Menara Perkebunan 2025, 93(2), 135-147
p-ISSN: 0125-9318/ e-ISSN: 1858-3768
http://dx.doi.org/10.22302/iribb.jur.mp.v93i2.663
Accreditation Number: 177/E/KPT/2024

In silico study: identification and characterization of heat shock protein 90 (HSP90) in Arabica coffee (*Coffea arabica* L.)

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Received 5 Aug 2025 / Revised 11 Oct 2025 / Accepted 20 Oct 2025

Abstract

The use of low-quality planting material and extreme weather conditions caused by global warming are major factors contributing to low Arabica coffee productivity in Indonesia. The development of new cultivars and the improvement of Arabica coffee adaptability play crucial roles in preventing productivity decline. This study aims to identify and characterize HSP90 proteins in Arabica coffee through in silico analysis, focusing on their adaptability to biotic and abiotic stress conditions. This study was conducted using DNA and HSP90 protein sequences from Arabica coffee retrieved from various databases. The analysis included assessments of physicochemical properties, gene structure analysis, protein subcellular localization prediction, *cis*-acting element analysis, protein interaction analysis, and phylogenetic analysis. The results identified a total of twenty *CaHSP90* genes distributed across 11 Arabica coffee chromosomes. Characterization revealed that the HSP90 protein family has diverse physicochemical properties, with varying sequence lengths and molecular weights. Most members are acidic, hydrophilic proteins localized in the cytoplasm. Analysis of the *CaHSP90* gene expression based on *cis*-acting elements and phylogenetics showed that HSP90 in Arabica coffee is expressed in response to biotic and abiotic stresses as well as defense against pathogens. The results of this study provide a foundation for the development of new Arabica coffee cultivars with improved resistance to biotic and abiotic stresses, and support the selection of candidate *CaHSP90* genes for breeding programs.

[Keywords: bioinformatics, biotic and abiotic stress, C3 plant, heat stress]

Introduction

Indonesia is one of the world's top four coffee producers, after Brazil, Vietnam, and Colombia. This position is largely supported by the productivity of its coffee plantations. Data from the Central Statistics Agency (Badan Pusat Statistik) in 2023 indicated that the area of coffee plantations in Indonesia increased by 0.05% between 2022 and 2023. However, this increase in plantation area was not accompanied by a corresponding increase in coffee production. Coffee production declined by 1.43% from 786.19 tons in 2021 to 774.96 thousand tons in 2022. This decline continued in 2023 at a greater rate of 2.10% bringing total production down to 758.73 thousand tons. This figure represents the largest decline in the last five years, surpassing the decline in the 2018-2019 period, which was only 0.47% (Badan Pusat Statistik, 2023). The use of low-quality planting materials and various biotic and abiotic factors, such as pest and disease attacks, temperature, and altitude, have contributed to reduced Arabica coffee productivity.

The decline in coffee productivity in Indonesia may also be attributed to extreme weather conditions caused by global warming. Extreme weather can affect plant growth and development due to the inhibition of starch biosynthesis (Oldroyd & Leyser, 2020; Tigchelaar et al., 2018). Global warming can also cause protein denaturation due to an increase in temperature of 10-15°C above optimal plant growth. These conditions allow plants to develop adaptive mechanisms to tolerate high temperatures by producing stress-related proteins known as heat shock proteins (Peng et al., 2024).

Heat shock proteins (HSPs) regulate responses to heat stress and are highly conserved across both cellular and organismal levels (Appiah et al., 2021). In C3 plants such as coffee, HSP production is particularly important for supporting photosynthesis by maintaining and protecting heat-sensitive proteins, including PSII (Chauhan et al., 2023; S. Hu et al., 2020). HSPs are classified into five families based on their molecular weight: HSP20, HSP60, HSP70/DnaK, HSP90, and HSP100/ClpB (Donato & Geisler, 2019; Wasilah et al., 2019).

Accounting for 1-2% of total cellular proteins, HSP90 is the most abundant HSP family found in prokaryotic and eukaryotic cytoplasm. This family

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Menara Perkebunan is a DOAJ-indexed journal and accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3125)
How to Cite: Mustafidah, Q. & Su'udi, M. (2025). In silico study: identification and characterization of heat shock protein 90 (HSP90) in Arabica coffee (*Coffea Arabica* L.). *Menara Perkebunan*, *93*(2), 135-147.
http://dx.doi.org/10.22302/iribb.jur.mp.v93i2.663

is involved in several physiological processes, including plant growth and development, biotic and abiotic stress responses, and the repair of damaged proteins. HSP90 supports normal cell survival under stress, maintains the conformation of other proteins, and acts as a negative feedback regulator of the heat stress response (Peng et al., 2024). Structurally, consists of three domains: an N-terminal ATP-binding domain, an M domain, and a C-terminal substrate-binding domain (Chiosis et al., 2023). HSP90 is abundantly expressed in the plant cytoplasm under normal physiological conditions but rapidly accumulates in the nucleus under heat stress (Appiah et al., 2021).

HSP90 has been identified in several plants, including *Arabidopsis thaliana* with seven HSP90 genes (Krishna & Gloor, 2001), *Oryza sativa* with nine genes (Hu et al., 2009), *Zea mays* with eleven genes (Magnard & Vergne, 1996), and *Solanum lycopersicum* with six genes (Liu et al., 2014). These studies, conducted using both in silico bioinformatics analyses and in vitro validation, have provided valuable insights into the functional mechanisms of HSP90 proteins in various plants. However, similar studies have not yet been conducted in Arabica coffee, despite its economic importance and vulnerability to temperature-related stress. Accordingly, this study aims to identify and characterize HSP90 genes in Arabica coffee through in silico analysis, focusing on their potential roles in adaptation to biotic and abiotic stress conditions.

Materials and Methods

This research was conducted in silico using DNA and protein sequences of HSP90 from Arabica coffee, obtained from the Phytozome database (*Coffea arabica* Geisha v1.0) (Goodstein et al., 2012). HSP90 sequences from *A. thaliana* (AtHSP90) were also retrieved from the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/protein/) and used for motif analysis using the MOTIF tool (https://www.genome.jp/tools/motif/). The resulting motifs served as queries for BLAST searches (with E-value cutoff 10e-4) against the Arabica coffee genome. Subsequent analyses included HSP90 identification, physicochemical properties assessment, gene structure and chromosomal localization, *cis*-acting element analysis, protein interactions and subcellular localization prediction, and phylogenetic analysis.

Data extraction and identification of HSP90

From BLAST search results, transcript ID, chromosome number, chromosome location data, base pair length of coding sequence (CDS), protein length, and Phytozome annotations were collected. When identical transcript IDs were detected, redundant or incomplete sequences were removed. The candidate *HSP90* genes were named *CaHSP90* (*Coffea arabica HSP90*) and numbered sequentially according to their chromosomal position. The genome, CDS, and peptide sequences of each gene were then retrieved from the Phytozome database for further analysis.

Physicochemical properties analysis

The physicochemical properties of CaHSP90 proteins were analyzed using the Expasy ProtParam (https://web.expasy.org/protparam/) (Gasteiger et al., 2005). Parameters included molecular weight (kDa), isoelectric point (pI), and Grand Average of Hydropathicity (GRAVY). Negative GRAVY values indicate hydrophilic proteins, whereas positive values indicate hydrophobic proteins.

Gene structure analysis

Gene structure analysis was performed by aligning the genomic and CDS sequences of each *CaHSP90* gene using the Gene Structure Display Server 2.0 (https://gsds.gao-lab.org/) (Hu et al., 2015). This analysis was performed to determine the number of exons and introns and the completeness of each *CaHSP90* gene.

Chromosomal localization analysis

HSP90 gene locations and distribution within chromosomes were visualized using the PhenoGram Plot tool (https://visualization.ritchielab.org/phenograms/plot). Input data included chromosome number, gene location, and the length of each chromosome in the Arabica coffee genome. This analysis was conducted to determine the distribution of the *HSP90* gene within each chromosome in Arabica coffee.

Cis-acting element analysis

Cis-acting element analysis was performed by retrieving the promoter regions (2000 bp upstream of the start codon) of each *CaHSP90* gene from the Phytozome database. The obtained sequences were then analyzed using the PlantCARE database (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002). The results were visualized with TBtools-II (Chen et al., 2023) to predict the regulation of *HSP90* gene expression in Arabica coffee.

Protein interaction analysis

Analysis of CaHSP90 protein interactions was performed using STRING software (https://string-db.org/) (Szklarczyk et al., 2019) based on the CaHSP90 protein sequences.

Protein subcellular localization prediction

Subcellular localization of CaHSP90s was predicted using CELLO v.2.5 (http://cello.life.nctu.edu.tw/) (Yu et al., 2004; Yu et al., 2006) and WoLF PSORT (https://wolfpsort. hgc.jp/) (Horton et al., 2007). CELLO results marked with an asterisk were retained, while all WoLF PSORT data and predicted values were included. Data were visualized as a heat map using TBtools-II (Chen et al., 2023).

Phylogenetic analysis

Evolutionary relationships of Arabica coffee HSP90s were analyzed through multiple sequence alignment with HSP90s from *A. thaliana, S. lycopersicum, Z. mays,* and *O. sativa*. Sequence alignment was performed using ClustalW (Thompson et al., 1994) and MEGA11 (Tamura et al., 2021). Phylogenetic trees were constructed using the maximum likelihood method with partial deletion parameters and a 1000-fold bootstrap method. The phylogenetic tree construction provides information on the evolutionary relationships of Arabica coffee HS90 with several other plants to identify orthologs between species and paralogs within species.

Results and Discussion

*Identification of the HSP90 gene in Arabica coffee (*Coffea arabica *L.)*

BLAST results on the *AtHSP90* motif against the Arabica coffee genome sequence identified 20 *HSP90* genes, designated *CaHSP90-1* to *CaHSP90-20* (Table 1). The twenty *CaHSP90* genes have varying sequence lengths with CDS ranging from 306 bp (*CaHSP90-1*) to 2466 bp (*CaHSP90-20*) (Table 1). The identified genes encode proteins ranging from 102 to 822 amino acids (aa), corresponding molecular weights range from 11.641 to 93.525 kDa, and isoelectric points (pI) ranging from 4.86 to 9.13. Based on the pI values, fifteen CaHSP90 proteins (pI < 7) were acidic and five were basic (pI > 7). The basic CaHSP90 proteins have different properties compared to HSP90s found in *A. thaliana*, tomatoes, and several other acidic plants (Sajad et al., 2022).

The GRAVY index of CaHSP90s ranged from -0.896 to -0.332, indicating that all members of CaHSP90 are hydrophilic (Zhang et al., 2021). Protein localization predictions indicate that most CaHSP90 proteins are localized in the cytoplasm, with several also detected in the nucleus and endoplasmic reticulum. This is consistent with the results of Appiah et al. (2021), which reported that HSP90 is mainly accumulated in the cytoplasm and plays an important role in regulating the response to heat stress.

CaHSP90 protein motif

Motif analysis of the 20 *CaHSP90* genes showed that all contained a histidine kinase-like ATPase or HATPase_c domain (Figure 1). The HATPase_c is a conserved protein domain found in several ATP-binding proteins, including histidine kinase, DNA gyrase B (GyrB), topoisomerase (Bellon et al., 2004), molecular chaperones HSP90 (Immormino et al., 2004), DNA mismatch repair protein, and phytochrome-like ATPase (Bettaieb et al., 2020). Based on research by Zhang et al. (2021), it was stated that protein sequences containing both HSP90 and HATPase_c domains were identified as HSP90.

HSP90 interacts with HATPase to bind and hydrolyze ATP (Bettaieb et al., 2020). Motif analysis revealed that the longest HSP90 domain was present in CaHSP90-18, spanning 540 aa, while the shortest was in CaHSP90-15, comprising only 40 aa (Figure 1).

CaHSP90 gene structure

Gene structure analysis was done to further investigate the structural characteristics of the *CaHSP90* gene family. Variations in gene structure between *HSP90* groups are associated with differences in their function in subcellular compartments (Bettaieb et al., 2020). Among the twenty identified *CaHSP90* genes, each exhibits a distinct exon-intron pattern (Figure 2).

Genes with complete structures consist of upstream, exon, intron, and downstream regions. Among the 20 *CaHSP90* genes, nine possess complete structures, while eleven are incomplete. The largest gene, *CaHSP90-6,* spans around 9 kb, whereas the smallest gene, *CaHSP90-12*, is only 315 bp. The largest number of exons is found in *CaHSP90-5* and *CaHSP90-6,* with a total of twenty exons, while the fewest are found in *CaHSP90-2, CaHSP90-7, CaHSP90-9, CaHSP90-10, CaHSP90-12,* and *CaHSP90-13,* each with only a single exon.

Exon-intron organization provides insights into gene evolution (Wang et al., 2022). In Arabica coffee, the variation in the gene structure among *CaHSP90* genes suggests possible evolutionary divergence within the family. The number of introns is largely related to the sensitivity of gene transcription regulation. Genes with fewer introns usually respond more rapidly to environmental stimuli (Appiah et al., 2021; Sajad et al., 2022).

Table 1. *HSP90*s in *Coffea arabica* L.

Transcript ID	Gene name	Chr	Location	AA	Molecular weight (kDa)	pI	GRAVY	Exon; Intron	Protein localization prediction	Phytozome annotations
CAG017222	*CaHSP90-1*	1	9951346-9952313	102	11,641	9.13	-0.477	2;1	nuclear (1.704), mitochondrial (1.152)	Heat shock protein 90 // Heat shock protein 90-1
CAG017223	*CaHSP90-2*	1	9952492-9953164	224	26,047	8.27	-0.467	1;0	nuclear (2.086), cytoplasmic (2.032)	Heat shock protein Hsp90 family // Ribosomal protein S5 domain 2-type fold
CAG027009	*CaHSP90-3*	2	1880918-1884147	706	81,000	4.90	-0.622	3;2	cytoplasmic (3.399)	Molecular chaperone HtpG (htpG, HSP90A)
CAG020662	*CaHSP90-4*	2	2031653-2034988	706	80,997	4.90	-0.621	3;2	cytoplasmic (3.376)	Molecular chaperone HtpG (htpG, HSP90A)
CAG031359	*CaHSP90-5*	2	45410575-45418062	798	90,847	5.35	-0.575	20;19	cytoplasmic (1.955), nuclear (1.795)	Heat shock protein 90kDa beta (HSP90B, TRA1)
CAG025028	*CaHSP90-6*	2	47058756-47067705	798	90,797	5.44	-0.580	20;19	nuclear (2.019), cytoplasmic (1.700)	Heat shock protein 90kDa beta (HSP90B, TRA1)
CAG038127	*CaHSP90-7*	3	26971839-26973009	390	45,042	8.64	-0.551	1;0	nuclear (2.522), cytoplasmic (1.903)	Heat shock protein Hsp90 family // Concanavalin A-like lectin/glucanase domain // Ribosomal protein S5 domain 2-type fold
CAG048341	*CaHSP90-8*	5	3118777-3120085	381	44,622	5.52	-0.743	2;1	cytoplasmic (2.386), nuclear (2.212)	Heat shock protein Hsp90 family // Ribosomal protein S5 domain 2-type fold
CAG051226	*CaHSP90-9*	5	42225190-42226267	359	40,616	4.86	-0.580	1;0	cytoplasmic (3.014)	Heat shock protein 90 // Subfamily not named
CAG051227	*CaHSP90-10*	5	42226482-42226974	164	18,795	5.18	-0.896	1;0	nuclear (2.581)	Heat shock protein 90 // Heat shock protein 90-2-related

Table 1. (*continued*)

Transcript ID	Gene name	Chr	Location	AA	Molecular weight (kDa)	pI	GRAVY	Exon; Intron	Protein localization prediction	Phytozome annotations
CAG047913	*CaHSP90-11*	5	43279035-43282725	700	80,063	4.98	-0.577	3;2	cytoplasmic (3.825)	Heat shock protein 90 // Subfamily not named
CAG062435	*CaHSP90-12*	7	15914539-15914854	105	11,791	8.95	-0.461	1;0	nuclear (1.713)	Heat shock protein 90 // Heat shock protein 90-1
CAG062436	*CaHSP90-13*	7	15914880-15915606	242	27,994	5.63	-0.493	1;0	cytoplasmic (4.094)	Heat shock protein 90 // Subfamily not named
CAG069612	*CaHSP90-14*	8	39491412-39495200	704	80,948	4.99	-0.625	4;3	cytoplasmic (3.571)	Molecular chaperone HtpG (htpG, HSP90A)
CAG072725	*CaHSP90-15*	8	42150086-42151935	369	41,163	8.29	-0.332	4;3	plasmamembrane (1.164), nuclear (1.158)	Heat shock protein Hsp90 family // Ribosomal protein S5 domain 2-type fold
CAG072730	*CaHSP90-16*	8	42166348-42169730	667	76,721	5.17	-0.587	4;3	cytoplasmic (3.843)	Molecular chaperone HtpG (htpG, HSP90A)
CAG000807	*CaHSP90-17*	10	1175362-1180488	768	88,457	5.17	-0.668	15;14	ER (3.351)	Heat shock protein 90 // Endoplasmin homolog
CAG003938	*CaHSP90-18*	10	1533161-1539627	816	93,525	4.94	-0.706	15;14	ER (4.256)	Heat shock protein 90 // Endoplasmin homolog
CAG008931	*CaHSP90-19*	11	31790321-31796274	822	93,387	5.10	-0.530	19;18	cytoplasmic (2.631)	Heat shock protein 90kDa beta (HSP90B, TRA1)
CAG011964	*CaHSP90-20*	11	33999410-34005741	800	90,665	4.98	-0.539	19;18	cytoplasmic (2.617)	Heat shock protein 90kDa beta (HSP90B, TRA1)

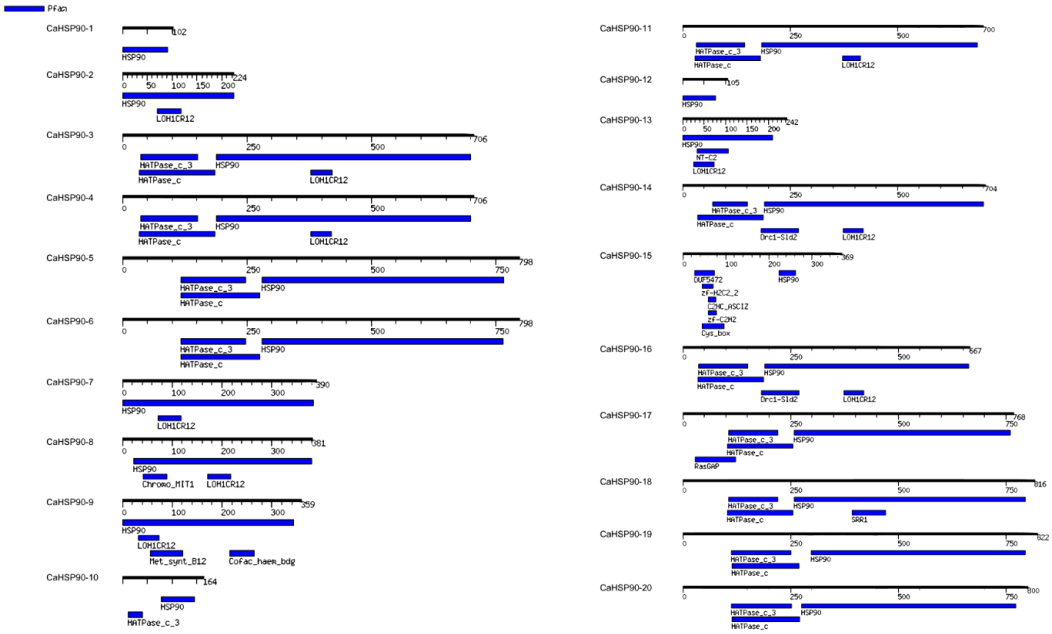

Figure 1. CaHSP90 protein motif

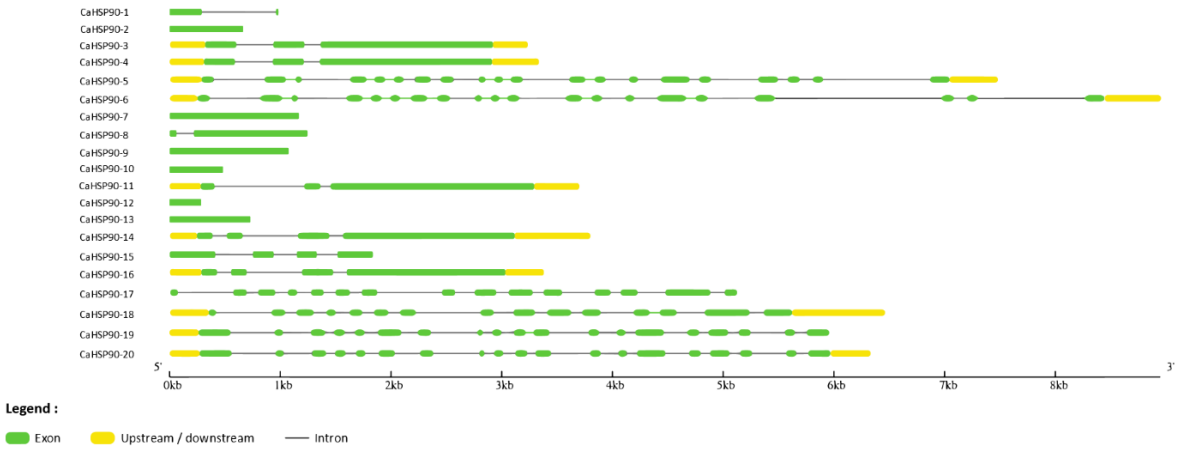

Figure 2. *CaHSP90* gene structure

Chromosomal localization of CaHSP90 genes

Arabica coffee has a tetraploid genome with 11 pairs of chromosomes. Localization data showed that one *CaHSP90* gene was located on chromosome 3; two genes each on chromosomes 1, 7, 10, and 11; three genes on chromosome 8; and four genes each on chromosomes 2 and 5. No genes were located on chromosomes 4 and 9 (Figure 3).

Compared to other species in the same subclass as Arabica coffee, the Asteridae, the distribution of *HSP90* genes in tobacco (*Nicotiana tabacum*) differs. The tobacco genome has nine HSP90s randomly distributed on its 12 chromosomes, while the remaining 12 HSP90s remain unassigned (Song et al., 2019).

Cis-acting element analysis of CaHSP90

Cis-acting element analysis was conducted to understand the regulation of *CaHSP90* expression in response to stress conditions under abiotic stress. The results revealed 51 types of *cis*-acting elements in the promoter regions of *CaHSP90* genes (Figure 4). Among them, the TATA box was detected across all *CaHSP90* promoters. This element plays a central role in transcription initiation by facilitating the binding of RNA polymerase II to the promoter via interaction with the TATA-binding protein (Savinkova et al., 2023).

Several light-responsive *cis*-acting elements were also identified, including MRE, G-box, ACE, Box 4, ATCT motif, GT1 motif, and GATA motif

(Bettaieb et al., 2020; Zhang et al., 2021). The MRE element was detected in *CaHSP90-7, CaHSP90-9, CaHSP90-13,* and *CaHSP90-15,* located 1,480 bp upstream of the start codon in *CaHSP90-15*. The ACE element was detected only in *CaHSP90-15* and *CaHSP90-18,* located 890 bp and 420 bp upstream of the start codon, respectively (Figure 4).

Other *cis*-acting elements identified in *CaHSP90* were LTR, ARE, MBS, and TC-rich repeats. LTR is known to be responsive to drought, salinity, and low temperature (Bettaieb et al., 2020; Xue et al., 2023). The element was found in *CaHSP90-3, CaHSP90-4, CaHSP90-5, CaHSP90-6, CaHSP90-19,* and *CaHSP90-20*. The ARE elements were detected in 18 *CaHSP90* genes, excluding *CaHSP90-3* and *CaHSP90-4.* The element is known to play an important role in responding to oxygen limitation. Additionally, TC-rich repeats, associated with defense and stress response, and the MYB Binding Site (MBS), involved in drought induction, were also present in several *CaHSP90* promoters (Bettaieb et al., 2020; Xue et al., 2023).

The promoter region analysis also revealed the presence of phytohormone-responsive elements, including AuxRR-core and TGA element (auxin-responsive), the GARE motif and P-box (gibberellin-responsive), ABRE (abscisic acid-responsive), TCA element (salicylic acid-responsive), and CGTCA and TGACG motifs (both methyl jasmonate-responsive) (Bettaieb et al., 2020).

Overall, the total of 51 *cis*-acting elements identified play key roles in growth, development, and abiotic or hormonal stress responses, suggesting the complex functions of *CaHSP90*s. The ABRE element, associated with abscisic acid responsiveness, has been identified in *A. thaliana* to regulate lignin deposition and secondary cell wall formation through phosphorylation of the NST1 wall protein (Liu et al., 2021). Collectively, these diverse regulatory elements may contribute to tissue-specific and stress-responsive expression patterns of *CaHSP90* genes in Arabica coffee, influencing both developmental processes and adaptive stress responses (Bettaieb et al., 2020).

Interactions between proteins in CaHSP90

The protein-protein interaction (PPI) network of HSP90s in Arabica coffee (Figure 5), showed that CaHSP90-1, CaHSP90-4, CaHSP90-12, CaHSP90-15, and CaHSP90-16 interact with CaHSP90-5, CaHSP90-6, CaHSP90-7, CaHSP90-17, CaHSP90-18, CaHSP90-19, and CaHSP90-20. Additionally, HSP90 proteins interact with several other proteins, including the cysteine- and histidine-rich domain-containing protein RAR1, the heat shock factor binding protein, the Aha1_N domain-containing protein, and the cochaperone protein p23 (Figure 5).

The RAR1 (required for Mla12 resistance) protein is involved in the plant immune response to pathogens. In plants, RAR1 plays a crucial role in R protein activity and facilitates he interaction between SGT1 and HSP90. RAR1 interacts with the N-terminal portion of HSP90, which contains the ATPase domain (Niikura & Kitagawa, 2019). The observation that RAR1 interacts with all CaHSP90 proteins suggests a conserved role for Arabica coffee HSP90s in pathogen defense and immune regulation.

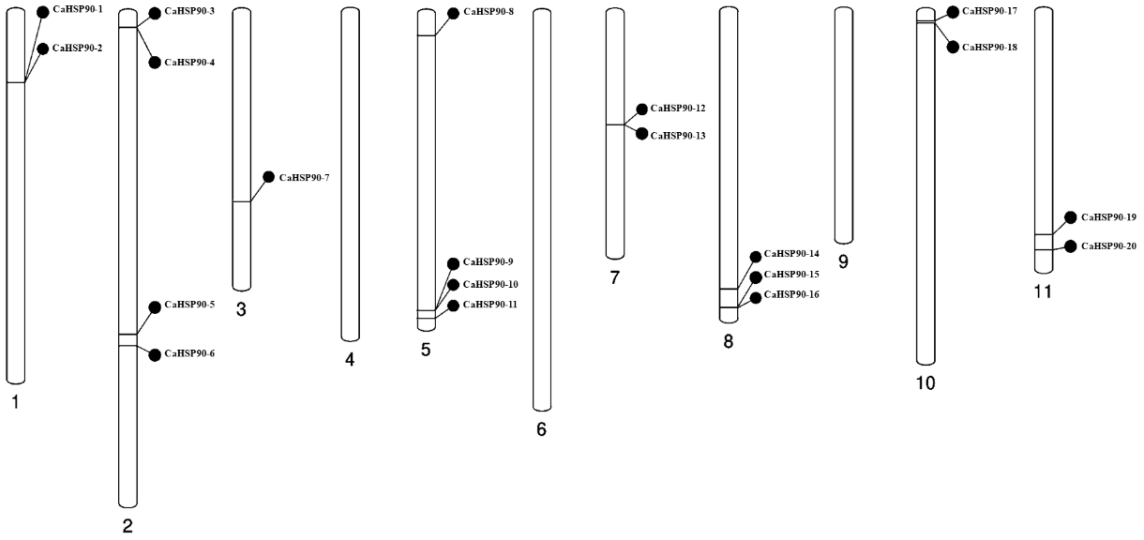

Figure 3. Chromosomal distribution of *CaHSP90* genes

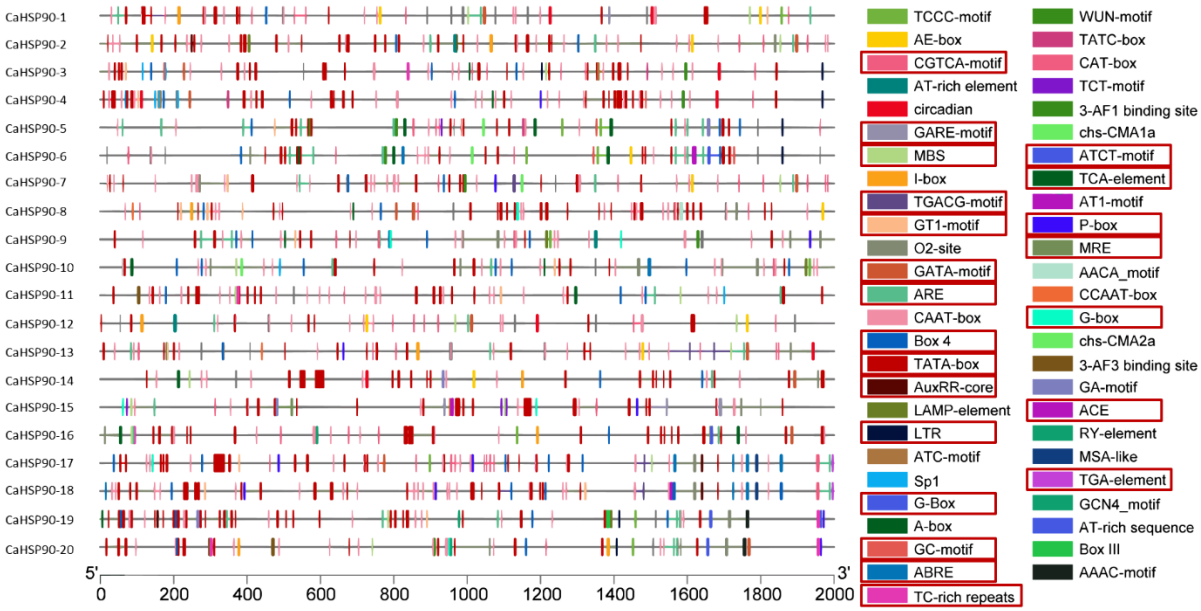

Figure 4. *Cis*-acting element CaHSP90. The most common cis-acting elements are marked with red boxes.

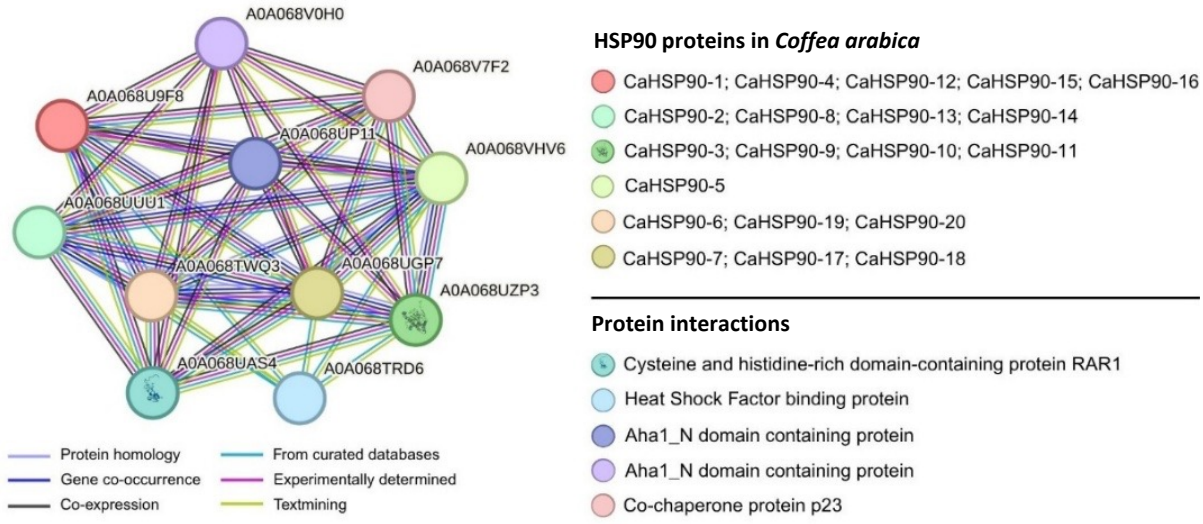

Figure 5. CaHSP90 protein interactions

In plants, the heat shock factor binding protein (HSBP) regulates the expression of the heat shock factor (*HSF*) gene under normal and recovery conditions. Based on the PPI analysis, HSBP interacts with all CaHSP90 proteins because it is related to HSF, which regulates HSP90 gene activation.

The Arabica coffee HSP90 proteins also interact with Aha1 (activator of HSP90 ATPase 1) as the only co-chaperone that accelerates the formation of its closed conformation. Aha1 comprises two domains, the C-terminal (Aha1_C) and N-terminal (Aha1_N), which interact with the HSP90 center domain of HSP90 proteins (Albakova, 2024; Mondol et al., 2023). Aha1_N (Figure 5) interacts with all CaHSP90 proteins because it is the main activator in increasing ATPase activity.

Another important interacting partner identified was p23/PTGES3, a co-chaperone that stabilizes the closed conformation of HSP90 and suppresses ATPase activity to regulate its chaperone cycle (Schopf et al., 2017). The interaction of p23 with all CaHSP90 proteins suggests its critical role in fine-

tuning HSP90's conformational dynamics during stress response and protein folding.

Subcellular localization of CaHSP90 proteins

The predicted subcellular localization of HSP90 proteins in Arabica coffee showed that most of the CaHSP90 proteins were localized in the nucleus with lower prediction scores, while a smaller proportion were detected in the cytoplasm with higher prediction scores (Figure 6). This is consistent with the study by Appiah et al. (2021), which stated that HSP90 is expressed in the cytoplasm. This was also shown in *A. thaliana* (Sarkar et al., 2009), *Brachypodium distachyon* (L.) P. Beauv (Zhang et al., 2017), which also indicates that the cytoplasm is the site of protein assembly, which may be the main site of HSP90 protein activity (Bettaieb et al., 2020).

Phylogenetic Analysis of CaHSP90

Phylogenetic analysis was conducted to examine evolutionary relationships among HSP90 proteins in *C. arabica* and other plant species, and to identify potential orthologous and paralogous relationships. The resulting tree grouped the proteins into five clades, with Group I having the largest number of members (15 genes) and Group III the fewest (three genes). Group I comprised four genes from *Z. mays*, three from *O. sativa*, two each from *A. thaliana* and *S. lycopersicum*, and four genes from *C. Arabica*. Group III contained two genes from *C. Arabica* and one from *O. sativa*.

Several orthologous relationships were identified between *C. arabica* and other species, including *CaHSP90-5* and *CaHSP90-6* with *AtHSP90-6*; *CaHSP90-17* and *CaHSP90-18* with *AtHSP90-7*; *CaHSP90-1* and *CaHSP90-15* with *Os09g36420*.1; *CaHSP90-11* with *Solycl2g015880.1.1* (*SlHSP90-7*); and *CaHSP90-3* and *CaHSP90-4, CaHSP90-9* and *CaHSP90*-13 with *AtHSP90*-1. Eight pairs of paralogous genes were also identified within C. arabica: *CaHSP90-19* and *CaHSP90-20*, *CaHSP90-5* and *CaHSP90-6*, *CaHSP90-17* and *CaHSP90-18*, *CaHSP90-8* and *CaHSP90-10*, *CaHSP90-2* and *CaHSP90-7*, *CaHSP90-14* and *CaHSP90-16, CaHSP90-3* and *CaHSP90-4,* and *CaHSP90-9* and *CaHSP90-13*. The phylogenetic clustering revealed clear separation between dicots (*C. arabica*, *A. thaliana*, and *S. lycopersicum*) and monocots (*O. sativa* and *Z. mays*), consistent with their taxonomic relationships. The presence of multiple paralogous pairs within *C. arabica* indicates gene duplication events during the evolution of the HSP90 family, contributing to species-specific diversification (Song et al., 2019).

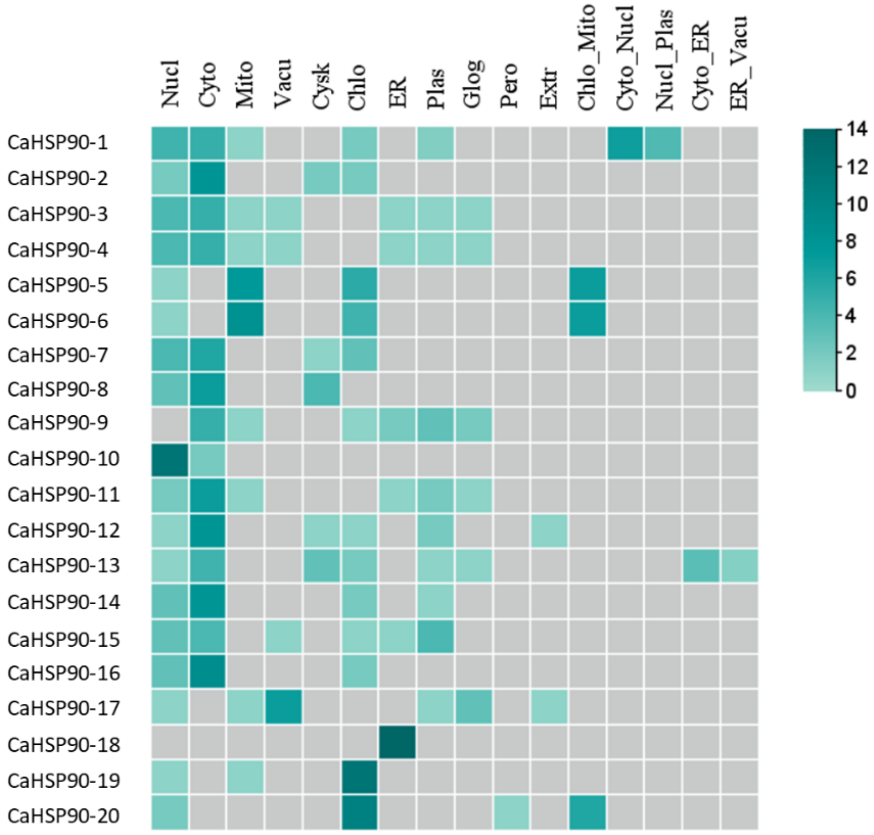

Figure 6. Prediction of the subcellular localization of CaHSP90 proteins

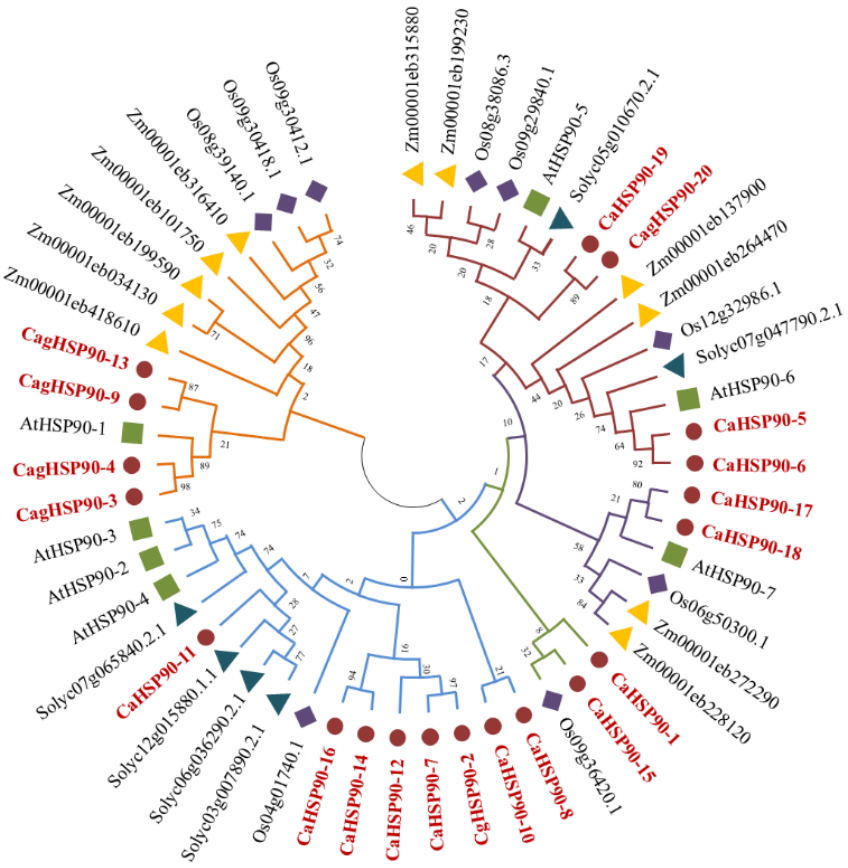

Figure 7. Evolutionary relationships of HSP90 in *C. Arabica, A. thaliana, S. lycopersicum, O. sativa,* and *Z. mays*; CaHSP90 is written in red.

Furthermore, several orthologous *CaHSP90* genes showed similarities to stress-responsive homologs in other species. *CaHSP90-19* and *CaHSP90-20* share similarity with *AtHSP90-5* and Solyc05g010670.2.1 (*SlHSP90-2*), which are associated with both drought and salinity tolerance (Song et al., 2009). *CaHSP90-5* and *CaHSP90-6* cluster with *AtHSP90-6,* which is linked to plant development (Luo et al., 2019; Zai et al., 2015). *CaHSP90-17* and *CaHSP90-18* share similarity with *AtHSP90-7*, which is associated with drought and salt stress tolerance (Song et al., 2009). Finally, *CaHSP90-3* and *CaHSP90-4, CaHSP90-9*, and *CaHSP90-13* group with *AtHSP90-1,* associated with osmotic stress response and ABA responses (Zai et al., 2015; Zhang et al., 2017).

Conclusion

This in silico study identified twenty *HSP90* genes in the *Coffea arabica* genome, providing the first comprehensive characterization of the *HSP90* gene family in this species. The identified CaHSP90 proteins displayed distinct physicochemical properties, reflecting functional diversity among family members. Most were found to be acidic, hydrophilic, and predominantly localized in the cytoplasm, consistent with their roles as molecular chaperones. *Cis*-acting element and phylogenetic analyses indicated that *CaHSP90* genes are regulated in response to various biotic and abiotic stresses, including light, drought, salinity, low temperature, and pathogen attack. These findings highlight the importance of the HSP90 family in stress adaptation and defense mechanisms in Arabica coffee. Overall, this study provides a molecular foundation for understanding the structure, regulation, and potential function of HSP90 genes in *C. arabica*. The identified candidate genes offer valuable targets for future breeding programs aimed at developing stress-tolerant Arabica coffee cultivars through molecular-assisted selection or genetic improvement strategies.

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