

Genome-wide identification and expression profiling of cowpea *HSP60* genes reveal key candidates for temperature stress responses

Prosper Ndericimpaye^{1,2,3}, Liting Zhang¹, Di Wang¹, Xilin Hou¹, Ying Li¹, Dong Xiao^{1,2*} and Tongkun Liu^{1,2*} 

¹ Sanya Research Institute, Nanjing Agricultural University, Nanjing 210095, China

² State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing 210095, China

³ Monitoring and Evaluation, Ministry of Environment, Agriculture and Livestock, Gitega, Burundi

* Correspondence: dong.xiao@njau.edu.cn (Xiao D); liutk@njau.edu.cn (Liu T)

Abstract

Heat and cold stress negatively affect the growth and yield of cowpea (*Vigna unguiculata*). Heat shock protein 60/chaperonins (HSP60/Cpn60) play a vital function in protecting plants against temperature stress. The *HSP60* genes in different species have been the subject of multiple investigations. Nevertheless, a systematic study of *HSP60* has not yet been conducted in cowpea. The present study was carried out to identify, characterize, and assess the expression trends for HSP60 proteins under temperature stressors. We identified 25 *HSP60* genes in the cowpea genome using bioinformatic techniques. These genes are segmentally duplicated and distributed on nine different chromosomes. The conserved domain (Cpn60_TCP1), evolutionary relationships, and motifs revealed that *VuHSP60s* are highly conserved and categorized into three groups. *VuHSP60* members underwent purifying selection, and their exon/intron organization displayed structural diversity. *VuHSP60s*' 2-kb promoter regions contained potential stress-defense and hormone-associated elements. Quantitative real-time polymerase chain reaction revealed that *VuHSP60s* were differentially expressed in cowpeas when exposed to temperature shocks. *VuHSP60-1* and *VuHSP60-12* were highly expressed under both heat and cold stress. *VuHSP60-3*, *VuHSP60-5.1*, *VuHSP60-5.2*, *VuHSP60-5.3*, *VuHSP60-11.2*, *VuHSP60-14.1*, and *VuHSP60-17* were significantly upregulated under high temperature, whereas *VuHSP60-4*, *VuHSP60-7*, *VuHSP60-8*, *VuHSP60-9.1*, *VuHSP60-9.2*, *VuHSP60-13*, *VuHSP60-15*, and *VuHSP60-16* were strongly induced by low temperature. Our results provide a preliminary understanding of the molecular mechanisms behind cowpea's temperature responses and point to potential genes for future functional verification and genetic improvement of cultivars that are resistant to extreme temperatures.

Keywords: Cowpeas, *HSP60* genes, Phylogenetic analysis, Temperature stress, Expression profiling

Citation: Ndericimpaye P, Zhang L, Wang D, Hou X, Li Y, et al. 2026. Genome-wide identification and expression profiling of cowpea *HSP60* genes reveal key candidates for temperature stress responses. *Vegetable Research* 6: e034 <https://doi.org/10.48130/vegres-0026-0029>

Introduction

Cowpea (*Vigna unguiculata*) is an annual herbaceous plant of the Fabaceae family. This crop is cultivated around the world for its seeds and immature pods, which are rich in protein, as well as for its economic value^[1]. Cowpea cultivation contributes enormously to sustainable agriculture and food security for millions of smallholder farmers in tropical and subtropical areas^[2]. However, the escalating volatility of global weather patterns has subjected cowpea to extreme temperature fluctuations that exceed its adaptive thresholds^[3]. Heat and cold shocks now represent major abiotic barriers to phytogeography, growth, and yield and are directly threatening the stability of global food production amid climate changes^[4]. When a plant is exposed to inadequate temperatures, ionic balance is disturbed, membrane stability is compromised, proteins are denatured, and ultimately yield and seed quality are reduced^[5,6]. Therefore, increasing cowpea's resistance to temperature stress offers both strategic and practical agricultural benefits for the production of climate-resilient crops.

Plants suffer irreversible damage from extreme temperatures during their life cycle. Thus, plants have established a range of physiological and biological defense mechanisms to cope with stressful environments^[7]. At the molecular level, multiple stress proteins are induced, such as heat shock proteins (*HSPs*). It has been reported that *HSPs* act as molecular chaperones to withstand the detrimental effects of thermal stress. *HSPs* are categorized according to their molecular weight into five major families: *HSP100*, *HSP90*, *HSP70*,

HSP60, and small *HSPs* (*sHSPs*)^[8]. Among these, proteins in the *HSP60* family, also known as "chaperonins" (Cpn60), are crucial for protein folding, refolding, managing aggregation, and trafficking to mitochondria and chloroplasts^[9]. *HSP60* chaperones are essential for organelle function, photosynthetic efficiency, development, and overall thermotolerance in plants. They are widely found in eukaryotes, eubacteria, and archaea. In plants, *HSP60s* are generally classified into Group I chaperonins, which resemble the bacterial Growth Relation Large (GroEL) system and act with co-chaperonins (Cpn10 or Cpn20)^[10]. *HSP60* proteins are divided into α (alpha) and β (beta) subunits in plant cells. They assemble into oligomeric complexes that facilitate adenosine triphosphate (ATP)-dependent protein folding. Another form, sometimes referred to as the CCT-type chaperonins, belongs to the Group II chaperonins, mainly found in the eukaryotic cytosol and involved in folding cytoskeletal and regulatory proteins^[11]. These classes ensure adequate protein homeostasis under stress conditions.

Recent studies showed that *HSP60s* are a key regulator of plant thermotolerance, working through several molecular processes. Studies on their function have shown that knocking down *HSP60* significantly reduces the plant's ability to withstand heat stress. The silencing of *CaHSP60-6* in pepper (*Capsicum annuum*) led to an increase in sensitivity to heat stress, as evidenced by increased electrolyte leakage, lipid peroxidation, reactive oxygen species accumulation, reduced chlorophyll content, and diminished antioxidant enzyme activity. In contrast, heat treatment resulted in an upregulation of 93% of the *HSP60* genes in both thermosensitive and

thermotolerant pepper lines^[12]. In rice (*Oryza sativa*), *OsHSP60-3B* is essential for the proper formation of starch granules within pollen, thus safeguarding male fertility under elevated temperatures. The *OsHSP60-3B* mutant displayed typical fertility under ideal temperature conditions; however, its fertility diminished with rising temperatures, leading to impaired pollen starch accumulation and altered pollen morphology. In plastids, *OsHSP60-3B* interacts with *FLOURY ENDOSPERM6* (*FLO6*) in a mechanism that stabilizes this essential enzyme for starch biosynthesis and reduces the levels of reactive oxygen species in the anthers^[13]. Comprehensive genome-wide analyses have demonstrated that *HSP60* genes are subject to extensive transcriptional regulation in response to temperature stress. Multiple *CsHSP60* genes in tea (*Camellia sinensis*) plants exhibited substantial upregulation in response to drought and heat^[14]. Chloroplast-localized *HSP60* proteins, by interacting with ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) activase, might mitigate its thermal inactivation, thus protecting the photosynthetic apparatus under heat stress^[15]. Levels of plastid Cpn60 increase in response to stress during seed germination and early development^[16]. Moreover, proteomic analyses of soybean (*Glycine max*) and azalea (*Rhododendron* spp.) identified *HSP60* as part of wide heat-primed molecular networks that improve thermotolerance via pre-activated transcriptional programs^[17,18]. Overall, these studies demonstrate the critical function that *HSP60* proteins play in various abiotic stress responses.

Cowpea, an important warm-season crop, is sensitive to temperature stress, particularly during germination and flowering^[19]. In subtropical zones, lower temperatures (below 15 °C) can inhibit or reduce germination rates by triggering imbibitional chilling injury and severe membrane fracturing. Following emergence, seedlings suffer from a rapid accumulation of toxic reactive oxygen species, which disrupt cell metabolism and lead to phenotypic wilting, chlorophyll degradation, and leaf chlorosis. Conversely, high daytime temperatures (above 35 °C) coupled with long photoperiods abort early floral buds, whereas elevated nocturnal temperatures 7–9 d before anthesis induce male sterility by impairing pollen viability, thereby preventing successful fertilization. This heat stress ultimately suppresses pod development and causes near-total fruit-setting failure, severely limiting subtropical yields^[20,21]. Despite the well-known importance of these genes for alleviating plant environmental stress, a comprehensive assessment of the *HSP60* family in cowpea has not been reported to date. Identifying these genes and their expression profiles is therefore essential to pinpointing the candidates responsible for thermal adaptation. In this work, we present a thorough analysis of the *HSP60* gene family in cowpea under heat and cold stress. Gene structure, conserved domains and motifs, chromosomal distributions, phylogenetic analysis, *cis*-acting elements in the 5' upstream region, and expression patterns were examined. The findings provide a vital baseline for functional validation and genetic enhancement of cowpea cultivars that are resistant to temperature stress.

Materials and methods

Genome data retrieval and detection of the *HSP60* family members in *V. unguiculata*

Genomes of cowpeas (*V. unguiculata* v1.2) and *Arabidopsis thaliana* (Araport11) were obtained from the Phytozome13 database (<https://phytozome-next.jgi.doe.gov>). *A. thaliana* *HSP60* proteins were taken from the NCBI database (www.ncbi.nlm.nih.gov) and used as queries in TBtools' BLAST to find their protein

homologs in the *V. unguiculata* genome. The Pfam database (<http://pfam.xfam.org>) provided the hidden Markov model (HMM) profile of the domain of each family in *A. thaliana*. We identified the domains in the cowpea proteome using HAMMER 3.0. After duplicates were removed, sequences lacking the Cpn60_TCP1 domain were also excluded using NCBI's Conserved Domain Database (CDD) (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and SMART (<http://smart.embl-heidelberg.de>)^[22,23]. In the *HSP60* family, the genes encoding a protein with the required domain were taken as candidates for further analysis. Hydropathy, instability index, molecular weight (MW), sequence size, and isoelectric point (pI) were among the physical and chemical properties of the chosen candidate genes, as determined by TBtools II. To determine the subcellular location of proteins inside eukaryotic sequences, we used the CELLO subcellular localization system (<https://cello.life.nctu.edu.tw>)^[24]. According to their chromosomal positions and distributions, these genes were systematically designated using the prefix 'Vu' for *V. unguiculata*.

Chromosome arrangement and gene duplication analysis

Using TBtools II software, we evaluated *HSP60* proteins from the Phytozome v13 database to establish their distribution on different chromosomes and to calculate the ratio of nonsynonymous to synonymous substitutions (Ka/Ks) for the gene duplication events. The Nei–Gojobori approach was used to remove any blank or missing values from the analysis^[25].

Evolutionary relationship and sequence alignment

The phylogenetic tree was constructed using sequences of *HSP60* proteins from three different species (*V. unguiculata*, *A. thaliana*, *G. max*, and *O. sativa*). A neighbor-joining (NJ) tree with a bootstrap value of 1,000 repetitions was constructed using the Molecular Evolutionary Genetic Analysis (MEGA12) program. To show the evolutionary tree, the Interactive Tree of Life (ITOL; <https://itol.embl.de>) was used^[26]. ClustalW was used to align the *HSP60* protein sequences in MEGA12.

Analysis of conserved motifs, gene structure, and miRNA prediction

In this study, the VuHSP60 protein sequences were used to assess the exon/intron architecture using the TBtools II software. NCBI's CDD (www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) was used to identify the conserved domains. MEME in TBtools was used to identify conserved protein motifs^[27]. In addition, we used the Plant miRNA Encyclopedia (PmiREN; <https://pmiren.com>) and Plant Small RNA Target Analysis Server (psRNATarget; www.zhaolab.org/psRNATarget) databases to predict miRNAs associated with VuHSP60 proteins. The miRNA–target gene network diagram was generated by www.bioinformatics.com.cn, an online platform for data analysis and visualization^[28].

Examination of synteny, collinearity, and *cis*-acting elements

For synteny and collinearity studies, orthologs of the cowpea *HSP60* genes were identified across four species (namely, *A. thaliana*, *G. max*, *Zea mays*, and *Medicago truncatula*) using genomic data retrieved from the Phytozome v13 database. TBtools II's one-step MCScanX method was used to examine the orthologs,

and the interspecific collinearity map was displayed using the Multiple Synteny Plot option^[29]. The 2,000-bp promoter sequences of the cowpea *VuHSP60* gene family were investigated using the PlantCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html>) to predict *cis*-acting regulatory elements^[30]. Data visualization was facilitated with TBtools II software.

Plant materials and temperature stress treatments

Low- and high-temperature-sensitive cowpea ('BBWQ') seeds were provided by Nanjing Agricultural University's Brassica System Biology Laboratory. These seeds were planted in pots with regular potting mix and grown in a plant growth chamber with the ideal controlled conditions: 25 °C, 70% relative humidity, and a 16-h light and 8-h dark photoperiod. At exactly 2 weeks after the sowing date, when the seedlings reached the early trifoliate stage, uniform plants were selected for stress treatments and while some seedlings were maintained under ideal conditions to serve as controls. For cold stress, the seedlings were kept at 10 °C for a full day with a cycle of 16 h of light and 8 h of darkness, whereas heat stress was induced by the exposure of seedlings to 40 °C during 24 h. Young leaf samples were harvested at 1, 3, 6, 12, and 24 h after the onset of treatment, with three distinct biological replicates at each time point. Leaves of the control plants were also collected. In preparation for the subsequent RNA extraction and analysis, all samples were promptly frozen in liquid nitrogen and kept at –80 °C.

Quantitative real-time polymerase chain reaction

Total RNA was extracted from the samples according to the manufacturer's instructions using the Total RNA Extraction Reagent (Vazyme Biotech Co., Ltd., Nanjing, China). A NanoDrop™ One spectrophotometer measured the RNA's purity and concentration at 260 nm. RNA integrity was further confirmed using 1.0% agarose gel electrophoresis. We used the HiScript® II 1st Strand cDNA Synthesis Kit (Vazyme Biotech Co., Ltd.) to generate the first strand of cDNA. The study used a Bio-Rad CFX96 Touch Real-Time PCR Detection System and 2× ChamQ Blue Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd., Nanjing, China). Each 20 µL reaction mixture included 10 µL of 2× SYBR Master Mix, double-distilled water (ddH₂O), 2.0 µL of the diluted cDNA template (1:10), and 0.4 µL of the primers. The thermal cycling conditions included an initial denaturation at 95 °C for 4 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s. The 2^{–ΔΔCT} technique was used to calculate the relative gene expression levels. The internal reference was the cowpea actin gene (Accession No. XM_028073549). Primer specificity was confirmed with NCBI Primer-BLAST, and primer pairs were generated using the Primer3 online tool based on the coding sequences (CDS) of cowpea *HSP60* genes (Supplementary Table S1). Supplementary Table S2 shows the primer sequences used in this study. Each quantitative real-time polymerase chain reaction (qRT-PCR) test consisted of three biological duplicates and three technical repetitions each^[31,32].

Expression data analysis and statistical methods

Analysis of variance (ANOVA) in SPSS version 23.0 was used for statistical analysis. Significant differences between the control and treatment groups, as well as at different time points, were established using Tukey's test at the 5% significance level ($p < 0.05$). Three biological replicates were used in each experiment, and each treatment's standard error of the mean (SE) was determined. The data were visualized using GraphPad Prism 10^[33].

Results

Detection and characterization of *HSP60s* in cowpea using *in silico* approaches

In total, 25 *HSP60* genes were identified in the current study through genome-wide scanning of *V. unguiculata*. These proteins were dispersed randomly over nine distinct chromosomes. At minimum, three genes were found on chromosomes 3, 7, and 11, among other chromosomes. Other chromosomes contain one or two genes of *HSP60* (Fig. 1). However, *VuHSP60-18* was not assigned to a specific chromosome and remains on an unplaced contig (Contig_339). According to their chromosomal positions and distributions, these *V. unguiculata* *HSP60* genes were named with the prefix 'Vu'. The *VuHsp60* genes are fully described in Table 1 below. The *HSP60s* ranged in molecular weight from 44.91 kDa (*VuHSP60-15*) to 71.76 kDa (*VuHSP60-18*) and in amino acid content from 414 (*VuHSP60-15*) to 680 (*VuHSP60-18*). The pIs ranged from 5.15 (*VuHSP60-17*) to 9.11 (*VuHSP60-9.1*). Of the 25 *HSP60s*, five were identified as basic ($pI > 7$), while the remaining 20 were classified as acidic ($pI < 7$). According to the grand average of hydropathy (GRAVY), the majority of *VuHSP60s* have negative GRAVY values, which suggest that they are hydrophilic, with the exception of *VuHSP60-3*, *VuHSP60-5.1*, *VuHSP60-5.2*, *VuHSP60-5.3*, and *VuHSP60-10*, which are hydrophobic, with GRAVY > 0. Moreover, 24 of the 25 *VuHSP60s* are stable, with instability indices ranging from 23.64 (*VuHSP60-14.1*) to 39.99 (*VuHSP60-2*), but only one protein is unstable with a value of 46.12 (*VuHSP60-11.1*). The aliphatic index of the majority of HSPs in cowpea ranges from 96.04 (*VuHSP60-13*) to 115.78 (*VuHSP60-3*). Subcellular localization predicted that *VuHSP60s* are mainly identified in the cytoplasm, mitochondria, and chloroplasts. The highest number of *HSP60s* are localized in the cytoplasmic compartment, with 13 genes, followed by the mitochondria, where 5 *HSP60* genes were counted (Table 1).

Gene duplication analysis showed that there were 93 paralogous pairs in the *VuHSP60* gene family (Supplementary Table S3). When genes are duplicated and positioned on distinct chromosomes, this scenario is known as segmental duplication. Segmental duplication was the most prevalent kind in *VuHSP60*, with 91 gene pairs. In *VuHSP60*, dispersed duplication was less common, occurring in two gene pairs. These results suggest that the evolution of this gene family in cowpea was significantly influenced by large-scale chromosomal duplication events. The predicted K_a , K_s , and K_a/K_s values for these gene pairs are shown in Supplementary Table S3. Since all K_a/K_s ratios are less than 1, these genes likely underwent strong purifying selection during the process of evolution. This suggests functional conservation and selective pressure to maintain protein stability and essential biological functions.

Figure 1 indicates the localization of *HSP60* gene family members. Megabases (Mb) indicate chromosomal size and the genes' locations. For loci exhibiting alternative splicing events, the base gene name represents the physical locus position, and only the primary transcript model coordinates were utilized for visualization of the map layout. The chromosome numbers of *V. unguiculata* are numbered from Vu01 to Vu10 and are indicated at the left of each bar. The arrangement of genes in cowpea chromosomes was developed by the TBtools II program.

Evolutionary relationships of *HSP60s* in *V. unguiculata*

We used the neighbor-joining method in MEGA 12 software to generate a rooted phylogenetic tree (Fig. 2). The full-length *HSP60*

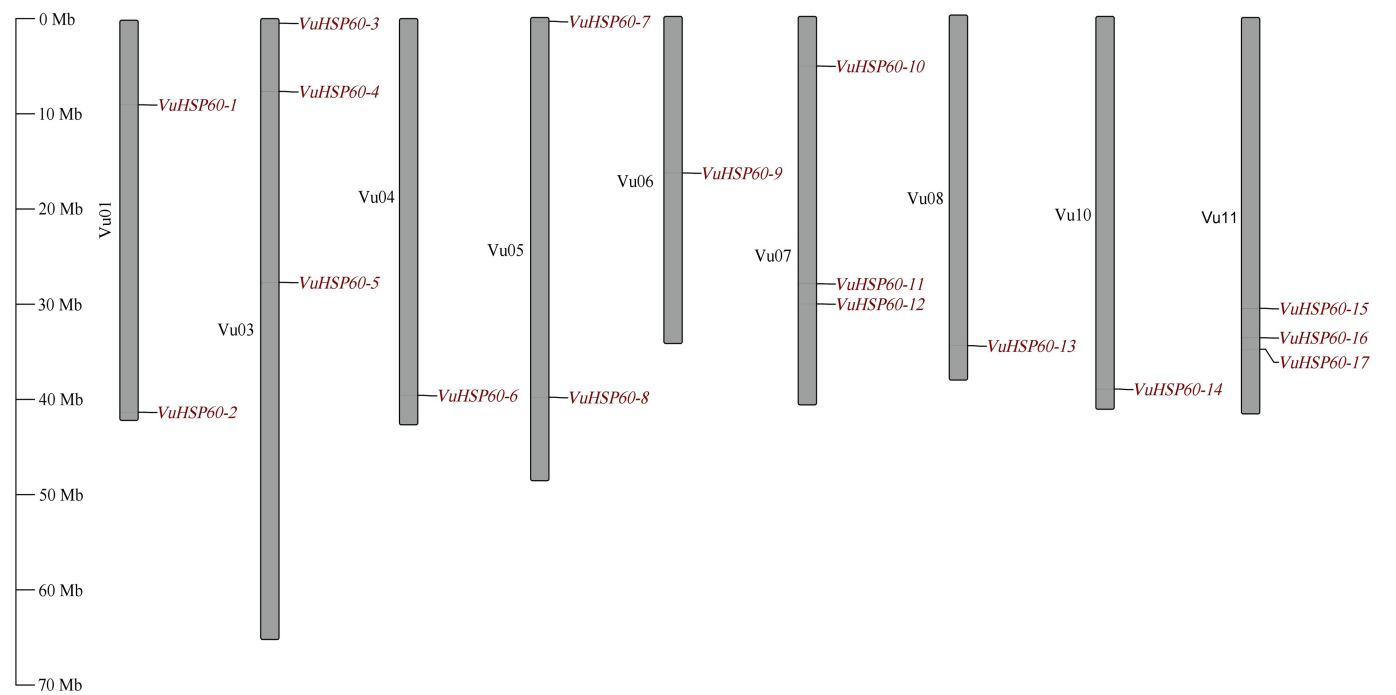


Fig. 1 *VuHSP60* genes' chromosomal positions.

Table 1. Basic characteristics of the *V. unguiculata* HSP60 gene family.

Gene ID	Gene name	Chr	aa	MW	pI	Ins I	Alip I	GRAVY	Localization
Vigun01g052500.1	<i>VuHSP60-1</i>	Vu01	593	62.90	5.8	33.06	99.31	−0.102	Ch
Vigun01g242300.1	<i>VuHSP60-2</i>	Vu01	535	59.15	5.61	39.99	97.53	−0.188	Cy
Vigun03g007100.1	<i>VuHSP60-3</i>	Vu03	533	57.53	6.85	34.25	115.78	0.145	Cy
Vigun03g090600.1	<i>VuHSP60-4</i>	Vu03	545	59.01	5.98	30.37	103.28	−0.043	Cy
Vigun03g196200.1	<i>VuHSP60-5.1</i>	Vu03	588	62.61	7.66	30.72	105.19	0.071	Mi
Vigun03g196200.2	<i>VuHSP60-5.2</i>	Vu03	485	51.67	6.08	26.04	108.82	0.105	Mi
Vigun03g196200.3	<i>VuHSP60-5.3</i>	Vu03	476	50.57	6.08	25.75	109.85	0.112	Mi, Cy
Vigun04g171700.1	<i>VuHSP60-6</i>	Vu04	555	60.31	5.97	39.26	101.8	−0.107	Cy
Vigun05g005000.1	<i>VuHSP60-7</i>	Vu05	535	59.03	5.6	32.08	100.75	−0.158	Cy
Vigun05g208400.1	<i>VuHSP60-8</i>	Vu05	527	57.16	5.55	35.11	101.48	−0.042	Cy
Vigun06g042300.1	<i>VuHSP60-9.1</i>	Vu06	611	66.11	9.11	33.58	101.18	−0.084	Mi, Ch
Vigun06g042300.3	<i>VuHSP60-9.2</i>	Vu06	539	58.20	8.81	31.42	102.56	−0.098	Ch
Vigun07g050000.1	<i>VuHSP60-10</i>	Vu07	637	67.81	6.28	25.6	101.98	0.019	Mi
Vigun07g166500.2	<i>VuHSP60-11.1</i>	Vu07	483	53.60	8.77	46.12	103.79	−0.17	Cy
Vigun07g166500.4	<i>VuHSP60-11.2</i>	Vu07	461	50.53	5.73	38.82	104.53	−0.06	Cy
Vigun07g166500.5	<i>VuHSP60-11.3</i>	Vu07	456	49.99	6.08	39.95	106.34	−0.083	Cy
Vigun07g166500.7	<i>VuHSP60-11.4</i>	Vu07	436	47.96	5.73	37.34	105.16	−0.072	Cy
Vigun07g185200.1	<i>VuHSP60-12</i>	Vu07	575	61.30	5.9	26.34	100.09	−0.087	Mi
Vigun08g177100.1	<i>VuHSP60-13</i>	Vu08	560	60.31	6.2	35.37	96.04	−0.082	Cy
Vigun10g173100.1	<i>VuHSP60-14.1</i>	Vu10	575	61.69	7.57	23.64	99.03	−0.142	Mi
Vigun10g173100.2	<i>VuHSP60-14.2</i>	Vu10	467	50.51	5.2	27.35	104.18	−0.104	Ch, Cy
Vigun11g107100.2	<i>VuHSP60-15</i>	Vu11	414	44.91	5.43	31.62	102.49	−0.015	Cy
Vigun11g128900.1	<i>VuHSP60-16</i>	Vu11	527	57.10	5.45	34.04	102.03	−0.028	Cy
Vigun11g139100.1	<i>VuHSP60-17</i>	Vu11	584	61.67	5.15	30.15	106.11	−0.026	Ch
VigunL051601.1	<i>VuHSP60-18</i>	Contig_339	680	71.76	5.27	27.1	108.34	−0.021	Cy, Mi, Ch

Note: Using TBtool II and CELLO, the following basic features of the *VuHSP60* gene family members were predicted from the *V. unguiculata* genome. aa, amino acids; chr, chromosome; MW, molecular weight; pI, isoelectric point; Ins I, instability index; Alip I, aliphatic index; GRAVY, grand average hydropathicity; Cy, cytoplasm; Mi, mitochondria; Ch, chloroplast.

protein sequences from three dicots (cowpea, *A. thaliana*, and soybean) and one monocot (rice) were used (Supplementary Table S4). According to their localization, this study categorized 98 HSP60 proteins into four groups (Group I, II, III, and IV). Group I, which is the biggest, contains 30 cytoplasmic proteins. Of these, 13 (52% of the total *VuHSP60s*) come from cowpea, nine come from *Arabidopsis*, eight come from rice, and 18 from soybean. Groups II and III

comprise mitochondrial and chloroplastic proteins, respectively; cowpea genes represent 28% of *VuHSP60s* in Group II and 20% in Group III and contain representatives from four plant species. Group IV is completely devoid of both cowpea (*V. unguiculata*) and soybean genes, containing exclusively orthologs from *A. thaliana* and rice. Furthermore, each group includes a mix of monocot and dicot proteins.

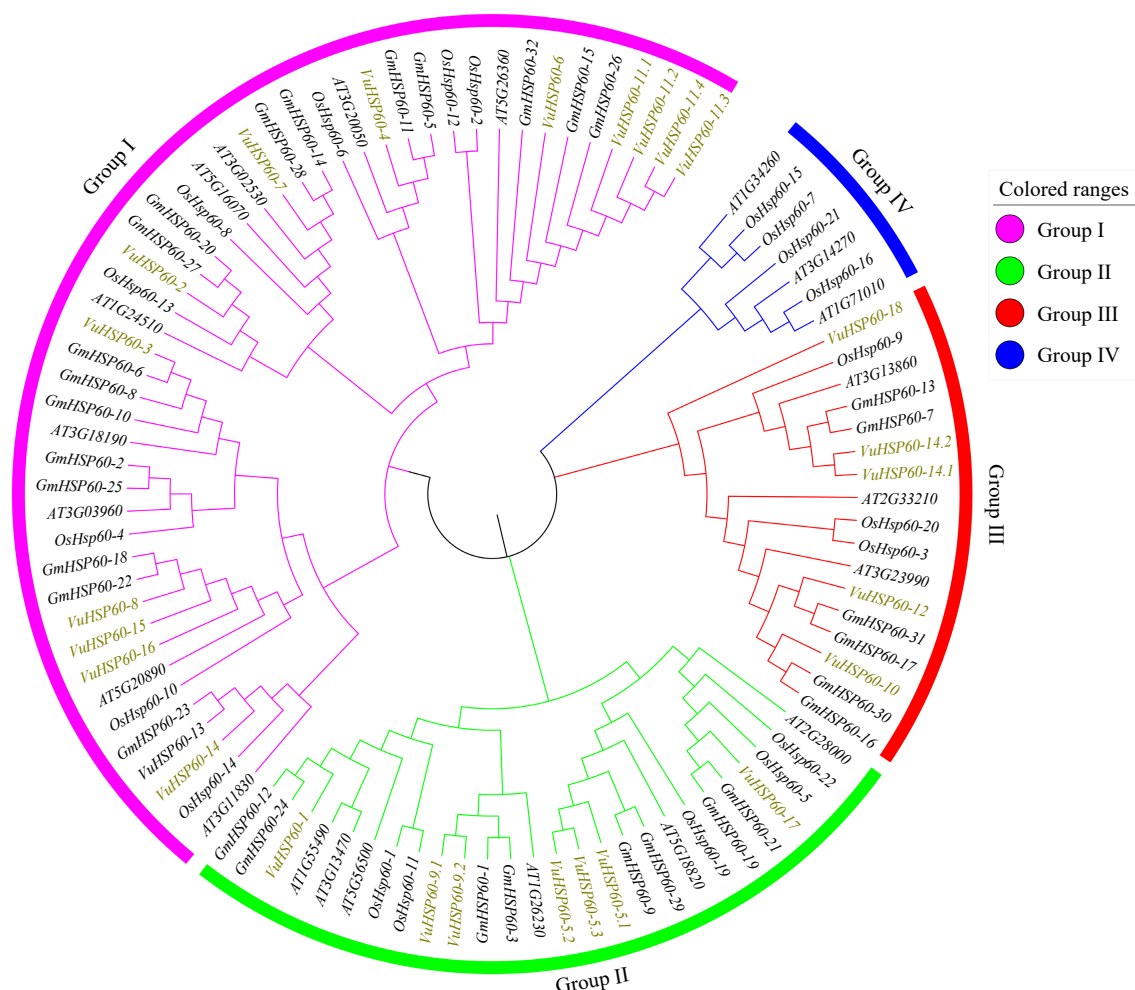


Fig. 2 Phylogenetic trees of the *VuHSP60* gene family.

MEGA12 software was used to generate the phylogenetic trees using the HSP60 protein sequences from cowpea, *A. thaliana*, soybean, and rice. The figure shows the *HSP60* genes' phylogenetic tree, which is divided into four groups (I, II, III, and IV) of 98 genes. The Interactive Tree of Life (iTOL) was used to illustrate the figure, with bootstrap values derived from 1,000 replicates.

Gene structure and conserved motif analysis

We investigated the structural variation of the *VuHSP60* genes by analyzing the exon–intron organization, domains, and motifs (Fig. 3). Closely related members within the same phylogenetic group exhibited similar gene architectures. Intron counts varied widely across the family, ranging from 0 to 17. The highest intron numbers (16–17) were observed in *VuHSP60-4*, *VuHSP60-14.1*, *VuHSP60-14.2*, *VuHSP60-10*, and *VuHSP60-12*, which encode proteins predicted by CELLO to be localized to the cytoplasm and mitochondria. *VuHSP60-3* differs from the other *VuHSP60* members in that it lacks introns. According to the results, the number of introns of the remaining *VuHSP60* genes range from 5 to 14. To further characterize the proteins, we used the MEME suite (integrated in TBtools) to identify the conserved motifs shared by related proteins in each subfamily. We found 10 potential motifs of 21–50 amino acids across the *HSP60* family (Supplementary Table S5). The arrangement and composition of motifs vary throughout the family and are correlated with phylogenetic clustering. Motifs 1 and 2, which are associated with the Cpn60_TCP1 domain, are present in all members of the

VuHSP60 family. Conserved domain annotation (Pfam, CDD, SMART) databases revealed a highly conserved domain architecture, confirming their classification as molecular chaperones.

The left panel (a) displays the phylogenetic tree of the three *VuHSP60* protein family. The full-length amino acid sequences of *VuHSP60* proteins were used to construct the rooted neighbor-joining phylogenetic tree with MEGA 12 (Supplementary Table S6). The bootstrap test replicates were set to 1,000. The second panel (b) illustrates the distributions of conserved motif proteins. The 10 possible motifs are marked by numbered frames in various colors, ranging from 1 to 10. Panel (c) represents the conserved domains that are present in *VuHSP60* proteins. The right panel (d) illustrates the exon–intron layout of the *VuHSP60* genes. Gray lines symbolize introns, and the yellow rectangles symbolize exons (CDS). Green boxes represent the upstream and downstream regions (untranslated regions, UTRs) of *VuHSP60* genes. The graphic also shows a description of each colored box.

In silico prediction of microRNAs potentially targeting the *VuHSP60* gene family

In silico prediction identified a total of 20 miRNAs predicted to target and regulate *VuHSP60* genes (Fig. 4). These miRNAs were classified into eight families involved in RNA cleavage and translation inhibition. Important miRNAs known to regulate *VuHSP60s* include Vun-miR3309, Vun-miR165, Vun-miR2111, Vun-miR394, and Vun-miR408. Vun-miR167 is involved in plant growth and targets



Fig. 3 Conserved motifs, gene structure, conserved domains, and phylogenetic relationship of *VuHSP60* proteins.

VuHSP60-5.1, *VuHSP60-5.2*, and *VuHSP60-5.3*. The stress-responsive *Vun-miR164* targets *VuHSP60-9.1* and *VuHSP60-9.2*. A multigene targeting network was observed, particularly for the plant growth regulator *Vun-miR396*, which participates in stress responses to drought, high salinity, and extreme temperatures and targets five specific genes: *VuHSP60-8*, *VuHSP60-15*, *VuHSP60-16*, *VuHSP60-14.1*, and *VuHSP60-14.2*.

Synteny and collinearity investigation of the *HSP60* genes from *V. unguiculata* and other species

To infer evolutionary conservation and genomic divergence, we used TBtools to perform synteny and collinearity analyses of the *HSP60* gene family in *V. unguiculata* with *A. thaliana*, *G. max*, *Z. mays*, and *M. truncatula* (Fig. 5). Syntenic blocks between *V. unguiculata* and three of the four comparison species were detected. Specifically, *G. max* and *V. unguiculata* showed 27 collinear gene pairs within the syntenic blocks, indicating strong microsynteny conservation despite legumes' diversification. In contrast, *V. unguiculata*

and *A. thaliana* showed nine collinear gene pairs, whereas *M. truncatula* showed only eight. These results demonstrate that genomic conservation is strictly higher within the tribe Phaseoleae and substantially reduced in both distant legumes and nonlegume dicots. Surprisingly, no collinear gene pairs were found between *Z. mays* and *V. unguiculata*, reflecting genomic alterations in specific lineages, along with the deep evolutionary divergence between dicots and monocots.

Cis-acting regulatory element analysis

To predict potential *cis*-regulatory elements, we uploaded 2,000-bp upstream promoter regions from *VuHSP60* genes to the PlantCARE database. The results revealed 37 distinct types of *cis*-regulatory elements (Fig. 6). These components participate in multiple biological processes, such as development, stress response, hormone signaling, light perception, and promoter activity. Of the 25 genes, 12 (48%) contained low-temperature responsive elements (LTRs), and 9 (36%) contained defense and stress responsive elements (TC-rich repeats). In terms of hormone-responsive elements, 17

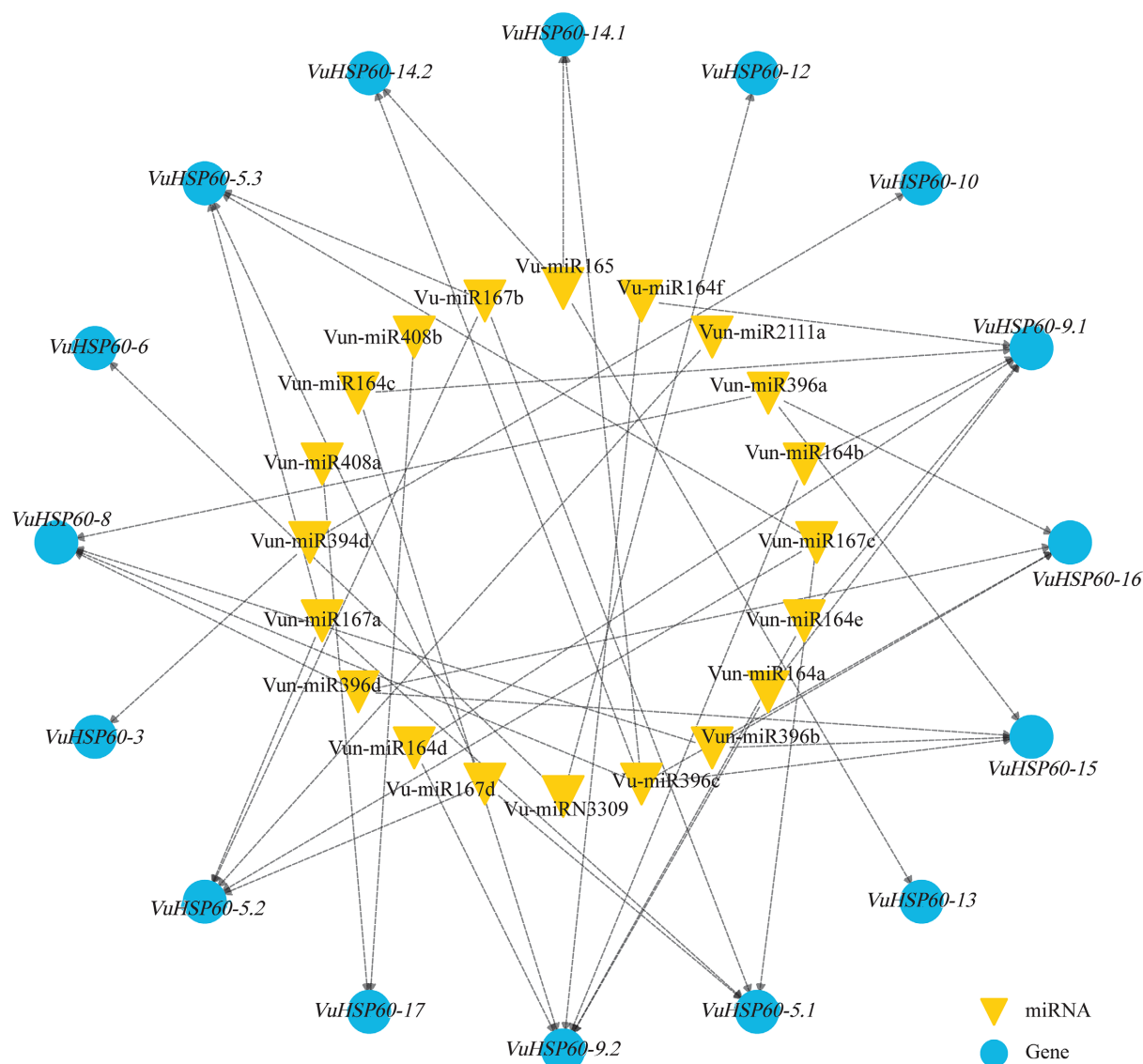


Fig. 4 Predicted miRNA-*VuHSP60* gene interaction network. Light blue circles represent *VuHSP60* genes, yellow triangles represent miRNAs, and gray lines show predicted targeting interactions.

(68%), 7 (28%), 11 (44%), 6 (24%), and 18 (72%) genes contained one or more abscisic acid-responsive elements (ABRE), auxin (indole-3-acetic acid; IAA)-responsive elements, gibberellin (GA)-responsive elements, salicylic acid (SA)-responsive elements, and methyl jasmonate (MeJA)-responsive elements. Other elements detected in 20 and two *VuHSP60* genes were anaerobic response elements (AREs) and wound-response elements (WUN motifs), respectively. The existence of LTR elements and hormones responding to environmental stresses points to the predicted role for the *VuHSP60* genes in mediating cowpea's adaptation to extreme temperatures.

Cis-regulatory elements were predicted by PlantCARE in the promoter sequences (2,000 bp) of the 25 *VuHSP60* genes that were analyzed. The various types of *cis*-acting elements are represented by distinct colored shapes. We used the TBtools software to visualize all components.

Relative expression levels of *VuHSP60*s under temperature stress

We investigated the expression levels of 25 members of the *HSP60* gene family in response to heat and cold stressors (Fig. 7).

The expression trends of nine genes, including *VuHSP60-1*, *VuHSP60-3*, *VuHSP60-5.1*, *VuHSP60-5.2*, *VuHSP60-5.3*, *VuHSP60-11.2*, *VuHSP60-12*, *VuHSP60-14.1*, and *VuHSP60-17*, exhibited a significant increase under high-temperature treatment in comparison with the control. *VuHSP60-12* and *VuHSP60-14.1* exhibited the highest expression levels among these genes, peaking at 1 h (6.64-fold) and 12 h (4-fold) after treatment, respectively, before gradually declining. The expression trends of *VuHSP60-5.1*, *VuHSP60-5.2*, and *VuHSP60-5.3* increased gradually and reached their highest point at 24 h post-treatment. *VuHSP60-1*, *VuHSP60-3*, *VuHSP60-11.2*, and *VuHSP60-17* showed different variations. Conversely, the remaining 16 family members displayed either nonsignificant changes or significant downregulation relative to the control. Under cold treatment, 10 genes (*VuHSP60-1*, *VuHSP60-4*, *VuHSP60-7*, *VuHSP60-8*, *VuHSP60-9.1*, *VuHSP60-9.2*, *VuHSP60-12*, *VuHSP60-13*, *VuHSP60-15*, and *VuHSP60-16*) were significantly upregulated. Notably, *VuHSP60-4*, *VuHSP60-7*, and *VuHSP60-15* exhibited the highest expression levels, reaching 8-, 7.5-, and 7.2-fold increases over the control, respectively. The remaining 15 *VuHSP60* genes showed differential expression levels with nonsignificant increases. In general, these distinct temporal

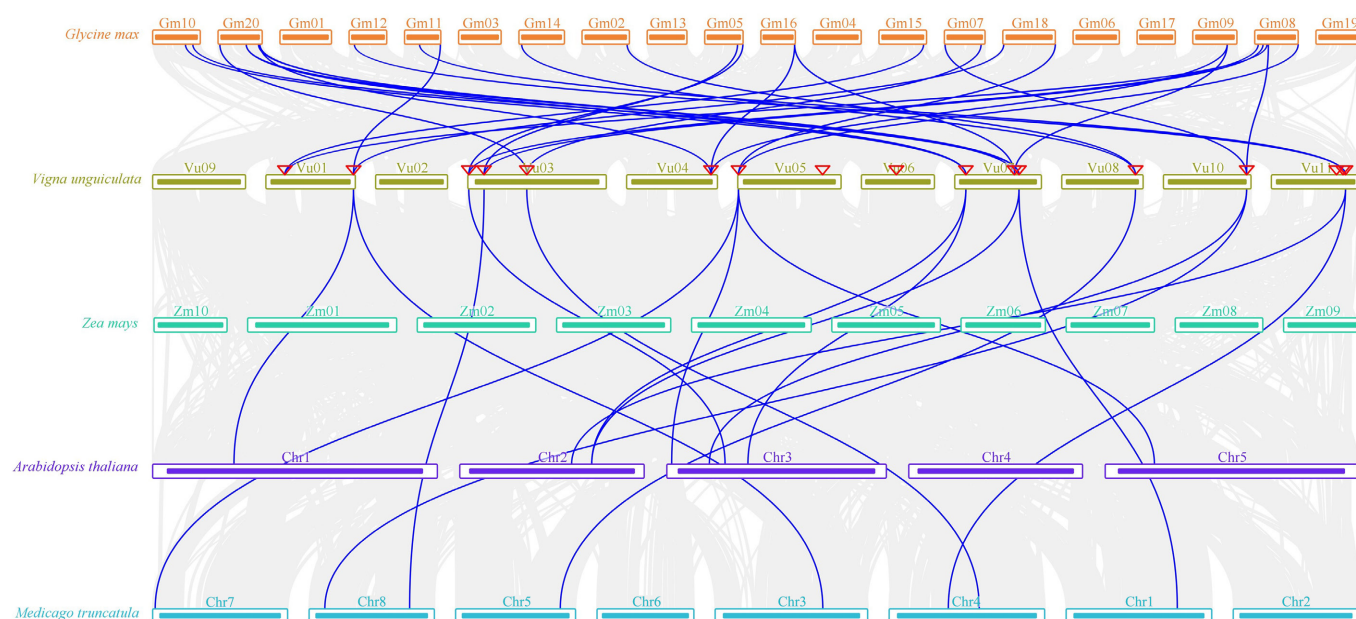


Fig. 5 Synteny and collinearity analysis. Blue lines indicate the collinear *HSP60* gene pairs within conserved syntenic blocks, while the faint gray lines in the background represent the genome-wide synteny (all other collinear gene pairs) in five species.

expression profiles demonstrate that specific *VuHSP60* genes actively participate in heat, cold, or dual thermal stress responses, indicating functional diversification within the gene family.

Figure 7 illustrates the qRT-PCR results of the leaf tissues of the 'BBWQ' cowpea cultivar under heat stress (40 °C) and cold stress (10 °C). Panels are categorized into Group I, II, and III according to the phylogenetic classification of the *VuHSP60* gene family. The $2^{-\Delta\Delta CT}$ method served to determine the expression levels; the bars represent the mean $\pm$ standard error. The Tukey test in SPSS 23.0 demonstrated statistically significant differences between the control and treatments. Asterisks indicate statistically significant differences compared with the control (CK) (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$), whereas the absence of asterisks shows no significant difference ($p \geq 0.05$). The graphs were generated using GraphPad Prism 10.

Discussion

Cowpea (*V. unguiculata*) is a globally important crop^[34], but its yield is severely constrained by specific phenological vulnerabilities, including cold-induced chlorosis during early vegetative establishment and acute heat-induced pollen sterility during blooming in subtropical regions^[35,36]. These thermal shocks cause protein denaturation and metabolic disruption of important chloroplast and mitochondrial bioenergetic pathways^[37]. According to earlier studies, *HSP60s* are essential for how plants respond to temperature stress^[38]. Therefore, this gene family has been studied in the genomes of several species. However, very little is known about *HSP60* genes in cowpea. In this study, 25 *HSP60* genes were detected in a systematic genome-wide analysis of *V. unguiculata* (Table 1), a number similar to those found in multiple plant species such as *O. sativa* (22)^[39], mung bean (*Vigna radiata*) (23)^[40], *Sorghum bicolor* (30)^[41], *A. thaliana* (29)^[42], *C. sinensis* (19)^[14], and Jerusalem artichoke (*Helianthus tuberosus*) (22)^[43]. The *VuHSP60* genes in this study have mostly experienced segmental duplication (97.8%) and minor dispersed duplication (2.2%) (Supplementary Table S3), indicating that the expansion of this gene family in *V. unguiculata* was

primarily driven by large-scale genomic events. These findings are consistent with those of other crops where segmental duplication has played a significant role in the spread of the *HSP60* gene family, including tea^[14], *S. bicolor*^[41], and soybean^[44]. Additionally, the Ka/Ks ratios of all duplicated *VuHSP60* gene pairs were less than 1, indicating that this family has evolved under purifying selection that maintains a functional conservation among the paralogs. The physicochemical characteristics of proteins, such as their MW and pI, provide important information about their functions^[45]. In the present study, the *VuHSP60* proteins exhibited diverse MW and pI values (Table 1). Most of them possessed high aliphatic indices, indicating enhanced thermostability, alongside hydrophilic and acidic properties^[46]. In molecular chaperones, these biochemical properties are typically associated with increased protein solubility and the prevention of thermal aggregation within aqueous cellular environments^[47]. Therefore, various profiles of *VuHSP60* proteins may reflect an evolutionary adaptation to preserve organellar structural integrity during the severe thermal variation characteristic of cowpea's subtropical environment.

Phylogenetic analysis revealed the closeness of evolution among *HSP60* sequences, suggesting a common ancestor and underscoring possible functional conservation within some clades^[48]. In previous studies, *HSP60* has been classified according to the predicted subcellular localizations. For example, the evolutionary study of *HSP60s* in the model plant *A. thaliana* partitioned them into the mitochondria and chloroplasts^[42]. Similarly, three groups of *VuHSP60* genes were identified; these genes are primarily predicted to localize in the cytoplasm, mitochondria, and chloroplasts (Fig. 2, Table 1), with a mix of monocot and dicot species, which suggests that these *HSP60* genes diverged before the monocot–dicot split. Group IV completely lacks both cowpea and soybean representatives (Fig. 2). This selective absence suggests a lineage-specific gene loss event or a distinct evolutionary divergence within the Fabaceae family that warrants further genomic investigation. According to the domain architecture analysis, all identified proteins contained the highly conserved Cpn60_TCP1 domain, which is essential for ATP-dependent protein folding activity. In addition, significant structural



Fig. 6 *Cis*-elements predicted in the promoter regions of *VuHsp60* genes.

diversity in gene architecture was observed, with intron counts ranging from 0 to 17 (Fig. 3). This architectural variability aligns with recent findings reported in other crops, including tea^[14] and cassava (*Manihot esculenta*)^[49]. This structural variation in *VuHSP60* genes reflects a history of intron loss and gain events. Low-intron variants are structurally optimized for rapid transcription by minimizing RNA splicing requirements, whereas multi-intron genes provide the architectural framework that supports alternative splicing.

In the current study, the predicted multigene network showed a centralized post-transcriptional regulatory framework for cowpea chaperonins. Screening based on psRNATarget categorized these interactions into RNA cleavage and translation inhibition mechanisms across eight miRNA families (Fig. 4). The network topology demonstrated that growth- and stress-associated regulators, particularly *Vun-miR396* and *Vun-miR164*, exhibit high sequence

complementarity with multiple *VuHSP60* paralogs simultaneously. This multitarget architecture suggests an evolutionary mechanism where single small RNA nodes synchronize the fine-tuning of multiple chaperonin loci^[50]. Ultimately, these *in silico* interactions provide a robust candidate pool for future experimental validation of cowpea's stress response networks.

Characterization of the upstream promoter regions revealed a diverse architectural layout of *cis*-regulatory elements across the *VuHSP60* gene family (Fig. 6). The diverse promoter profiles predicted by the PlantCARE database suggest a highly integrated transcriptional network for the *VuHSP60* family. The high abundance of predicted ABREs and MeJA-responsive elements suggest that these chaperonin loci are computationally located within core phytohormone-mediated stress cascades^[51,52]. Rather than operating in isolation, the predicted coexistence of these

Group I

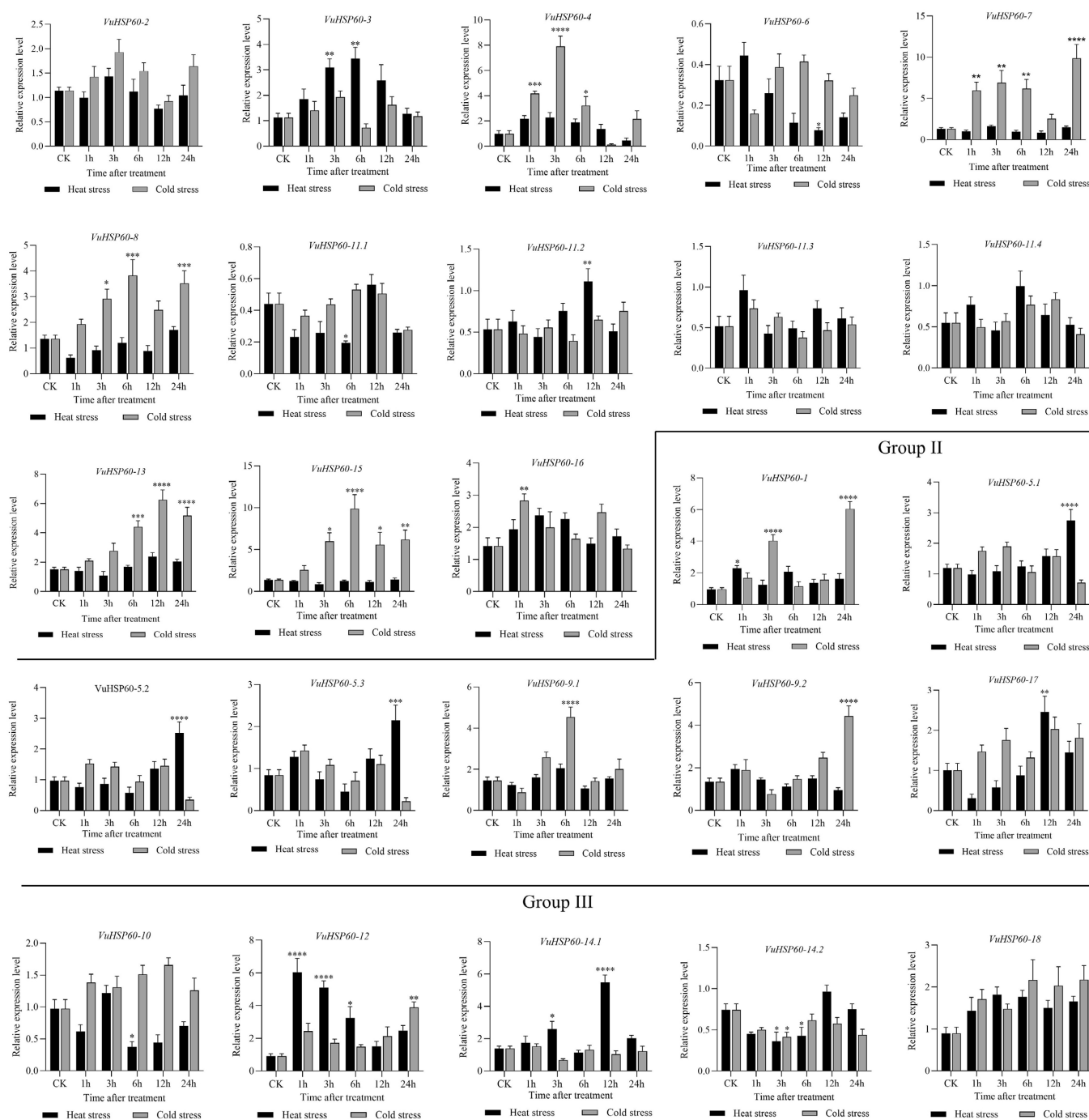


Fig. 7 Expression levels of 25 *VuHSP60* genes.

hormone-responsive components alongside specialized motifs, such as LTRs, defense-related TC-rich repeats, and AREs, points to potential functional diversification at the promoter level. This specialized architectural layout reveals a structural framework that supports the potential for differential or selective transcript activation among individual *VuHSP60* paralogs. Finally, the present *in silico* multi-stress framework provides a mechanistic hypothesis of cowpea's molecular chaperone system to coordinate its transcription under environmental fluctuations, establishing a defined target pool for future functional validation under low and high temperature and hormone treatments.

According to the qRT-PCR expression profiling, *VuHSP60* gene family members exhibit differential transcriptional regulation under heat and cold stress (Fig. 7), consistent with findings reported in *S. bicolor*^[41] and *C. sinensis*^[14]. The significant upregulation of nine genes under heat stress and 10 under cold stress highlights distinct transcriptional variations within this gene family. Although the majority of these genes displayed strict stimulus-specific expression (seven exclusively under heat and eight exclusively under cold), *VuHSP60-1* and *VuHSP60-12* exhibited dual responsiveness to both treatments. This overlapping accumulation suggests that these two specific family members are transcriptionally activated by upstream

signaling networks common to both heat and cold stress. Similar transcriptionally distinct profiles within chaperone families have been documented in other legumes such as *V. radiata*, where specific *VrHSP60* genes displayed pronounced, stress-specific expression patterns during thermal stress. For example, *VrHSP-60.22* showed differential transcription depending on the stress type, exhibiting responsiveness to both heat and cold stress, whereas other chaperones in mungbean demonstrated strict single-stimulus induction^[40]. Similarly, cowpea displays specific upregulation of *VuHSP60* genes exclusively under high- and low-temperature conditions. Collectively, these expression variations identify specific *VuHSP60* candidates that are responsive to temperature stress; however, further functional studies are required to determine whether these transcript-level changes translate to altered protein abundance or functional differences.

This study establishes the first genome-wide characterization of the *HSP60* gene family in cowpea and thus provides a detailed baseline for chaperone architecture in legumes. The data also reveal structural conservation across paralogs by showing that family expansion was driven by segmentally duplicated events under purifying selection. The different transcriptional profiles obtained by qRT-PCR showed that *VuHSP60-1* and *VuHSP60-12* are candidate genes that are responsive to high and low temperatures. These *in silico* and expression datasets provide precise, high-priority targets to guide future functional validation and molecular breeding strategies for climate-resilient cultivars.

Conclusions

This work presents the complete genomic analysis of the *HSP60/Cpn* gene family in cowpea, characterizing a structurally diverse group of 25 members shaped by segmental duplication and purifying selection. The differential and overlapping expression profiles of these genes at severe temperatures suggest their specific potential functions in maintaining cellular integrity. Instead of a mere roster of stress-responsive candidates, this work identified *VuHSP60-1* and *VuHSP60-12* as candidate genes for future functional studies. In addition, protein interactions, and multi-omics approaches should be integrated to further investigate the specific regulatory networks and translate these findings into robust vegetable cultivars suited to increasing climatic variability.

Ethical statements

Not applicable.

Author contributions

The authors confirm their contributions to the paper as follows: study conception and design, experiment, data analysis, and manuscript writing: Ndericimpaye P; experiment conduction: Zhang L, Wang D; data validation and curation, manuscript review, and editing: Liu T, Hou X, Li Y, Xiao D. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Both the manuscript and the supplementary materials indicate the data that support the findings of this study. These data are also available in the Phytozome v13 (<https://phytozome-next.jgi.doe.gov>) and National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov) databases.

Acknowledgments

This work was funded by the Sanya City Science and Technology Innovation Special Project (2022KJCX80), the National Natural Science Foundation of China (32372698, 32573023), and the Priority Academic Program Development of Jiangsu Higher Education Institutions. All data analysis was supported by the Bioinformatics Center of Nanjing Agricultural University.

Conflict of interest

The authors declare that they have no conflict of interest.

Supplementary information accompanies this paper online at: <https://doi.org/10.48130/vegres-0026-0029>.

Dates

Received 8 May 2026; Revised 30 July 2026; Accepted 14 August 2026; Published online 10 October 2026

References

- [1] Bondok AAT, Saker HM, Abo-marzoka SA, Khalil M, Rady AMS, et al. 2024. Cowpea (*Vigna unguiculata* L.) morpho-physiological and yield responses to chemical, organic, and biofertilizers at various watering levels utilizing drip irrigation system. *Plant Production Science* 27:110–124
- [2] Kim DK, Ochar K, Iwar K, Ha BK, Kim SH. 2025. Cowpea (*Vigna unguiculata* L.) production, genetic resources and strategic breeding priorities for sustainable food security. *Frontiers in Plant Science* 16:1562142
- [3] Kumar V, Sandhu SS, Prabhjyot-Kaur, Kaur S, Walia SS, et al. 2025. Impact of heat stress on growth and fodder yield of C3 (cowpea) and C4 (pearl millet) crops: insights from field and modelling experiments. *Journal of Agronomy and Crop Science* 211:e70063
- [4] Mweta JM, Kanyairita GG, Quarcoo F, Makwinja F, Abugri DA, et al. 2025. Effect of weather conditions on phytochemical profiles in organically grown cowpea (*Vigna unguiculata* L. Walp). *Plants* 14:3179
- [5] Mahajan S, Thakur P, Das S, Sharma R, Manuja S, et al. 2025. Impression of contemporary heat stress complexities in agricultural crops: a review. *Plant Growth Regulation* 105:1805–1823
- [6] Waraich EA, Ahmad R, Halim A, Aziz T. 2012. Alleviation of temperature stress by nutrient management in crop plants: a review. *Journal of Soil Science and Plant Nutrition* 12:221–244
- [7] Zhang Y, Xu J, Li R, Ge Y, Li Y, et al. 2023. Plants' response to abiotic stress: mechanisms and strategies. *International Journal of Molecular Sciences* 24:10915
- [8] Kang Y, Lee K, Hoshikawa K, Kang M, Jang S. 2022. Molecular bases of heat stress responses in vegetable crops with focusing on heat shock factors and heat shock proteins. *Frontiers in Plant Science* 13:837152
- [9] Singh MK, Shin Y, Han S, Ha J, Tiwari PK, et al. 2024. Molecular chaperonin HSP60: current understanding and future prospects. *International Journal of Molecular Sciences* 25:5483
- [10] Guglielmotti DM, Pujato SA, Quiberoni A, Suarez VB. 2022. Hsp60 gene as a reliable target for taxonomical identification and discrimination of *Leuconostoc* species of dairy origin. *International Dairy Journal* 126:105227
- [11] Carrascosa JL, Llorca O, Valpuesta JM. 2001. Structural comparison of prokaryotic and eukaryotic chaperonins. *Micron* 32:43–50
- [12] Haq SU, Khan A, Ali M, Gai W, Zhang H, et al. 2019. Knockdown of CaHSP60-6 confers enhanced sensitivity to heat stress in pepper (*Capsicum annuum* L.). *Planta* 250:2127–2145
- [13] Lin S, Liu Z, Sun S, Xue F, Li H, et al. 2023. Rice heat shock protein60-3b maintains male fertility under high temperature by starch granule biogenesis. *Plant Physiology* 192:2301–2317
- [14] Zheng A, Tian C, Zhou C, Yang N, Wen S, et al. 2025. Genome-wide identification and characterization of CsHSP60 gene family associated

- with heat and drought responses in tea plants (*Camellia sinensis*). *Plant Physiology and Biochemistry* 222:109758
- [15] Salvucci ME. 2008. Association of Rubisco activase with chaperonin-60beta: a possible mechanism for protecting photosynthesis during heat stress. *Journal of Experimental Botany* 59:1923–1933
- [16] Holland N, Belkind A, Holland D, Pick U, Edelman M. 1998. Stress-responsive accumulation of plastid chaperonin 60 during seedling development. *The Plant Journal* 13(3):311–316
- [17] Ahsan N, Donnart T, Nouri MZ, Komatsu S. 2010. Tissue-specific defense and thermo-adaptive mechanisms of soybean seedlings under heat stress revealed by proteomic approach. *Journal of Proteome Research* 9:4189–4204
- [18] Wang X, Li Z, Liu B, Zhou H, Elmongy MS, et al. 2020. Combined proteome and transcriptome analysis of heat-primed *Azalea* reveals new insights into plant heat acclimation memory. *Frontiers in Plant Science* 11:1278
- [19] Mohammed SB, Ongom PO, Togola A, Boukar O. 2024. Enhancing cowpea tolerance to elevated temperature: achievements, challenges and future directions. *Agronomy* 14:513
- [20] PS B, Biradar M, Meena VS, Tripathi K, Babar R, et al. 2026. Multi-trait stability selection drives genetic gains in cowpea (*Vigna unguiculata* [L.] Walp.) under high-temperature stress. *BMC Plant Biology* 26:519
- [21] Biradar M, Tripathi K, Meena VS, Basavaraj PS, Kumari J, et al. 2025. Morpho-physiological traits underpinning high-temperature stress tolerance in cowpea (*Vigna unguiculata* L. Walp.). *Genetic Resources and Crop Evolution* 72:6097–6110
- [22] Sheng Y, Yu H, Pan H, Qiu K, Xie Q, et al. 2022. Genome-wide analysis of the gene structure, expression and protein interactions of the peach (*Prunus persica*) TIFY gene family. *Frontier in Plant Science* 13:792802
- [23] Sami A, Haider MZ, Shafiq M, Sadiq S, Ahmad F. 2024. Genome-wide identification and *in-silico* expression analysis of CCO gene family in sunflower (*Helianthus annuus*) against abiotic stress. *Plant Molecular Biology* 114:34
- [24] Rahim RA, Chowdhury M, Hasi MA, Haque F, Robin AHK. 2025. Genome wide identification and characterization of LIM domain associated gene superfamily in common bean (*Phaseolus vulgaris* L.). *International Journal of Genomics* 2025:5584901
- [25] Xie X, Lin C, Yu F, Li H, Xiao J, et al. 2025. Genome-wide identification and expression analysis of the shaker K⁺ channel gene family in cassava (*Manihot esculenta* Crantz) under potassium stress. *Plants* 14:2213
- [26] Chen C, Cai Y, He B, Zhang Q, Liang D, et al. 2024. Genome-wide identification, evolution, and expression analysis of the DIR gene family in *Schima superba*. *International Journal of Molecular Sciences* 25:7467
- [27] Cheng X, Xu S, Yao S, Zhong Q. 2025. Genome-wide identification and expression pattern analysis of the YTH domain in *Rosa chinensis* 'Old Blush' and validation of the m⁶A binding capability of Rcdf1A. *Industrial Crops and Products* 226:120604
- [28] Guo Z, Kuang Z, Wang Y, Zhao Y, Tao Y, et al. 2020. PmiREN: a comprehensive encyclopedia of plant miRNAs. *Nucleic Acids Research* 48:D1114–D1121
- [29] Li C, Li S, Xu J, Han Z, Li W, et al. 2025. Genome-wide identification and functional characterization of the CP12 gene family in cotton reveals its critical role in heat stress response. *Frontiers in Plant Science* 16:1707567
- [30] Jia C, Shi Y, Wang H, Zhang Y, Luo F, et al. 2024. Genome-wide identification and expression analysis of SMALL AUXIN UP RNA (SAUR) genes in rice (*Oryza sativa*). *Plant Signaling & Behavior* 19:2391658
- [31] Ren Y, Ma R, Wang Z, Li L, Xie M, et al. 2026. Genome-wide analysis identifies ScTCP6 as a stress responsive gene in rye. *Current Issues in Molecular Biology* 48:266
- [32] Wang Z, Cui R, Gao J, Jia L, Yan C, et al. 2025. Genome-wide identification, stress-responsive expression analysis, and key candidate gene mining of the SAP gene family in cucumber and watermelon. *Scientia Horticulturae* 353:114475
- [33] Wang Z, Yuan Y, Rehman F, Wang X, Wu T, et al. 2024. Genome-wide identification and characterization of bHLH gene family in *Hevea brasiliensis*. *Forests* 15:2027
- [34] Kim DK, Iwar K, Ochar K, Park SY, Go EB, et al. 2024. Cowpea (*Vigna unguiculata*) cultivation and breeding in the republic of Korea: advances and future perspectives. *Agronomy* 14(11):2679
- [35] Ehlers JD, Hall AE. 1997. Cowpea (*Vigna unguiculata* L. Walp.). *Field Crops Research* 53(1-3):187–204
- [36] Giorno F, Wolters-Arts M, Mariani C, Rieu I. 2013. Ensuring reproduction at high temperatures: the heat stress response during anther and pollen development. *Plants* 2:489–506
- [37] Suzuki N. 2023. Fine tuning of ROS, redox and energy regulatory systems associated with the functions of chloroplasts and mitochondria in plants under heat stress. *International Journal of Molecular Sciences* 24(2):1356
- [38] Sadura I, Janeczko A. 2024. Are heat shock proteins important in low-temperature-stressed plants? a minireview. *Agronomy* 14(6):1296
- [39] Wang H, Charagh S, Dong N, Lu F, Wang Y, et al. 2024. Genome-wide analysis of heat shock protein family and identification of their functions in rice quality and yield. *International Journal of Molecular Sciences* 25:11931
- [40] Singh P, Pandey B, Purwar S, Singh CM, Pratap A, et al. 2025. Exogenous application of γ -aminobutyric acid alleviates temperature stress in mungbean (*Vigna radiata*) and its wild non-progenitor (*Vigna glabrescens*) by regulating heat shock protein genes. *BMC Plant Biology* 25(1):1651
- [41] Nagaraju M, Kumar A, Jalaja N, Rao DM, Kavi Kishor PBK. 2021. Functional exploration of chaperonin (HSP60/10) family genes and their abiotic stress-induced expression patterns in *Sorghum bicolor*. *Current Genomics* 22:137–152
- [42] Hill JE, Hemmingsen SM. 2001. *Arabidopsis thaliana* type I and II chaperonins. *Cell Stress & Chaperones* 6:190–200
- [43] Ren W, Ding B, Dong W, Yue Y, Long X, et al. 2024. Unveiling HSP40/60/70/90/100 gene families and abiotic stress response in Jerusalem artichoke. *Gene* 893:147912
- [44] Mohammadi S. 2024. Identification of HSP60 family gene in the soybean genome and their functional analysis in response to abiotic stresses. *Crop Biotechnology* 13:11–28
- [45] Xu M, Fu J, Ni Y, Zhang C. 2024. Genome-wide analysis of the MYB gene family in pumpkin. *Peer Journal* 12:e17304
- [46] Paya G, Bautista V, Camacho M, Esclapez J, Bonete MJ. 2023. Comprehensive bioinformatics analysis of the biodiversity of Ism proteins in the *Archaea* domain. *Microorganisms* 11(5):1196
- [47] de Marco A, Deuerling E, Mogk A, Tomoyasu T, Bukau B. 2007. Chaperone-based procedure to increase yields of soluble recombinant recombinant proteins produced in *E. coli*. *BMC Biotechnology* 7:32
- [48] Brocchieri L, Karlin S. 2000. Conservation among HSP60 sequences in relation to structure, function, and evolution. *Protein Science* 9:476–486
- [49] Wang C, Ran F, Zang Y, Liu L, Wang D, et al. 2023. Genome-wide identification and expression analysis of heat shock protein gene family in cassava. *The Plant Genome* 16(4):e20407
- [50] Ying SY, Chang DC, Lin SL. 2008. The microRNA (miRNA): overview of the RNA genes that modulate gene function. *Molecular Biotechnology* 38:257–268
- [51] Zheng Y, Wang X, Cui X, Wang K, Wang Y, et al. 2023. Phytohormones regulate the abiotic stress: an overview of physiological, biochemical, and molecular responses in horticultural crops. *Frontiers in Plant Science* 13:1095363
- [52] Yoshida T, Fabregas N, Fernie AR. 2025. Unveiling links between phytohormone and abiotic stress signaling. *Plant Hormones* 1:e025



Copyright: © 2026 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.