chr06	178,338	182,462	+	4,125
<i>Ga07G2203</i>	Chr07	89,108,505	89,113,619	–	5,115

(Continued)

TABLE 2 Continued

Gene ID	Chromosome	Start	End	Strand	Length (bp)
Ga08G1356	Chr08	92,103,514	92,111,427	–	7,914
Ga12G0240	Chr12	1,914,986	1,917,873	+	2,888
Ga13G1166	Chr13	59,371,166	59,376,339	+	5,174
Ga13G1641	Chr13	102,610,798	102,613,481	+	2,684
Gorai.001G220600	Chr01	44,749,309	44,754,987	–	5,679
Gorai.002G103000	Chr02	13,118,219	13,121,608	–	3,390
Gorai.002G122800	Chr02	17,649,893	17,654,527	+	4,635
Gorai.003G155600	Chr03	42,463,639	42,467,252	–	3,614
Gorai.004G033900	Chr04	2,773,498	2,776,641	–	3,144
Gorai.004G034000	Chr04	2,786,127	2,789,058	–	2,932
Gorai.004G138600	Chr04	38,870,293	38,873,451	–	3,159
Gorai.005G148100	Chr05	40,579,037	40,585,408	+	6,372
Gorai.007G238900	Chr07	32,618,140	32,619,777	–	1,638
Gorai.008G274600	Chr08	55,290,618	55,294,359	–	3,742
Gorai.010G003000	Chr10	124,875	135,837	+	10,963
Gorai.010G173700	Chr10	50,606,514	50,609,166	–	2,653
Gorai.013G098300	Chr13	17,479,456	17,484,611	+	5,156
Gorai.013G150300	Chr13	41,037,208	41,040,239	+	3,032

and *Triticum aestivum* were exclusively present in clade III with *Gossypium* species whereas clade I and II contained only cotton genes. *Ghir_A08G003100* and *Ga02G1769* were present in clade I, whereas *Ghir_D08G03190*, *Ghir_DO8G003210* of *G. hirsutum*, *Gorai.004G033900* and *Gorai.004G034000* of *G. raimondii* were present in clade II [Figure 3A](#). A similar pattern was observed in the phylogenetic tree of cotton species as shown in [Figure 3B](#).

Chromosomal mapping

To determine the division of *HSP90* genes on different chromosomes of cotton, we constructed the chromosomal map with information taken from three cotton genomes as demonstrated in [Figure 4](#). The results showed that most of the 56 genes were scattered irregularly across 13 chromosomes, while one gene *Ghir_A08G026410* was located on a scaffold. *G. hirsutum* was attributed with 13 genes distributed on A-subgenome whereas 14 genes were located on D-subgenome. Moreover, chromosomes *A03* and *D08* were found to have the highest number of *HSP90* genes, having 3 genes on each chromosome, followed by chromosomes *A01*, *A08*, *A13*, *D01*, *D06*, and *D13*, with 2 genes each; the lowest number of genes 1 was found on chromosomes *A05*, *A06*, *A07*, *A12*, *D02*, *D03*, *D07*, *D11* and *D12*. In *G. arboreum*, chromosome 01 had the highest number of gene 3, followed by 2 genes on chr 02, chr06, and chr13 the lowest number of gene 1 was found on chromosomes 03, chr05, chr07, chr08, and chr12. In *G. raimondii*, the highest

number of genes were found on chromosome 04, with 3 genes, and the lowest number of genes only one *HSP90* member was found on chromosomes chr01, chr03, chr05, chr07, and chr08. Moreover, to explore the selection pressure we calculated the *Ka*, *Ks*, and *Ka/Ks* values of identified genes. The *ka/Ks* > demonstrates a positive selection, whereas *Ka/Ks* = 1 denotes neutral selection and *Ka/Ks* <1 corresponds to the negative selection pressure. In the current study, the *Ka/Ks* values of all the *HSP90* genes were < 1, suggesting that these gene pairs evolved under negative selection pressure in upland cotton as given in [Supplementary Table S3](#).

Gene structure, conserved motifs, and Cis-regulatory elements

To determine the gene structures and evolutionary tree of *HSP90* genes, the intron-exon structures of 56 cotton genes were analyzed using the TBtools software, as shown in [Figures 5A, B](#). The analysis revealed that one gene *Ghir_A03G002800* had only one exon, while the gene *Gorai.010G003000* had the highest number of exons i.e., 22. Forty motifs were identified using (<http://meme-suite.org/>). Motifs 1 and 2 were found most conserved out of all 40 motifs as shown in [Figure 5C](#). The *cis*-acting elements in the promoter region play important roles in the plant responses to stress, and, participate in the responses to drought, ABA, and other stresses ([Yazaki and Kikuchi, 2005](#); [Nakashima and Yamaguchi-Shinozaki, 2013](#)). The twelve most abundant *cis*-acting elements

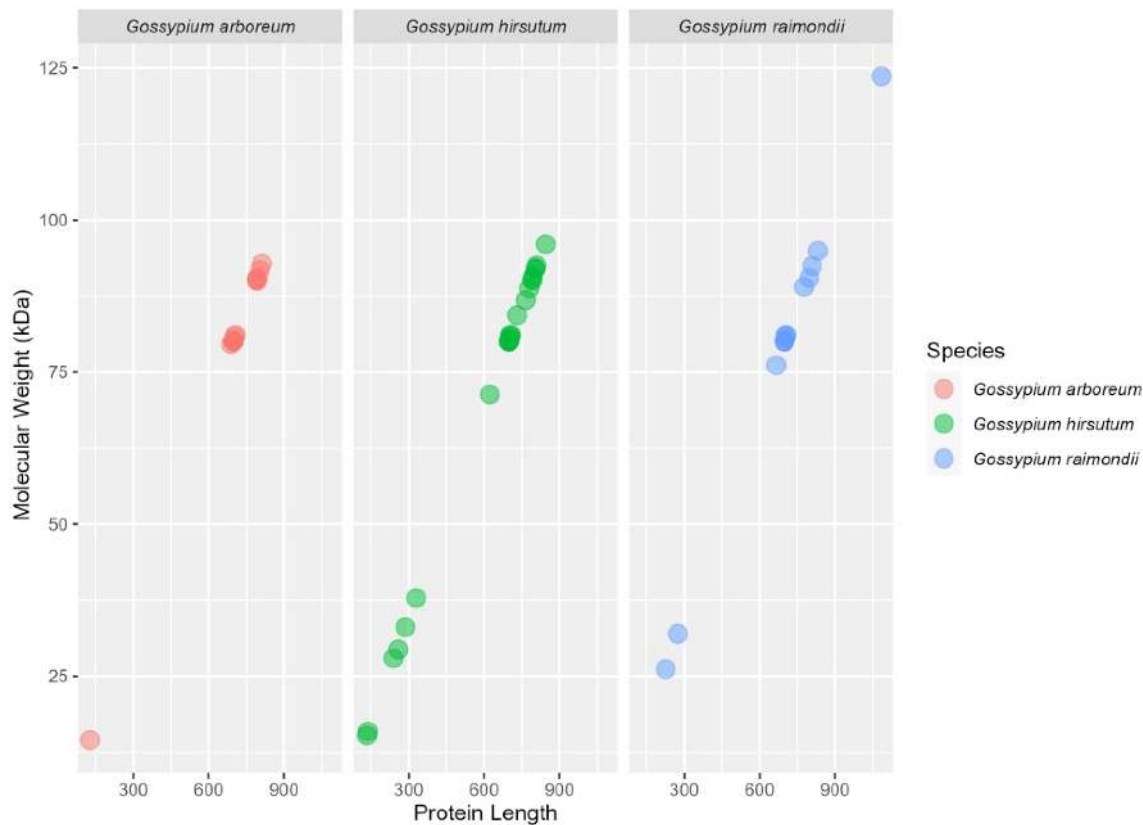


FIGURE 2
The plot depicts the estimation of the amino acids length on X-axis and molecular weight of HSP90 proteins on Y-axis.

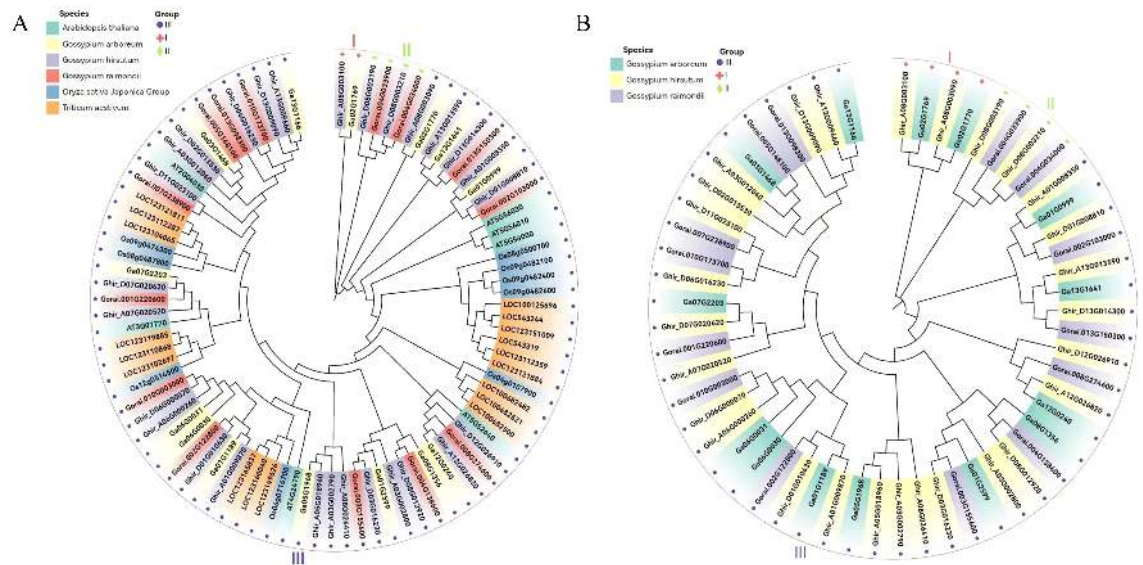
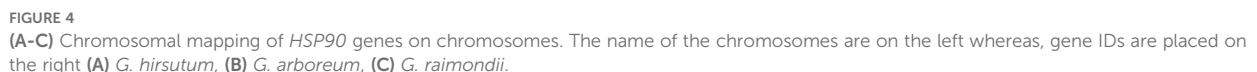
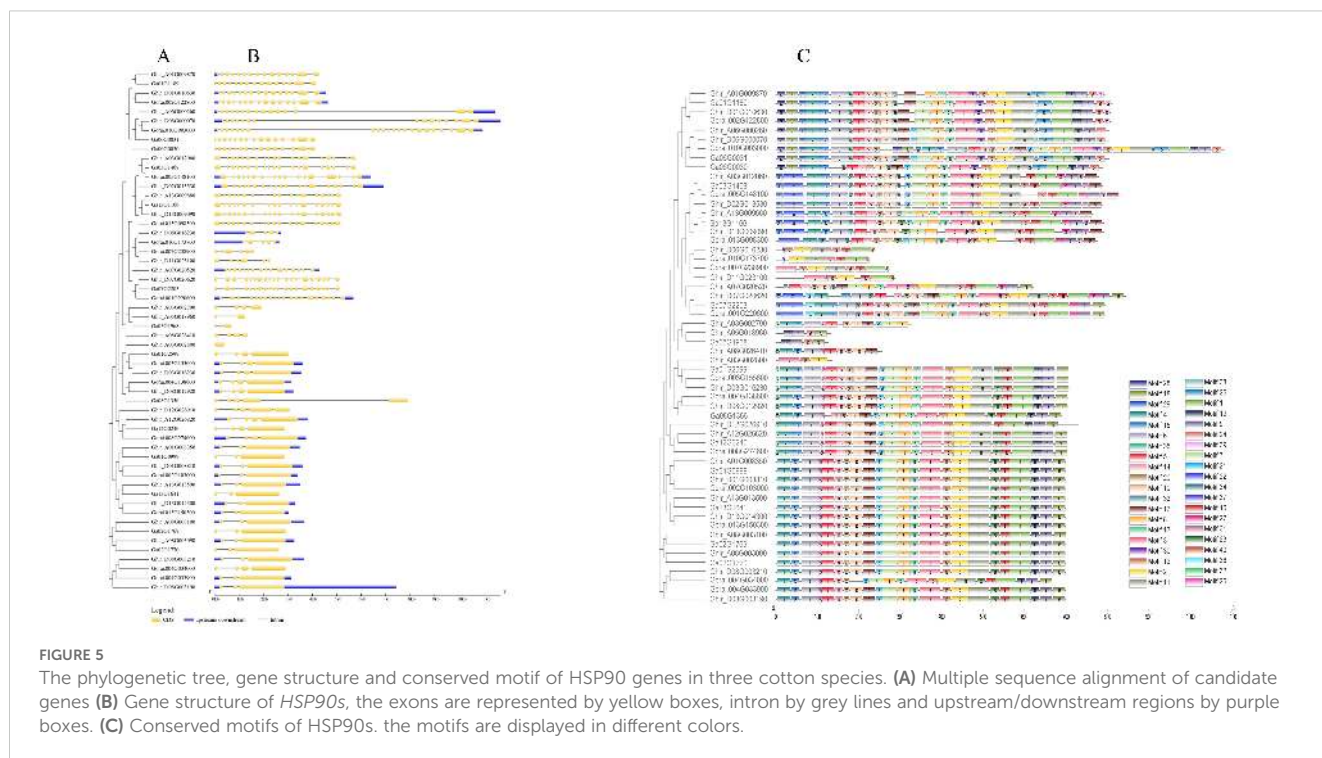


FIGURE 3
Phylogenetic analysis. (A) Neighbor-joining phylogenetic tree of 56 HSP90 genes of the three cotton species, model plant *Arabidopsis thaliana*, *Oryza sativa*, and *Triticum aestivum*. (B) Phylogenetic tree of three cotton species.



Transcriptome profiling and qRT-PCR analysis

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transcripts, and can usually be used as transcript frequency or gene expression level (Zhao et al., 2021). The cluster analysis and heat-map visualization based on the level of expression of transcripts divided *HSP90* genes into three groups A, B, and C. In A group which includes 28 genes, 6 were differentially expressed at 1, 3, 6, 12, and 24 hours after heat and salt stress application (Figure 7A). Notably, *Ghir_D08G003210* and *Ghir_A08G003090* depicted early differential expression following 1-hour heat treatment. Moreover, *Ghir_A03G012060* and *Ghir_D02G013530* were highly expressed after 6 and 12 hours of heat stress. Interestingly, *Ghir_D08G003210* and *Ghir_A08G003090* were also expressed during salt stress at 3, 6, 12, and 24 hours of salt stress. In the B group, *Ghir_A13G013590* revealed high expression after 24-hour salt stress. In the C group, *Ghir_A12G026820* exhibited high expression under 1h salt and 24h heat stress but the remaining members in this group showed significantly low expression. To validate these findings, ten genes were selected for qRT-PCR analysis. Among them, *Ghir_A03G002790* was significantly upregulated at 48h salt treatment in salt-tolerant cultivar as compared to salt-sensitive cultivar (Figure 7B). Similarly, *Ghir_D03G016230*, *Ghir_A07G020520*, *Ghir_D02G013530*, and *Ghir_D12G026910* depicted significant differential expression between salt-tolerant and salt-sensitive cotton cultivars. Finally, based on these qRT-PCR results, we selected two differentially expressed genes *Ghir_D03G016230* and *Ghir_D02G013530* for functional validation.

Effect of gene silencing on ROS scavenging enzymes (SOD, POD, CAT), and lipid peroxidation marker MDA

Reactive oxygen species (ROS) scavenging enzymes such as SOD (Superoxide Dismutase) POD (Peroxidase) and, CAT (Catalase) play

an imperative role in the plant's defense mechanism against oxidative stress (Fujita and Hasanuzzaman, 2022). The silencing of *Ghir_D02G013530* and *Ghir_D03G016230* exhibited a significant difference in the expression of the scavenging enzymes and lipid peroxidation marker under salt stress at different time intervals. Figures 7C, D exhibits the contents of SOD, POD, CAT, and MDA in cotton seedlings subjected to the silencing of upregulated genes. The silencing of *Ghir_D02G013530* exhibited an overall decrease of 23.43% in SOD content, 13.29% in POD content, and 49.53% in CAT contents of leaf tissue under salt stress. Moreover, a 93.71% increase in MDA content was observed. The lowest SOD, POD, and CAT contents -30.65%, -22.89%, and -85.61%, were found at 3h, 12h, and 24h time intervals respectively. Likewise, the highest MDA content 167.47% increase was found at a 3h time interval. Similarly, the silencing of *Ghir_D03G016230* resulted in an average decrease of 24.50% in SOD expression, 16.32% in POD expression, and 7.30% in CAT expression in leaf tissue of the cotton seedlings under salt stress. Whereas, an overall 112.22% increase in MDA content was observed in *Ghir_D03G016230* silenced plants, moreover highest differences of SOD, POD, and CAT contents -38.47%, -34.94%, and -15.45% were found at 48h, 12h, and 3h time intervals. In addition, leaf relative water contents and chlorophyll contents significantly reduced in cotton plants subjected to the silencing of *Ghir_D03G016230* and *Ghir_D02G013530* and subsequently treated with salt stress Figure 8.

Discussion

Climate change is exacerbating the detrimental impact of high temperatures on crop growth, necessitating an intensified focus on investigating plant responses to heat stress to ensure optimal plant

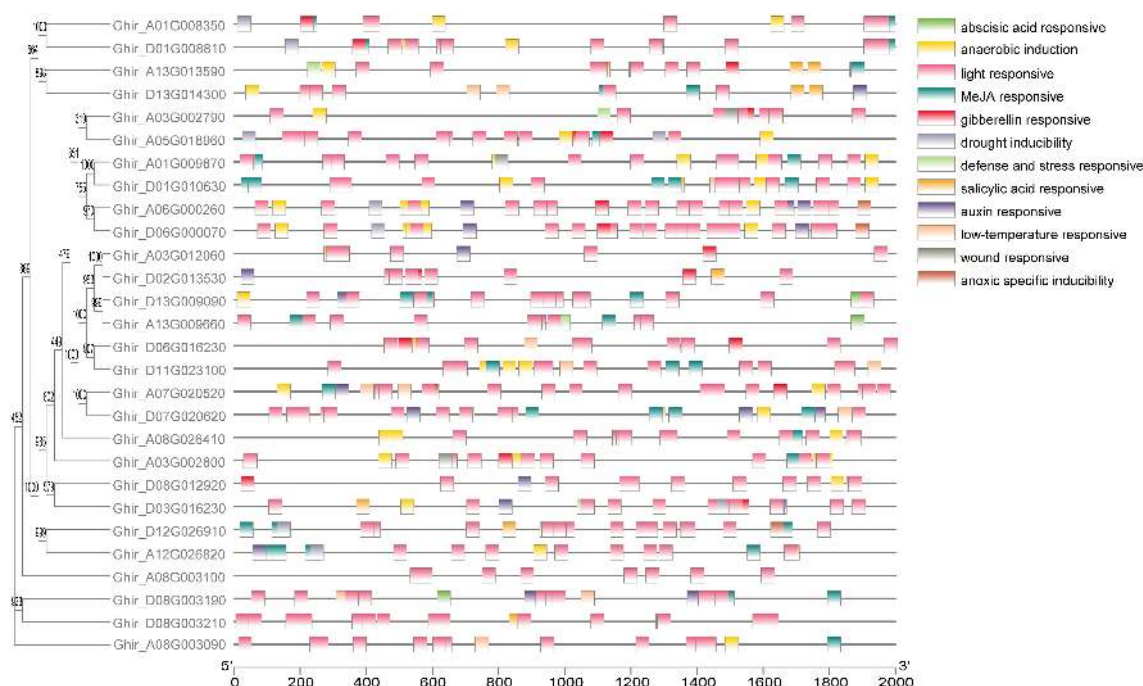


FIGURE 6

Cis-acting elements on promoter regions of *HSP90* genes in *G. hirsutum*. The evolutionary tree and cis-acting elements of *HSP90*s. Different colors represent cis-acting elements mediating stress tolerance pathway.

development (Shinozaki et al., 2006). Elevated temperatures, specifically a 5°C increase compared to normal conditions, impede physiological and biochemical reactions in organisms, leading to the inhibition of protein synthesis and mRNA transcription due to heat stress (Mittler et al., 2012). Under high-temperature stress, the expression of stress-related genes is often altered, with heat shock proteins (HSPs) being crucial for temperature acclimation. Heat shock proteins, such as HSP90, are rapidly synthesized in response to heat stress to facilitate temperature tolerance (Pearl and Prodromou, 2000; Tissières et al., 1974). In addition to heat stress, salt stress poses significant challenges to plant growth. Salinity, a prominent abiotic stress factor, primarily disrupts cellular homeostasis and ion distribution, leading to osmotic stress. Excessive salinity adversely affects multiple stages of plant development, causing growth retardation, biomass reduction, and leaf senescence (Munns and Tester, 2008; Parida and Das, 2005). Although heat shock protein HSP90 has been characterized in numerous plant species, limited information is available regarding HSP90 in cotton. Hence, in this study, we conducted a comprehensive analysis to identify and genetically examine the *HSP90* gene family in *Gossypium hirsutum*. Furthermore, we investigated the expression patterns of these genes in response to salt and heat stress, thereby shedding light on their potential functions in *G. hirsutum*.

In contrast to the previously identified *HSP90* gene families in other plant species, such as 7 in *A. thaliana* and 9 in rice (Krishna and Gloor, 2001), a significant increase in the number of *HSP90* genes was observed in three cotton species, with a total of 56 genes identified. This substantial expansion in gene count could be

attributed to the polyploidization event that occurred in *Gossypium hirsutum* following the hybridization of *G. arboreum* and *G. raimondii* (Wang et al., 2018). We identified 14 *HSP90* genes each from *Gossypium arboreum* and *G. raimondii*, and 28 from *G. hirsutum*. This observation aligns with the known formation of allotetraploid *G. hirsutum* through the hybridization of *G. arboreum* and *G. raimondii* (Hu et al., 2019). The theoretical isoelectric points of all the proteins of *G. arboreum* were < 7, whereas all proteins of *G. hirsutum* were ascribed to have isoelectric points < 7 except for one protein. Only two proteins of *G. raimondii* have isoelectric point > 7. These results indicated that the *HSP90* proteins of all the *Gossypium* species were acidic (Xia, 2007). Moreover, the grand average of hydropathy values of all proteins of cotton species was negative, indicating that all proteins were hydrophilic Table 1. The phylogenetic analysis of *Gossypium* and other plant species revealed that *HSP90* genes in subgroup III have retained higher similarities in function and structure with non-cotton species. Moreover, subgroups I and II have likely undergone more lineage-specific evolution, indicating diversification among cotton species, probably adapting to certain specific environmental conditions and functional specialization exclusive to cotton as shown in Figure 3A. The phylogenetic analysis of three *Gossypium* species Figure 3B, further corroborated the results of a combined tree of cotton and other species. the phylogenetic tree of cotton species suggested that *HSP90* genes among cotton species were not only conserved throughout evolution as subgroup III consists of genes of three cotton species showing a shared evolutionary pathway. More interestingly, it was observed that subgroup II has two genes from D subgenome of *G. hirsutum* grouped together with two genes of

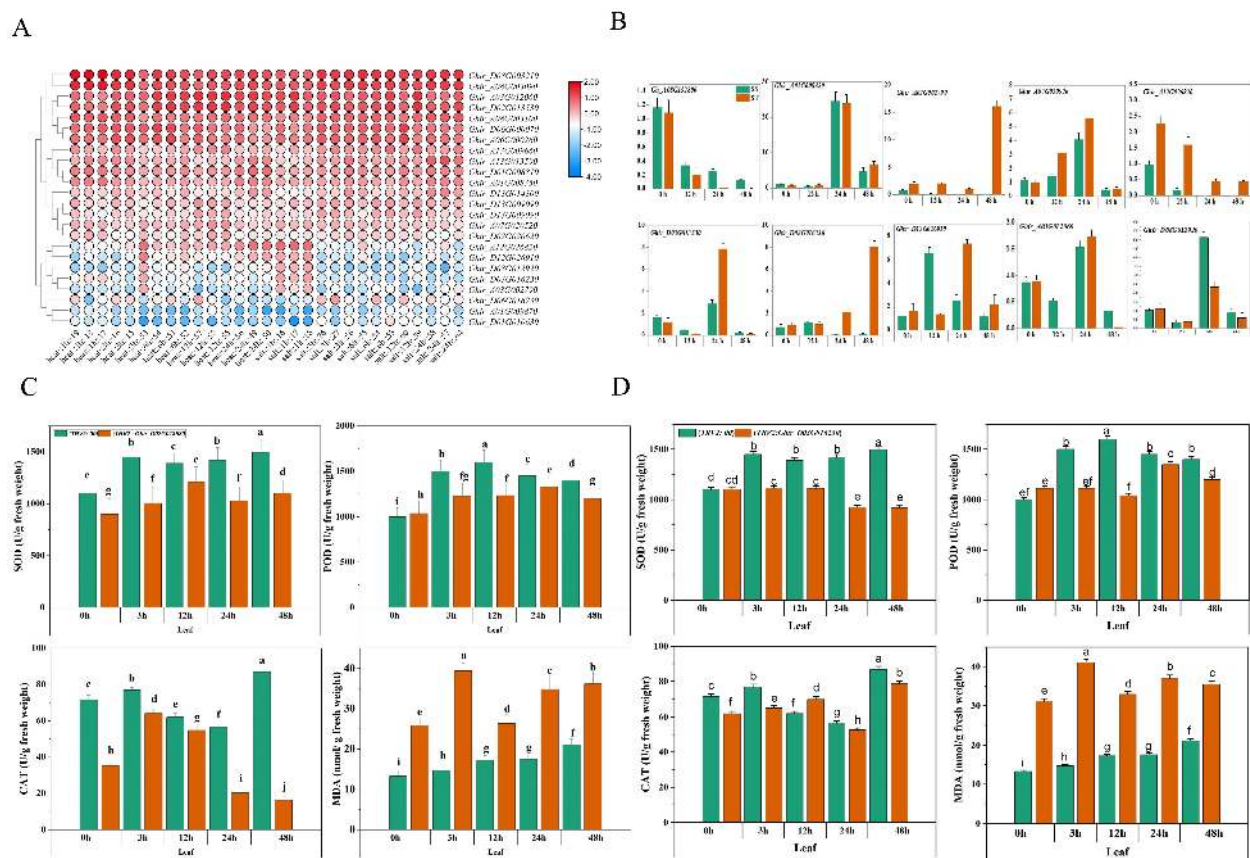


FIGURE 7

RNA expression profiling and qRT-PCR of *HSP90* genes of *G. hirsutum*. (A) Heatmap showing expression of *HSP90* genes under heat and salt stress at different time intervals. (B) qRT-PCR of ten genes under salt stress (C) Comparative expression of SOD, POD, CAT and MDA in leaf tissues of *Ghir_D03G016530* and (D) *Ghir_D02G013230* silenced plants of *G. hirsutum*. Different letters represent significant difference at $p < 0.05$.

G. raimondii, which is an indication of the contribution of the special function genes from *raimondii* to *hirsutum*. These genes confirm that they have diverged from their progenitor (*G. raimondii*) to upland cotton. *G. hirsutum* is an allotetraploid species ($2n = 4x = 52$) that originated from a hybridization event between A-genome species like *G. arboreum* or *G. herbaceum* and D genome progenitor-like *G. raimondii*. Comparative genomics analysis in previous studies has reported evidence of D-genome contribution from *G. raimondii* to *G. hirsutum*. Moreover, it can be inferred that during the inheritance of these genes there might still be some subtle evolutionary modifications that assisted *G. hirsutum* to adapt to new ecological niches or changing environmental patterns as compared to *G. raimondii*. These insights support our hypothesis that certain *HSP90* genes mediating heat tolerance also play a key role in salt tolerance. Overall, It was found that the *HSP90* genes in *Gossypium* species exhibited significant similarity and a monophyletic distribution within three phylogenetic subgroups, indicating a conserved evolutionary pattern of these genes.

In the current study, an examination of the gene structure in different groups revealed that the number of exons and the exon-intron arrangements of the *HSP90* genes within each group were highly similar as shown in Figure 5B. Notably, three motifs (1, 2, and 3) as depicted in Figure 5C, were identified as being highly

conserved, which might be due to selective pressure throughout the evolutionary time. They were present in all *HSP90* genes across the three *Gossypium* species. Furthermore, the distribution of these motifs exhibited a consistent pattern among all *HSP90* genes. The highly conserved motifs often indicate the crucial functional elements within genes. These motifs play key functional roles in several important processes such as DNA binding, enzymatic actions, and protein-protein interactions. The findings of conserved motifs are indicative of their key roles necessary for the adaptations and proper functioning of the plant. Motifs which are part of the regulatory regions, may be critical for the desired expression of the genes mediating key developmental phases and stress responses. These analyses provide evidence supporting the reliability of the evolutionary classification of *HSP90* genes.

Excessive salinity, in particular, is a significant variable that adversely affects cotton production globally (Sharif et al., 2019). The analysis of *cis*-regulatory elements (CREs) revealed that the *HSP90* genes possess a substantial enrichment of important *cis*-regulatory elements that play a crucial role in mitigating environmental stresses. In the current study, a diverse array of CREs such as abscisic acid-responsive elements (ABRE), anaerobic induction, light, Methyl jasmonate (MeJA), gibberellin, drought, defense, salicylic acid, auxin, low-temperature, wound responsive CREs

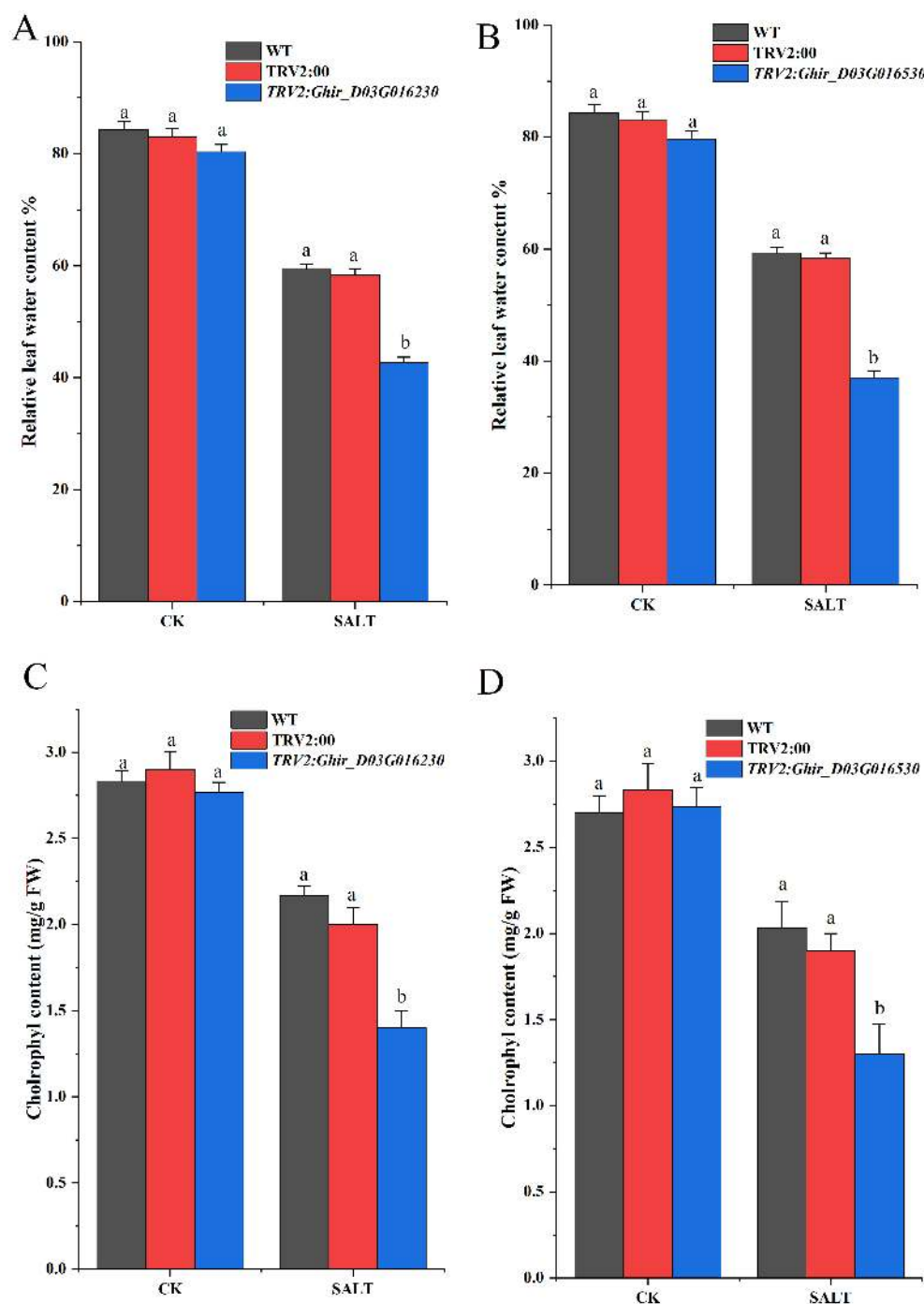


FIGURE 8

Quantitative determination of chlorophyll and leaf water contents. (A,B) Relative leaf water content of wild-type, control and *Ghir_D02G013230* and *Ghir_D03G016530* silenced plants (C,D) chlorophyll contents of wild-type, control and *Ghir_D02G013230* and *Ghir_D03G016530* silenced plants after 8-days of salt stress exposure. Letters depict significant differences (two-tailed, $p < 0.01$). each experiment was conducted in triplicate.

were found across the three cotton species Figure 6. Among these *cis*-regulatory elements, several CREs play pertinent roles in the salt and heat stress tolerance of cotton plants. Among these CREs, ABREs and MeJA-responsive elements play a key role in salt tolerance in plants. Previous studies have reported that the ABA hormone accumulates under salt stress and binds to the receptors initiating the ABA signaling pathway (Feng et al., 2019). This

binding results in the activation of basic leucine zipper bZIP transcription factors called ABRE binding factors ABFs (Shrestha et al., 2021). The ABFs resultantly bind to the ABREs present in the promoter region of their respective genes, thereby inducing their transcription (Bhat et al., 2024). Several ABFs encode proteins crucial for the salt tolerance mechanisms such as biosynthesis of proline and glycine betaine osmolytes to maintain osmotic balance.

Moreover, activation of the antioxidant system and signaling of stress-responsive signaling cascades aid in the salt tolerance of plants. Similarly, MeJA enhances salt tolerance of plants in several ways such as regulating antioxidant systems and phytohormones levels, inducing stress-responsive genes thereby protecting cell organelles and interacting with other signaling pathways (Delgado et al., 2021; Yin et al., 2023; Zhang et al., 2023). The MeJA induces the activities of reactive oxygen species scavenging enzymes such as SOD, POD, and CAT to alleviate oxidative damage caused by salt stress. Moreover, a study reported that MeJA increases the levels of auxin and cytokinins to enhance plant growth and delay senescence under salt stress (Zhang et al., 2023). These findings further support our results of comparatively elevated levels of SOD, POD, and CAT in salt-tolerant genotype HN2019-2520 than salt-sensitive genotype HN2019-252, and the potential role of MeJA responsive elements in regulating salt tolerance mechanism.

Multiple studies have provided evidence of the involvement of *HSP90* genes in response to abiotic stress (Panaretou, 1998; Pearl and Prodromou, 2000). Song et al. studied the dynamic expression levels of *Nicotiana tabacum* L. *HSP90* genes under various abiotic stress conditions, including salt, ABA, drought, cold, and heat stresses. The results demonstrated that the expression of *NtHSP90-4*, *NtHSP90-5*, and *NtHSP90-9* was up-regulated. Furthermore, the expression of *NtHSP90* genes was induced by salt, ABA, drought, cold, and heat stresses, indicating their potential involvement in abiotic stress responses (Song et al., 2019).

The heatmap of publicly available data of *HSP90* genes exhibited upregulation of most of the genes under heat and salt stress at different time intervals, which is indicative of the fact that *HSP90* genes play a key role in salt and heat stress tolerance. Moreover, the qRT-PCR analysis of key candidate genes exhibited differential expression of the *HSP90* genes in salt-tolerant and sensitive genotypes, which is an indication of the activity of *HSP90* genes in the salt tolerance mechanism. A previous study of *HSP90* genes in *Brassica napus* L. confirmed that *HSP90* genes play a critical role in biotic and abiotic stress, particularly under salt stress (Wang et al., 2022). Zhang et al. reported that expression of *HSP90* genes was prolonged or increased under salt stress in *Brachypodium distachyon* L (Zhang et al., 2017).

In the present study, an analysis of expression patterns revealed that the expressions of *Ghir_D08G003210*, *Ghir_A08G003090*, *Ghir_A13G013590*, and *Ghir_A12G026820* were induced by salt stress. Additionally, in the qRT-PCR analysis, *Ghir_A07G020520*, *Ghir_D02G013530*, *Ghir_D03G016230*, and *Ghir_D12G026910* exhibited significant differential expression between salt-sensitive and salt-tolerant genotypes of *G.hirsutum*. Significantly higher expression of certain *HSP90* genes and elevated accumulation of SOD, POD and CAT enzymes in salt tolerant genotype explain the level of tolerance of cotton plants under salt stress. Moreover, the silencing of candidate genes *Ghir_D02G013530*, and *Ghir_D03G016230* revealed that the expression of SOD, POD, and CAT was intertwined with the expression of potential candidate genes of *HSP90s*. The silencing of *Ghir_D02G013530* and *Ghir_D03G016230* significantly reduced the accumulation of

ROSs scavenging enzymes under salt stress and elevated the accumulation of MDA in salt tolerant genotype. It is a clear indication of the damage caused by salt stress and no/low expression of the *HSP90* candidate genes. Moreover, reduction in leaf relative water contents and chlorophyll contents paraded that silencing of the candidate genes significantly reduced the ability of cotton seedlings to tolerate the effects caused by the salt stress. These findings provide important insights into the potential role of *HSP90* genes in the salt tolerance mechanism of cotton plants. However, further functional validation of candidate genes and the production pattern of SOD, POD, CAT, and MDA in a large collection of germplasm could help to understand the markers of salt tolerance mechanism in cotton plants. In agreement with the previous reports of the potential role of *HSP90* genes in heat stress tolerance, the results of the current study revealed that *HSP90* genes not only play a key role in heat tolerance but also the salt tolerance mechanism of plants due to shared genes of multiple stress tolerance pathways.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author/s.

Author contributions

ZH: Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing; QF: Data curation and Formal analysis, Writing-original draft, Writing - review & editing. XM: Resources, Investigation, Software, Writing - original draft, Writing - review & editing. DQ: Formal analysis, Software, Writing - original draft. YQ: Validation, Visualization, Writing - original draft, Writing - review & editing. ZY: Visualization, Data curation, Writing - original draft, Writing - review and editing. ME: Resources, Writing - original draft, Writing - review and funding. EK: Visualization, Resources, Writing - original draft, Writing -review and funding. YM: Writing - original draft, Writing - review & editing. JR: Supervision, Writing - original draft, Writing - review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

References

- Alici, E. H., and Arabaci, G. (2016). Determination of SOD, POD, PPO and cat enzyme activities in *Rumex obtusifolius* L. *Annu. Res. Rev. Biol.* 11, 1–7. doi: 10.9734/ARRB/2016/29809
- Al-Wahaibi, M. H. (2011). Plant heat-shock proteins: A mini review. *J. King. Saud. Univ. - Sci.* 23, 139–150. doi: 10.1016/j.jksus.2010.06.022
- Bailey, T. L., Williams, N., Misleh, C., and Li, W. W. (2006). MEME: discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* 34, W369–W373. doi: 10.1093/nar/gkl198
- Bandurska, H. (2022). Drought stress responses: coping strategy and resistance. *Plants* 11, 922. doi: 10.3390/plants11070922
- Bhat, B. A., Mir, R. A., Mir, W. R., Hamdani, S. S., and Mir, M. A. (2024). Transcription factors-golden keys to modulate the plant metabolism to develop salinity tolerance. *Plant Stress* 11, 100409. doi: 10.1016/j.stress.2024.100409
- Caplan, A. J., Jackson, S., and Smith, D. (2003). Hsp90 reaches new heights. *EMBO Rep.* 4, 126–130. doi: 10.1038/sj.embor.embor742
- Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., et al. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13, 1194–1202. doi: 10.1016/j.molp.2020.06.009
- Chou, K.-C., and Shen, H.-B. (2008). Cell-PLoc: a package of Web servers for predicting subcellular localization of proteins in various organisms. *Nat. Protoc.* 3, 153–162. doi: 10.1038/nprot.2007.494
- Delgado, C., Mora-Poblete, F., Ahmar, S., Chen, J.-T., and Figueroa, C. R. (2021). Jasmonates and plant salt stress: molecular players, physiological effects, and improving tolerance by using genome-associated tools. *Int. J. Mol. Sci.* 22, 3082. doi: 10.3390/ijms22063082
- Feng, R.-J., Ren, M.-Y., Lu, L.-F., Peng, M., Guan, X., Zhou, D.-B., et al. (2019). Involvement of abscisic acid-responsive element-binding factors in cassava (*Manihot esculenta*) dehydration stress response. *Sci. Rep.* 9, 12661. doi: 10.1038/s41598-019-49083-3
- Finn, R. D., Coghill, P., Eberhardt, R. Y., Eddy, S. R., Mistry, J., Mitchell, A. L., et al. (2015). The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Res.* 44, D279–D285. doi: 10.1093/nar/gkv1344
- Fujita, M., and Hasanuzzaman, M. (2022). Approaches to enhancing antioxidant defense in plants. *Antioxidants* 11, 925. doi: 10.3390/antiox11050925
- Gao, X., Britt, R. C. Jr., Shan, L., and He, P. (2011). Agrobacterium-mediated virus-induced gene silencing assay in cotton. *JoVE* 54, e2938. doi: 10.3791/2938
- Goldberg, T., Hecht, M., Hamp, T., Karl, T., Yachdav, G., Ahmed, N., et al. (2014). LocTree3 prediction of localization. *Nucleic Acids Res.* 42, W350–W355. doi: 10.1093/nar/gku396
- Horton, P., Park, K.-J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. J., et al. (2007). WoLF PSORT: protein localization predictor. *Nucleic Acids Res.* 35, W585–W587. doi: 10.1093/nar/gkm259
- Hsu, A.-L., Murphy, C. T., and Kenyon, C. (2003). Regulation of aging and age-related disease by DAF-16 and heat-shock factor. *Science* 300, 1142–1145. doi: 10.1126/science.1083701
- Hu, Y., Chen, J., Fang, L., Zhang, Z., Ma, W., Niu, Y., et al. (2019). *Gossypium barbadense* and *Gossypium hirsutum* genomes provide insights into the origin and evolution of allotetraploid cotton. *Nat. Genet.* 51, 739–748. doi: 10.1038/s41588-019-0371-5
- Hu, W., Hu, G., and Han, B. (2009). Genome-wide survey and expression profiling of heat shock proteins and heat shock factors revealed overlapped and stress specific response under abiotic stresses in rice. *Plant Sci.* 176, 583–590. doi: 10.1016/j.plantsci.2009.01.016
- Hu, B., Jin, J., Guo, A. Y., Zhang, H., Luo, J., and Gao, G. (2015). GSDS 2.0: an upgraded gene feature visualization server. *Bioinformatics* 31, 1296–1297. doi: 10.1093/bioinformatics/btu817
- Huang, L.-Z., Zhou, M., Ding, Y.-F., and Zhu, C. (2022). Gene networks involved in plant heat stress response and tolerance. *Int. J. Mol. Sci.* 23, 11970. Available at: <https://www.mdpi.com/1422-0067/23/19/11970>.
- Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A., and Khan, N. A. (2015). Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci.* 6. doi: 10.3389/fpls.2015.00462
- King, A. M., and MacRae, T. H. (2015). Insect Heat Shock Proteins During Stress and Diapause. *Annual Review of Entomology* 60(60), 59–75. doi: 10.1146/annurev-ento-011613-162107
- Krishna, P., and Gloor, G. (2001). The Hsp90 family of proteins in *Arabidopsis thaliana*. *Cell Stress Chaperones* 6, 238–246. doi: 10.1379/1466-1268(2001)006<0238:thfopi>2.0.co;2
- Lachowicz, J., Lemus, T., Borenstein, E., and Queitsch, C. (2014). Hsp90 promotes kinase evolution. *Mol. Biol. Evol.* 32, 91–99. doi: 10.1093/molbev/msu270
- Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and clustal X version 2.0. *Bioinformatics* 23, 2947–2948. doi: 10.1093/bioinformatics/btm404
- Liu, J. J., and Ekramoddoullah, A. K. M. (2006). The family 10 of plant pathogenesis-related proteins: Their structure, regulation, and function in response to biotic and abiotic stresses. *Physiol. Mol. Plant Pathol.* 68, 3–13. doi: 10.1016/j.pmpp.2006.06.004
- Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-ΔΔCT} method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262
- Magwanga, R. O., Lu, P., Kirungu, J. N., Dong, Q., Cai, X., Zhou, Z., et al. (2019). Knockdown of Cytochrome P450 Genes Gh_D07G1197 and Gh_A13G2057 on Chromosomes D07 and A13 Reveals Their Putative Role in Enhancing Drought and

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2025.1574604/full#supplementary-material>

Salt Stress Tolerance in *Gossypium hirsutum*. *Genes* 10, 226. Available at: <https://www.mdpi.com/2073-4425/10/3/226>.

Mittler, R., Finka, A., and Goloubinoff, P. (2012). How do plants feel the heat? *Trends Biochem. Sci.* 37, 118–125. doi: 10.1016/j.tibs.2011.11.007

Mukhopadhyay, I., Nazir, A., Saxena, D. K., and Chowdhuri, D. K. (2003). Heat shock response: hsp70 in environmental monitoring. *J. Biochem. Mol. Toxicol.* 17, 249–254. doi: 10.1002/jbt.10086

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681. doi: 10.1146/annurev.arplant.59.032607.092911

Nakashima, K., and Yamaguchi-Shinozaki, K. ABA signaling in stress-response and seed development. *Plant Cell Rep.* (2013) 32, 959–970. doi: 10.1007/s00299-013-1418-1

Panaretou, B., Prodromou, C., Roe, S. M., O'Brien, R., Ladbury, J. E., Piper, P. W., et al. (1998). ATP binding and hydrolysis are essential to the function of the Hsp90 molecular chaperone *in vivo*. *The EMBO Journal* 17(16), 4829–4836. doi: 10.1093/emboj/17.16.4829

Parida, A. K., and Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Saf.* 60, 324–349. doi: 10.1016/j.ecoenv.2004.06.010

Pearl, L. H., and Prodromou, C. (2000). Structure and *in vivo* function of Hsp90. *Curr. Opin. Struct. Biol.* 10, 46–51. doi: 10.1016/S0959-440X(99)00047-0

Picard, D. (2002). Heat-shock protein 90, a chaperone for folding and regulation. *Cell. Mol. Life Sci. CMLS.* 59, 1640–1648. doi: 10.1007/PL00012491

Prasinos, C., Krampis, K., Samakovli, D., and Hatzopoulos, P. (2004). Tight regulation of expression of two Arabidopsis cytosolic Hsp90 genes during embryo development. *J. Exp. Bot.* 56, 633–644. doi: 10.1093/jxb/eri035

Pratt, W. B., and Toft, D. O. (2003). Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp. Biol. Med.* 228, 111–133. doi: 10.1177/153537020322800201

Qin, Q., Zhao, Y., Zhang, J., Chen, L., Si, W., and Jiang, H. (2022). A maize heat shock factor ZmHsf11 negatively regulates heat stress tolerance in transgenic plants. *BMC Plant Biol.* 22, 406. doi: 10.1186/s12870-022-03789-1

Queitsch, C., Sangster, T. A., and Lindquist, S. (2002). Hsp90 as a capacitor of phenotypic variation. *Nature* 417, 618–624. doi: 10.1038/nature749

Razzaque, S., Elias, S. M., Haque, T., Biswas, S., Jewel, G. M. N. A., Rahman, S., et al. (2019). Gene Expression analysis associated with salt stress in a reciprocally crossed rice population. *Sci. Rep.* 9, 8249. doi: 10.1038/s41598-019-44757-4

Rychlik, W. (2007). OLIGO 7 Primer Analysis Software. In A. Yuryev (Ed.), *PCR Primer Design Humana Press*. 35–59. doi: 10.1007/978-1-59745-528-2_2

Robert, J. (2003). Evolution of heat shock protein and immunity. *Dev. Comp. Immunol.* 27, 449–464. doi: 10.1016/S0145-305X(02)00160-X

Rodriguez, R. J., Henson, J., Van Volkenburgh, E., Hoy, M., Wright, L., Beckwith, F., et al. (2008). Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.* 2, 404–416. doi: 10.1038/ismej.2007.106

Schmedes, A., and Hölmer, G. (1989). A new thiobarbituric acid (TBA) method for determining free malondialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. *J. Am. Oil Chemists' Soc.* 66, 813–817. doi: 10.1007/BF02653674

Sharif, I., Aleem, S., Farooq, J., Rizwan, M., Younas, A., Sarwar, G., et al. (2019). Salinity stress in cotton: effects, mechanism of tolerance and its management strategies. *Physiol. Mol. Biol. Plants* 25, 807–820. doi: 10.1007/s12298-019-00676-2

Sha Valli Khan, P. S., Nagamallaiiah, G. V., Dhanunjay Rao, M., Sergeant, K., and Hausman, J. F. (2014). "Chapter 2 - abiotic stress tolerance in plants: insights from proteomics," in *Emerging Technologies and Management of Crop Stress Tolerance*. Eds. P. Ahmad and S. Rasool (Springer Singapore: Academic Press), 23–68. doi: 10.1016/B978-0-12-800875-1.00002-8

Shinozaki, F., Minami, M., Chiba, T., Suzuki, M., Yoshimatsu, K., Ichikawa, Y., et al. (2006). Depletion of hsp90 β Induces multiple defects in B cell receptor signaling*. *J. Biol. Chem.* 281, 16361–16369. doi: 10.1074/jbc.M600891200

Shrestha, A., Cudjoe, D. K., Kamruzzaman, M., Siddique, S., Fiorani, F., Léon, J., et al. (2021). Absciscic acid-responsive element binding transcription factors contribute to proline synthesis and stress adaptation in Arabidopsis. *J. Plant Physiol.* 261, 153414. doi: 10.1016/j.jplph.2021.153414

Song, Z., Pan, F., Yang, C., Jia, H., Jiang, H., He, F., et al. (2019). Genome-wide identification and expression analysis of HSP90 gene family in *Nicotiana tabacum*. *BMC Genet.* 20, 35. doi: 10.1186/s12863-019-0738-8

Sudhir, K., Glen, S., and Koichiro, T. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution.* (2016) 33 (7), 1870–1874. doi: 10.1093/molbev/msw054

Tissi eres, A., Mitchell, H. K., and Tracy, U. M. (1974). Protein synthesis in salivary glands of *Drosophila melanogaster*: Relation to chromosome puffs. *J. Mol. Biol.* 84, 389–398. doi: 10.1016/0022-2836(74)90447-1

Trono, D., and Pecchioni, N. (2022). Candidate genes associated with abiotic stress response in plants as tools to engineer tolerance to drought, salinity and extreme temperatures in wheat: an overview. *Plants* 11, 3358. Available at: <https://www.mdpi.com/2223-7747/11/23/3358>.

Ullah, A., Sun, H., Hakim, Y., and Zhang, X. (2018). A novel cotton WRKY gene, GhWRKY6-like, improves salt tolerance by activating the ABA signaling pathway and scavenging of reactive oxygen species. *Physiol. Plant.* 162, 439–454. doi: 10.1111/plp.12651

Wang, L., Liu, F., Ju, L., Xue, B., Wang, Y., Wang, D., et al. (2022). Genome Structures and Evolution Analysis of Hsp90 Gene Family in *Brassica napus* Reveal the Possible Roles of Members in Response to Salt Stress and the Infection of *Sclerotinia sclerotiorum*. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.854034

Wang, W., Vinocur, B., Shoseyov, O., and Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* 9, 244–252. doi: 10.1016/j.tplants.2004.03.006

Wang, M., Wang, P., Lin, M., Ye, Z., Li, G., Tu, L., et al. (2018). Evolutionary dynamics of 3D genome architecture following polyploidization in cotton. *Nat. Plants* 4, 90–97. doi: 10.1038/s41477-017-0096-3

Xia, X. (2007). "Protein isoelectric point," in *Bioinformatics and the Cell: Modern Computational Approaches in Genomics, Proteomics and Transcriptomics*. Springer Cham, Switzerland 207–219.

Xiong, L., and Ishitani, M. (2006). "Stress Signal Transduction: components, pathways and network integration," in *Abiotic stress tolerance in plants*. Eds. A. K. Rai and T. Takabe (Springer Netherlands), 3–29. doi: 10.1007/1-4020-4389-9_1

Yasir, M., He, S., Sun, G., Geng, X., Pan, Z., Gong, W., et al. (2019). A Genome-wide association study revealed key SNPs/genes associated with salinity stress tolerance in upland cotton. *Genes* 10, 829. Available at: <https://www.mdpi.com/2073-4425/10/10/829>.

Yasir, M., Kanwal, H. H., Hussain, Q., Riaz, M. W., Sajjad, M., Rong, J., et al. (2022). Status and prospects of genome-wide association studies in cotton. *Front. Plant Sci.* 13. doi: 10.3389/fpls.2022.1019347

Yazaki, J., Kishimoto, N., Nagata, Y., Ishikawa, M., Fujii, F., Hashimoto, A., et al. (2003). Genomics Approach to Absciscic Acid- and Gibberellin-responsive Genes in Rice. *DNA Research* 10(6), 249–261. doi: 10.1093/dnares/10.6.249

Yin, Y., Yang, T., Li, S., Li, X., Wang, W., and Fan, S. (2023). Transcriptomic analysis reveals that methyl jasmonate confers salt tolerance in alfalfa by regulating antioxidant activity and ion homeostasis. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1258498

Zhang, X., Goatley, M., Wang, K., Conner, J., Brown, I., and Kosiarski, K. (2023). Methyl jasmonate enhances salt stress tolerance associated with antioxidant and cytokinin alteration in perennial ryegrass. *Grass. Res.* 3. doi: 10.48130/GR-2023-0006

Zhang, M., Shen, Z., Meng, G., Lu, Y., and Wang, Y. (2017). Genome-wide analysis of the *Brachypodium distachyon* (L.) P. Beauv. Hsp90 gene family reveals molecular evolution and expression profiling under drought and salt stresses. *PLoS One* 12, e0189187. doi: 10.1371/journal.pone.0189187

Zhao, Y., Li, M.-C., Konat , M. M., Chen, L., Das, B., Karlovich, C., et al. (2021). TPM, FPKM, or normalized counts? A comparative study of quantification measures for the analysis of RNA-seq data from the NCI patient-derived models repository. *J. Trans. Med.* 19, 269. doi: 10.1186/s12967-021-02936-w

Zuehlke, A., and Johnson, J. L. (2010). Hsp90 and co-chaperones twist the functions of diverse client proteins. *Biopolymers* 93, 211–217. doi: 10.1002/bip.21292