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Dr. Mostafa Haghpanah,
Kohgiluyeh and Boyerahmad Agricultural and
Natural Resources Research and Education
Center, Dryland Agricultural Research
Institute, Agricultural Research, Education and
Extension Organization (AREEO), Gachsaran,
Iran

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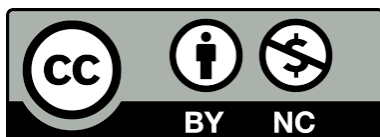
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Correspondence

Dr. Parviz Heidari
heidarip@shahroodut.ac.ir

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Structure, evolution, and expression analysis of *HSP100* genes in *Phaseolus vulgaris*

Parviz Heidari^{1*}, Zahra Khalili², Elnaz Ramezani², Maryam Maghsodi²,
Fatemeh Mahdavi Meighan²

¹ Shahrood University of Technology

² Faculty of Agriculture, Shahrood University of Technology, Shahrood, Iran

Abstract: Heat shock proteins (HSPs) are integral to plant responses to stress, with HSP100 constituting a significant family involved in protein disaggregation and thermotolerance. This study investigated the members of *HSP100* gene family in *Phaseolus vulgaris*, *Pisum sativum*, *Glycine max*, and *Oryza sativa*, disclosing variations in gene number (ranging from 8 to 17 members), molecular weight (between 101 and 119 kDa), exon count (ranging 3 to 12), and isoelectric point (from 5.83 to 8.81). Phylogenetic analysis separated HSP100 family members into four main groups, each of which showed specific subcellular localizations (nucleus, chloroplast, mitochondria, and cytoplasm-nucleus). Predictions of phosphorylation sites indicated interspecies variability in members of the HSP100 family. Prediction of duplication events in *P. vulgaris* revealed that segmental duplication events have occurred more in *HSP100* family members. Expression analyses revealed that PHAVU-008g035600g and PHAVU-001g243900g are more responsive to salinity stress. Interaction networks revealed that HSP100s associate with SCF ubiquitin ligase components, esterases, CLP proteases, and HSP21, all enriched in ATP-dependent activities. Our findings revealed a deeper understanding of the evolutionary and expression diversity of HSP100 proteins within the plants.

Keywords: Heat shock proteins; plant gene family; gene expression; bean genome.

Introduction

Heat shock proteins (HSPs) and ion transporters constitute an essential component of the plant stress response mechanism. They contribute to the protection of cellular structures and the maintenance of homeostasis under adverse conditions (Timperio et al., 2008; Hosseini Pouya et al., 2025). HSPs prevent the aggregation of denatured proteins by binding to damaged proteins, preventing the formation of toxic aggregates, and helping to restore the normal structure of proteins. In the event of irreversible damage, HSPs direct defective proteins to degradation systems such as the proteasome or Clp complexes (Parsell and Lindquist, 1993; Pickart and Cohen, 2004). These functions are crucial for maintaining protein homeostasis under stress conditions, making HSPs a key cell defense mechanism. These proteins are ubiquitous in both prokaryotes and eukaryotes (Wang et al., 2004). HSPs act as innate biomarkers and play a pivotal role in genetic diversity and facilitating the survival of organisms exposed to biotic and abiotic stressors (Abasi et al., 2024; Pan et al., 2024). In plant cells, HSPs provide cellular protection by modulating the synthesis and transport of newly synthesized proteins, as well as by repairing denatured proteins under stress and non-stress conditions (Sung et al., 2001; Al-Whaibi, 2011). Furthermore, these proteins are integral components of cell signaling pathways that ultimately affect the expression levels of specific genes during stress (Divya et al., 2019; Izumi, 2019; Pan et al., 2024). The HSP family includes a diverse range of protein types classified by their molecular weight and sequence homology. This classification includes small HSPs, chaperonins (Hsp60), Hsp70, Hsp90, and Hsp100, each of which exhibits main functional roles in the stress response. The molecular weights of these proteins can vary considerably, ranging from 10 kDa to over 100 kDa (McLoughlin et al., 2016; Nitnavare et al., 2016; Zhao et al., 2018; Heidari et al., 2024).

HSP100 proteins, especially ClpBs, are involved in the repair of proteins that have aggregated under severe stresses such as heat. These proteins use ATP energy to repair denatured proteins and restore them to a functional state. In plants, bacteria, and yeast, the absence of ClpB results in severe heat

sensitivity (Mishra and Grover, 2016). It was stated that HSP100/ClpB proteins can be induced in response to cold and salinity stresses (Singla et al., 1997). In *Arabidopsis thaliana*, HSP101 is essential for plant survival against heat stress. Deletion of the *HSP101* gene results in the plant's inability to recover from abiotic stresses such as heat stress (Hong and Vierling, 2001). HSP101 and HsSA32 proteins are up-regulated in rice tissues, including reproductive organs such as spikelets, in response to heat stress (Lin et al., 2014). Besides, HSP100 is also induced in response to other abiotic stresses such as drought, salinity, and oxidative stress. These proteins help plants resist adverse environmental conditions by controlling the activity of antioxidant enzymes and reducing damage to cellular proteins (Zheng et al., 2025). HSPs and HSP100 may interact with chloroplast membranes and protect photosynthetic processes from heat stress (Sun et al., 2002). Although *HSP100* genes have been studied in some plants, they have not yet been investigated in important plants such as beans. In this study, all members of the *HSP100* gene family (including HSPs with an equal molecular weight greater than 100 kDa) in the genomes of *Phaseolus vulgaris*, *Pisum sativum*, *Glycine max*, and *Oryza sativa* were identified and investigated. The hypothesis of this study was that members of this gene family have structural, evolutionary, and expression differences from each other.

Materials and Methods

Identification of HSP100 genes

The sequence of the known HSP100 protein in *Arabidopsis* was used as a reference against the genomes of *Phaseolus vulgaris*, *Pisum sativum*, *Glycine max*, and *Oryza sativa* in the BLASTP program with an E-value $\leq 1e-2$. All identified sequences were submitted to CDD (<https://www.ncbi.nlm.nih.gov/cdd/>) and the pfam database to confirm having the ClpA/ClpB, AAA lid domain. All non-redundant genes were targeted for sequence analysis.

Sequence analysis and structural characterization

Physicochemical properties such as protein isoelectric point (pI), molecular weight (MW), instability index, number of amino acids (AA), and GRAVY (Grand Average of Hydropathy) were

predicted using the ProtParam tool (Gasteiger et al., 2003). Besides, the subcellular localization of HSP100 proteins was predicted using the DeepLoc 2.0 server (<https://services.healthtech.dtu.dk/services/DeepLoc-2.0/>).

Phylogenetic analysis

The amino acid sequences of HSP100 proteins in *Phaseolus vulgaris*, *Pisum sativum*, *Glycine max*, and *Oryza sativa* were aligned using the Clustal Omega tool (Sievers and Higgins, 2018). The aligned file was introduced to the IQ-tree online software (Nguyen et al., 2015) and phylogenetic relationships were examined based on the maximum-likelihood method with 1000 bootstrap replications. Phylogeny tree was illustrated by using the iTOL online tool (Letunic and Bork, 2019).

Chromosomal localization and gene duplication

The positions of each HSP100 in *P. vulgaris* were collected from the Ensembl Plants database (Bolser et al., 2016) and information was uploaded to TBtools software (Chen et al., 2020) to construct the chromosome localization. Besides, the gene duplication events between HSP100 in *P. vulgaris* were determined according to similarity in aligned sequences. The paired sequences with more than 70% similarity were identified as a duplicated gene.

RNA-seq data analysis

High-throughput sequencing studies of *P. vulgaris* in Gene Expression Omnibus (GEO) were investigated. Two important studies were factored into this analysis. The first study (GEO accession: PRJNA574280) was conducted on fungal (*Sclerotinia sclerotiorum*) infection. The second study (GEO accession: GSE156113) was related to the expression profile in root and leaf tissues in two cultivars, including TR43477, as a salt-susceptible cultivar, and Ispir, as a salt-tolerant cultivar (Niron et al., 2020). To analyze raw data, FastQC was used to read the Fastq file quality, and Cutadapt was used to remove the adapters (Martin, 2011). Then, STAR was applied to align the cleaned reads based on the reference genome (Dobin et al., 2013) and the number of reads mapped to each gene was determined using the featureCounts (Liao et al., 2014). In the final step, the expression level of each

gene was calculated from the count table using the DESeq2 package (Love et al., 2014).

Interaction network of HSP100

To better understand how HSP100 proteins interact with other proteins, an interaction network was drawn based on the available information for the model plant, Arabidopsis. Initially, the sequences of HSP100 proteins in *P. vulgaris* were introduced into the STRING database (Mering et al., 2003), and the interaction network was drawn based on the degree of homology. The number of nodes in the first and second layers was set to 20 and 5, respectively. The Gene Ontology (GO) analysis was also performed based on the molecular functions of the network members.

Results

HSP100 genes characterization

Results showed that HSP100 family had 12 members in *P. vulgaris*, 8 members in *P. sativum*, 17 members in *G. max*, and 9 members in *O. sativa* (Table S1). The molecular weight of the members of this family varied from 101-119 kDa in *P. vulgaris*, from 101 to 110 kDa in *P. sativum*, 101-119 kDa in *G. max*, and 101-116 kDa in *O. sativa*. For the number of exons, the members of this gene family had significant differences, such that some members had fewer exons and some had more exons.

The number of exons was between 3 and 12 in *P. vulgaris*, *G. max* and *O. sativa*, and in HSP100 from *P. sativum* it was between 6 and 12 (Figure 1a). However, most of the studied HSP100s had 10 exons. For the isoelectric (pI), there were differences between the studied HSP100 proteins, and they had a range of 5.83 to 8.81 and the greatest changes were observed between HSP100s belonging to *P. sativum* (Figure 1b). The GRAVY index indicated that all studied HSP100s were hydrophilic, although HSP100 from *O. sativa* plants showed greater diversity than from dicotyledonous plants (Figure 1c). According to the instability index, most members of the HSP100 family in *P. vulgaris*, *G. max* and *O. sativa* were predicted as unstable proteins, while HSP100s from *P. sativum* were more stable (Figure 1d).

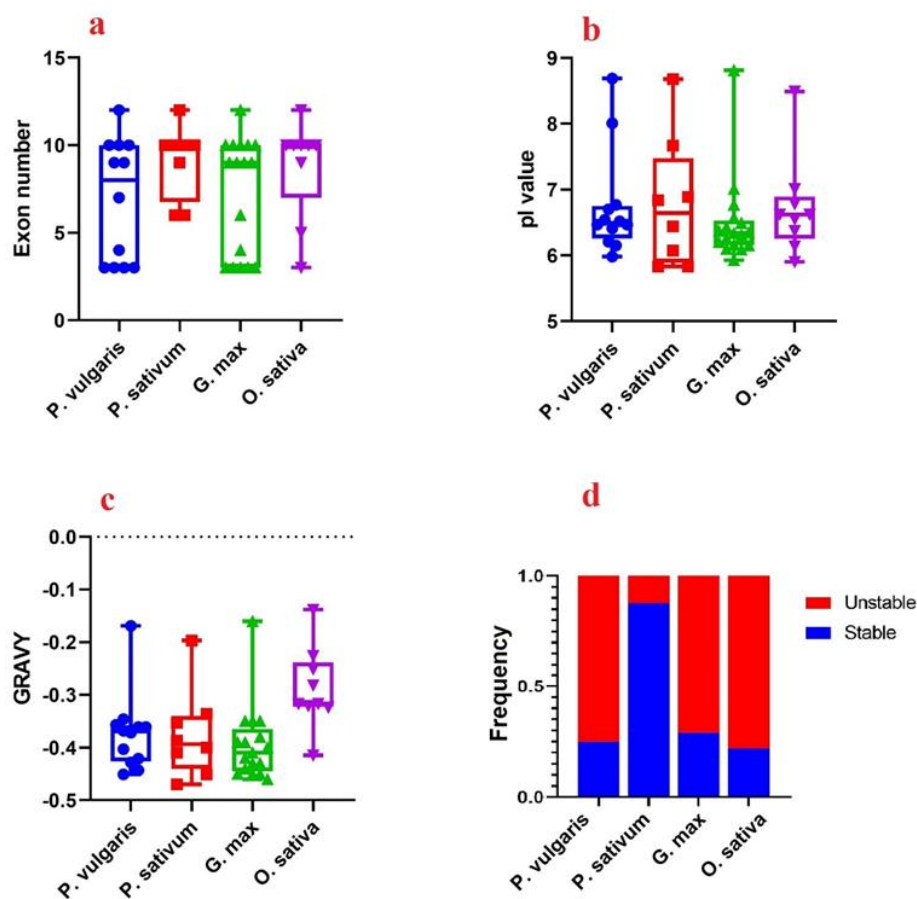


Figure 1. Physicochemical properties, exon number (a), pI (b), GRAVY value (c), and instability index (d) of HSP100 genes in *P. vulgaris*, *P. sativum*, *G. max*, and *O. sativa*.

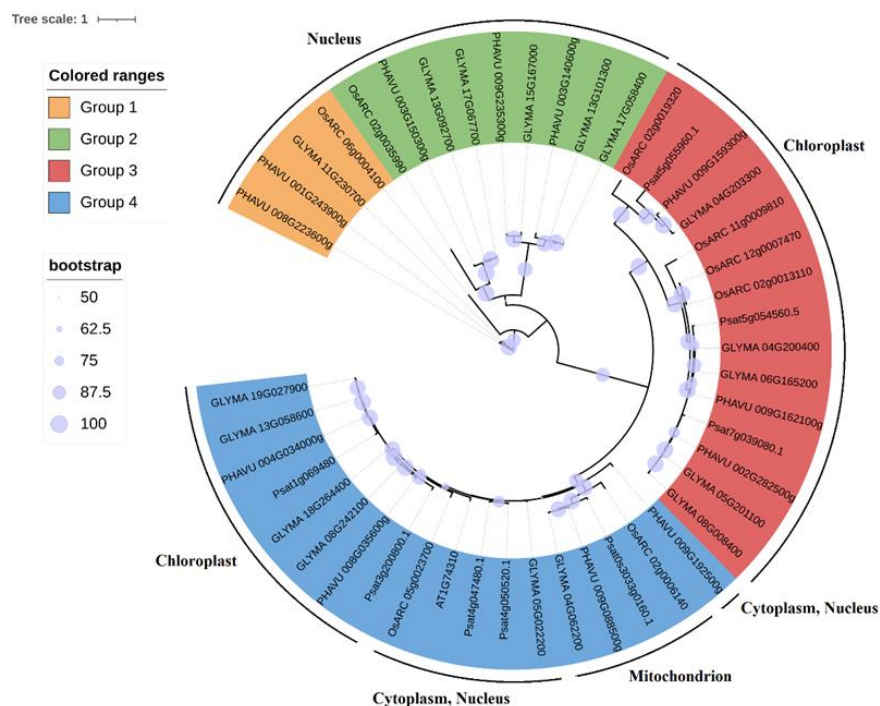


Figure 2. Phylogenetic analysis of HSP100 family members in *P. vulgaris*, *P. sativum*, *G. max*, *A. thaliana*, and *O. sativa*. Subcellular localization of studied proteins is shown around the phylogeny tree.



Figure 3 Prediction of phosphorylation events in HSP100 family members in *P. vulgaris* (a), *P. sativum* (b), and *G. max* (c).

Phylogenetic analysis

Phylogenetic analysis of *HSP100* genes in *P. vulgaris*, *P. sativum*, *G. max*, *A. thaliana*, and *O. sativa* was performed based on the ML method. Based on genetic distance, HSP100s were placed into four phylogenetic groups (Figure 2). Two members of *P. vulgaris* HSP100s were placed in group 1, three members in group 2, three members in group 3, and four members in group 4. Members of group 1 showed a greater genetic distance than other orthologs, and members of groups 3 and 4 had a smaller phylogenetic distance from each other. Based on the results, the subcellular localization of HSP100 proteins in groups 1 and 2 was all predicted to be in the nucleus, and members of phylogenetic group 3 in the chloroplast (Figure 1 and Table S1). Besides, members of phylogenetic group 4 had a variety of locations, with some of them located in mitochondria, some in chloroplasts, and some in the cytoplasm-nucleus. Based on this, it was determined that HSP100s are more active in chloroplasts.

Phosphorylation modification prediction

Phosphorylation is a post-translational modification that plays a significant role in the

activity and lifespan of protein. In this study, regions with high phosphorylation potential were predicted in members of the HSP100 family of *P. vulgaris*, *P. sativum*, and *G. max* (Figure 3). The results showed that members of this gene family differ from each other based on the potential for phosphorylation modifications. Among the members of the HSP100 family in *P. vulgaris*, PHAVU-001G243900G had 37 phosphorylation sites, and PHAVU-009G192500G had 11 phosphorylation sites (Figure 3a). In *P. sativum*, the phosphorylation sites varied from 13 (Psat4g050520.1) to 33 (Psat3g200800.1) (Figure 3b), and in *G. max*, they ranged from 12 (Glyma-05g022200) to 33 (Glyma-04g062200) (Figure 3c). Results reveal that HSP100s are diverse based on potential phosphorylation sites.

Chromosome location and duplication events

Examination of the HSP100 location in the *P. vulgaris* genome showed that members of this family are distributed on 6 chromosomes (Figure 4). Five *HSP100* genes are located on chromosome 9, and 2 *HSP100* genes are located on chromosomes 3 and 8, respectively. Examination of duplication events between members showed that duplications

occurred between the genes *PHAVU-002g282500g* and *PHAVU-009g162100g* and between *PHAVU-004g034000g* and *PHAVU008g035600g*. Both duplication events were of the segmental type.

Expression profile of HSP100 genes

Available RNA-Seq data of *P. vulgaris* in response to salt stress were analyzed. The results showed that HSP100 family members are less induced in the root. *PHAVU-009g088500g*, *PHAVU-008g035600g*, and *PHAVU-001g243900g* showed increased expression in response to salt stress in both studied cultivars (Figure 5a). The *PHAVU-009g192500g* gene showed increased expression specifically in the sensitive genotype, while the gene *PHAVU-009g159300g* showed differential expression between the sensitive and resistant genotypes in leaf tissue. In response to the stress of fungal infection (*Sclerotinia sclerotiorum*), the genes *PHAVU-008g035600g* and *PHAVU-001g243900g* showed increased expression, and the gene

PHAVU-003g140600g showed a significant decrease in expression (Figure 5b). It seems that *PHAVU-008g035600g* and *PHAVU-001g243900g* are induced more in response to stress than other HSP100s.

Interaction network of HSP100 proteins

An interaction network of HSP100 family members based on available data of model plant, *A. thaliana* was created (Figure 6). HSP100 showed strong interaction with components of SCF (ASK-cullin-F-box) E3 ubiquitin ligase complexes, including SKIP25 and MAX2. Also, esterases such as KAI2 and D14 had interaction with HSP100 proteins. Besides, CLP proteases showed strong interaction with HSP100 family members. Moreover, it was disclosed that HSP21 also interacts with HSP100 proteins. According to GO enrichment analysis for molecular function terms, hydrolase activity, ATP-dependent activity and ATPase binding were linked with HSP100 interaction network.

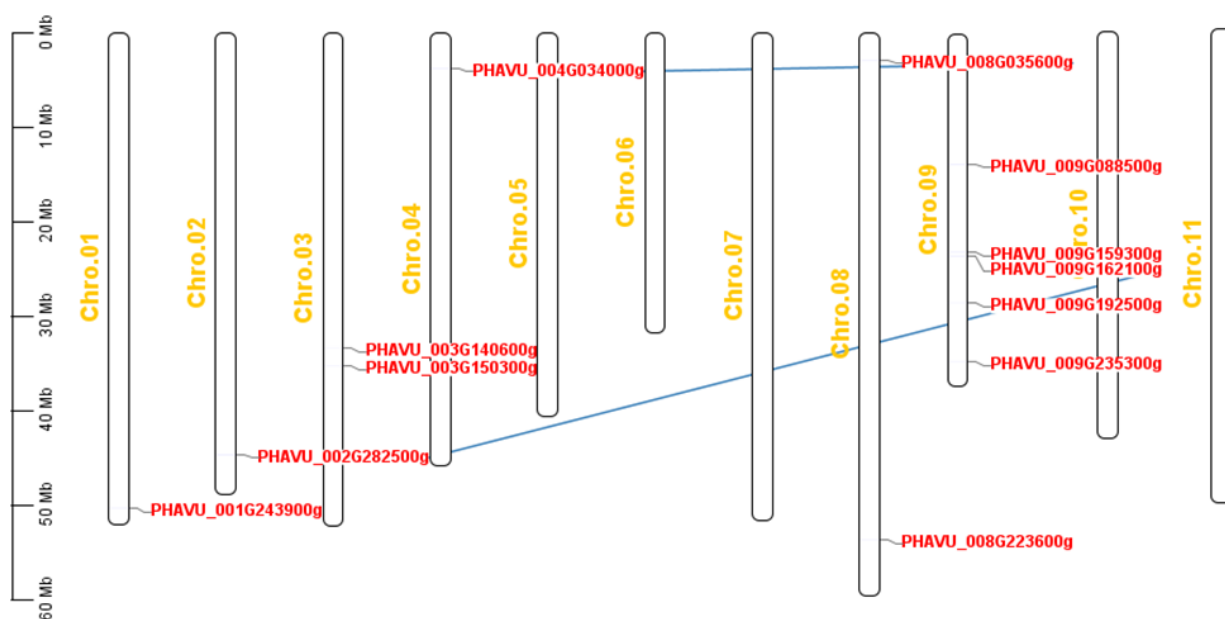


Figure 4 location of HSP100 gene on *P. vulgaris* genome. Duplicated genes connected by blue lines.

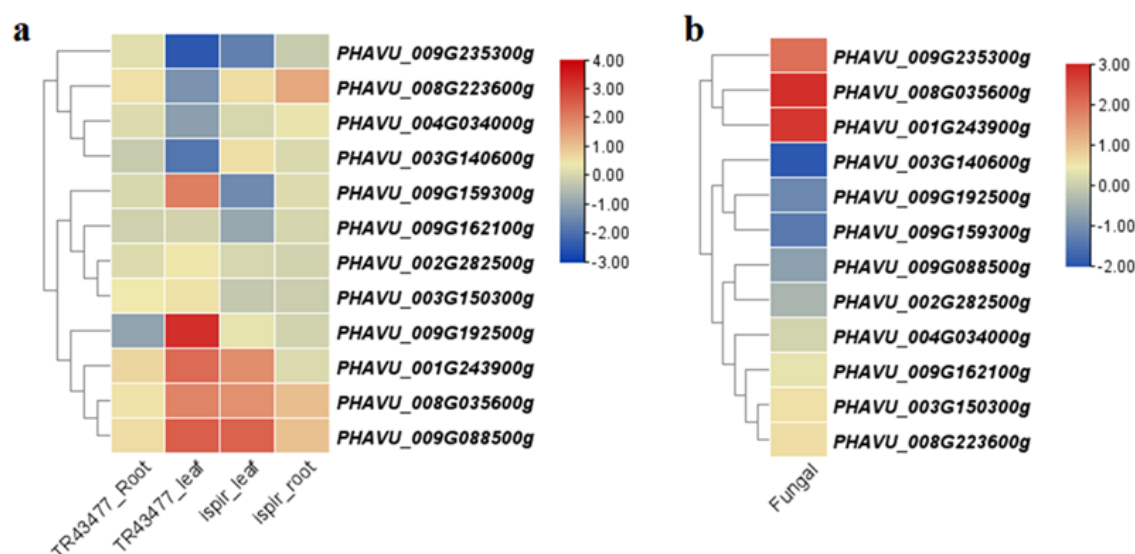


Figure 5. Expression levels of HSP100 family members in *P. vulgaris* in response to salt stress of the salt sensitive cultivar TR43477 and salt tolerant cultivar Ispir (a), and response to fungal (*Sclerotinia sclerotiorum*) infection (b).

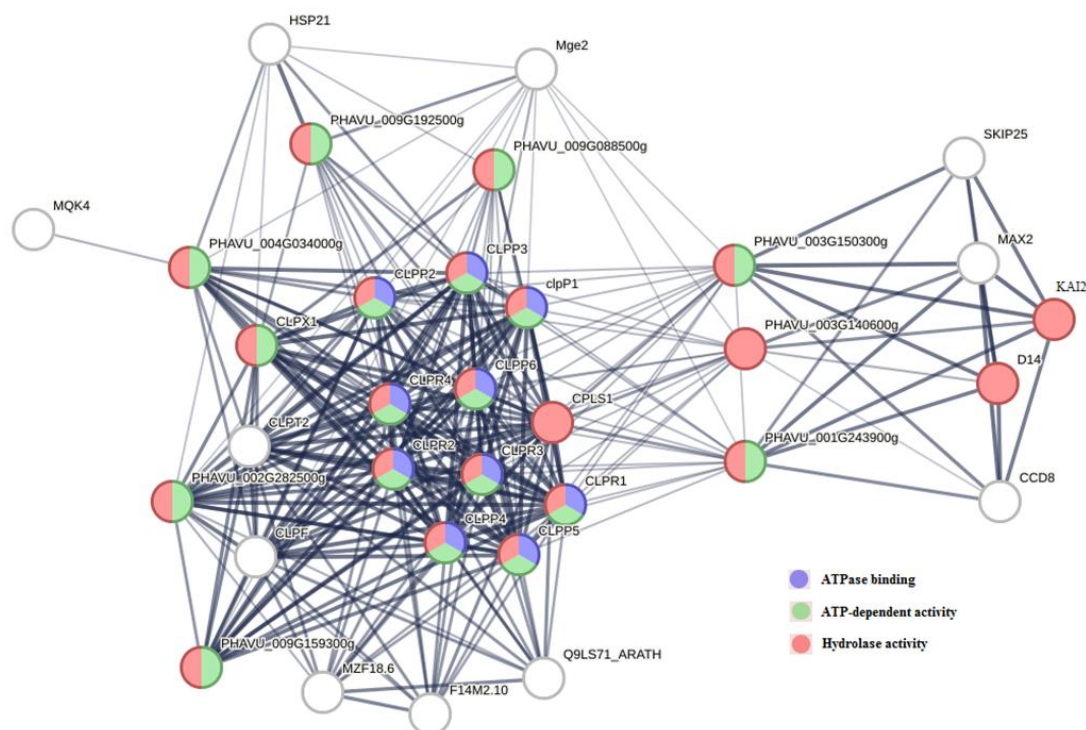


Figure 6. Interaction network of HSP100 family members based on available data in the model plant, *Arabidopsis*.

Discussion

Protein disaggregation is facilitated by chaperonic proteins that are part of the Caseinolytic Protease (Clp) protein family. ClpB/HSP100 proteins exhibit

a remarkable prevalence across various organisms, including yeast, bacteria, and plants. The expression levels of these proteins are modulated by thermal stress and developmental signals (Mishra and Grover, 2016). Both in vivo and in vitro studies

provide evidence that HSP100/ClpB proteins play a role in the ATP-dependent disassembly of protein aggregates present in the cytosol or nucleus that arise during thermal stress (Glover and Lindquist, 1998; Goloubinoff et al., 1999; Hong and Vierling, 2001). Bacteria possess a single form of ClpB, while yeast contains two variants. In contrast, plants harbor multiple isoforms of these proteins, which are distributed among distinct cellular compartments (such as the cytoplasm/nucleus, chloroplasts, or mitochondria) (Mishra and Grover, 2016). Based on the importance of these proteins, members of the *HSP100* gene family have not been well studied in plants. In the current study, 12 members of *HSP100* family were identified in *P. vulgaris* genome. Besides, 8 members in *P. sativum*, 17 members in *G. max* were also recognized for the first time. Previously in barley, 8 members and 6 members in *Aeluropus littoralis* in *HSP100* gene family have been identified (Chaudhary et al., 2019; Arab et al., 2023; Hashemipetroudi et al., 2023). It was stated that polyploidy level and genome size can affect the number of gene family members in plant species (Ahmadizadeh and Heidari, 2025; Heidari et al., 2025). *HSP100* family members showed diversity in terms of exon number and isoelectric point. Most *HSP100* genes were found to have 10 exons in their structure and pI ranged from 5.83 to 8.81. Exon number might affect the speed of expression process in plants (Heidari et al., 2023; Shahidi et al., 2024). It seems that the diverse structure of *HSP100* genes has been reflected by evolutionary pressures.

Phylogenetic analysis has categorized members of the *HSP100* family into four distinct groups. According to the findings, the subcellular localization of *HSP100* proteins in groups 1 and 2 is exclusively predicted to occur within the nucleus.. In contrast, those in phylogenetic group 3 are anticipated to reside in the chloroplast. The results indicate that *HSP100*s derived from *P. sativum* exhibit inactivity within the nucleus. This phenomenon may be attributed to the duplicative events among members of this family in *P. sativum*, where the emergent members were shaped by their ancestral forms. Conversely, it appears that the diversification within this gene family transpired more significantly following the divergence of monocots and dicots, albeit additional research is

warranted. Furthermore, members of phylogenetic group 4 exhibited a range of localizations, with certain members identified in mitochondria, others in chloroplasts, and some distributed between the cytoplasm and nucleus. Hence, it was concluded that *HSP*s demonstrate heightened activity within chloroplasts.

Phosphorylation, a post-translational modification (PTM), significantly affects the stability, function, and localization of target proteins (Cohen, 2002). Our study disclosed a significant diversity in phosphorylation sites among the *HSP100* family members, suggesting the existence of distinct regulatory mechanisms in different species. For example, *P. vulgaris* showed a wide range of phosphorylation sites (ranging from 11 to 37), with PHAVU-001G243900G harboring the highest count (37 sites), highlighting its potential for broad regulation through kinase-mediated signaling. In a similar vein, *P. sativum* and *G. max* demonstrated variations in their sites (13–33 and 12–33, respectively), reinforcing the notion that phosphorylation may precisely modulate *HSP100* functionality in a species-dependent manner.

The RNA-Seq analysis conducted under salt stress conditions disclosed that the majority of *P. vulgaris* *HSP100* genes showed minimal induction in root tissues, whereas PHAVU-009g088500g, PHAVU-008g035600g, and PHAVU-001g243900g showed increased expression in both sensitive and resistant cultivars. Particularly, PHAVU-009g192500g was specifically induced in the sensitive genotype, suggesting its potential involvement in stress responses. The differential expression of PHAVU-009g159300g in leaf tissues between the studied genotypes also suggests that genetic background plays a key role in the regulation of stress-responsive *HSP100*s. In response to fungal infection (*S. sclerotiorum*), PHAVU-008g035600g and PHAVU-001g243900g were upregulated, whereas PHAVU-003g140600g was downregulated, highlighting that some *HSP100* members may have specialized functions in pathogen defense mechanisms.

The observed variation in phosphorylation could affect their ATPase activity and substrate interactions, similar to other heat shock proteins (Kampinga et al., 2009). Furthermore, the diverse expression patterns under salt and fungal stress suggest that *HSP100* genes may be involved in main

stress-response pathways, possibly through interactions with other signaling elements such as MAP kinases or calcium-dependent protein kinases (CDPKs) (Van Bentem et al., 2006). The interaction network of HSP100 family members in *A. thaliana* revealed significant connections with key protein complexes and metabolic regulators, thereby elucidating their functional roles in cellular processes. The prominent interactions observed between HSP100 proteins and components of the SCF (ASK-cullin-F-box) E3 ubiquitin ligase complexes, particularly SKIP25 and MAX2, suggest a potential regulatory function in protein degradation and response pathways related to stress (Stirnberg et al., 2007; Nelson et al., 2011). The SCF complex is known for its role in ubiquitin-mediated proteolysis, which is critical for maintaining protein homeostasis during stress conditions (Hua and Vierstra, 2011). The interaction between HSP100 and these components may represent a collaborative mechanism in the targeted turnover of proteins, potentially enhancing stress resistance.

In addition, the association of HSP100 with esterases such as KAI2 and D14 is particularly noteworthy, given that these proteins are involved in strigolactone signaling and plant growth processes (Waters et al., 2012). This interaction suggests that HSP100 may be involved in modulating hormone signaling pathways, thereby affecting stress adaptation and growth regulation. Furthermore, the strong interaction between HSP100 and CLP proteases highlights their shared role in protein quality control, particularly in chloroplasts, where CLP proteases are essential for maintaining proteostasis (Nishimura et al., 2016). This observation is consistent with the Gene Ontology (GO) enrichment analysis, which highlights the importance of hydrolase activity, ATP-dependent functionality, and ATPase binding in the HSP100 interaction network. Overall, these findings highlight the key role of HSP100 proteins in ATP-induced proteostasis mechanisms, inducing processes such as protein degradation, and unfolding (Agarwal et al., 2002).

Conclusion

Current study characterized the *HSP100* gene family in *Phaseolus vulgaris*, *Pisum sativum*, *Glycine*

max, and *Oryza sativa*, disclosing variations in gene copy number, molecular weight, exon structure, physicochemical characteristics, and subcellular localization. According to phylogenetic analysis, HSP100 proteins are classified into four groups, with their unique subcellular localization suggesting functional diversification. Predictions of phosphorylation sites showed considerable variability in post-translational modification potential in HSP100 members, potentially impacting their regulatory functions in response to stress conditions. Several duplication events observed in *P. vulgaris* indicated segmental duplications affecting the expansion of the HSP100 family. Expression patterns in response to salt stress and fungal infection revealed genotype- and tissue-specific induction of *PHAVU-008g035600g* and *PHAVU-001g243900g*. Interaction analysis demonstrated the involvement of HSP100 in ubiquitin-proteasome pathways, ATP-dependent processes, and chaperone networks. These findings increase our understanding of the structural and functional diversity of HSP100 in legumes and cereals and provide a foundation for further research into their roles in stress adaptation and potential applications in crop improvement strategies.

Supplementary Materials

The supplementary material for this article can be found online at: <https://jpmb-gabit.sanru.ac.ir/article/729343.html>.

Supplementary Table S1. Physiochemical properties of HSP family members in *Phaseolus vulgaris*, *Pisum sativum*, *Glycine max*, and *Oryza sativa*.

Author contributions

Conceptualization, P.H.; methodology, P.H.; software, Z.K., E.R., M.M. and F.M.M.; validation, Z.K.; formal analysis, Z.K., E.R., M.M. and F.M.M.; investigation, P.H.; writing—original draft preparation, P.H. and Z.K.; writing—review and editing, P.H. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest statement

The authors declare no conflict of interest.

References

- Abasi, F., Raja, N.I., Ehsan, M., Ali, H., and Shahbaz, M. (2024). Heat and wheat: adaptation strategies with respect to heat shock proteins and antioxidant potential; an era of climate change. *Int. J. Biol. Macromol.* 256: 128379.
- Agarwal, M., Katiyar-Agarwal, S., and Grover, A. (2002). Plant Hsp100 proteins: structure, function and regulation. *Plant Sci.* 163(3): 397-405.
- Ahmadizadeh, M., and Heidari, P. (2025). "Roles of Polyploidy in the Metabolic and Genetic Improvement of Medicinal Plants," in *Biotechnology, Multiple Omics, and Precision Breeding in Medicinal Plants*. CRC Press), 465-474.
- Al-Whaibi, M.H. (2011). Plant heat-shock proteins: a mini review. *J. King Saud Univ. Sci.* 23(2): 139-150.
- Arab, M., Najafi Zarrini, H., Nematzadeh, G., Heidari, P., Hashemipetroudi, S.H., and Kuhlmann, M. (2023). Comprehensive analysis of calcium sensor families, CBL and CIPK, in *Aeluropus littoralis* and their expression profile in response to salinity. *Genes* 14(3): 753.
- Bolser, D.M., Staines, D.M., Perry, E., and Kersey, P.J. (2016). "Ensembl plants: integrating tools for visualizing, mining, and analyzing plant genomic data," in *Plant Genomics Databases: Methods and Protocols*. Springer), 1-31.
- Chaudhary, R., Baranwal, V.K., Kumar, R., Sircar, D., and Chauhan, H. (2019). Genome-wide identification and expression analysis of Hsp70, Hsp90, and Hsp100 heat shock protein genes in barley under stress conditions and reproductive development. *Funct. Integr. Genom.* 19(6): 1007-1022.
- Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., and Xia, R. (2020). TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* 13(8): 1194-1202.
- Cohen, P. (2002). The origins of protein phosphorylation. *Nat. Cell Biol.* 4(5): E127-E130.
- Divya, K., Bhatnagar-Mathur, P., Sharma, K.K., and Reddy, P.S. (2019). "Heat shock proteins (Hsps) mediated signalling pathways during abiotic stress conditions," in *Plant Signal. Mol.*: Elsevier), 499-516.
- Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29(1): 15-21.
- Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R.D., and Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 31(13): 3784-3788.
- Glover, J.R., and Lindquist, S. (1998). Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins. *Cell* 94(1): 73-82.
- Goloubinoff, P., Mogk, A., Zvi, A.P.B., Tomoyasu, T., and Bukau, B. (1999). Sequential mechanism of solubilization and refolding of stable protein aggregates by a bichaperone network. *Proc. Natl. Acad. Sci. USA* 96(24): 13732-13737.
- Hashemipetroudi, S.H., Mohammadi, S., and Fatemi, F. (2023). Identification and expression analysis of HSP100 gene family in *Aeluropus littoralis*. *J. Plant Mol. Breed.* 11(2): 66-77.
- Heidari, P., Ahmadizadeh, M., and Rezaee, S. (2025). Genome-wide analysis of the ammonium transporters gene family in *Phaseolus vulgaris* and its roles in response to drought and salinity stress. *Sci. Rep.* 15(1): 22069.
- Heidari, P., Puresmaeli, F., Vafaei, Y., Ahmadizadeh, M., Ensani, M., and Ahmadiania, H. (2023). Comparative analysis of phospholipase D (PLD) gene family in *Camelina sativa* and *Brassica napus* and its responses in *Camelina* seedlings under salt stress. *Agronomy* 13(10): 2616.
- Heidari, P., Rezaee, S., Hosseini Pouya, H.S., and Mora-Poblete, F. (2024). Insights into the Heat Shock Protein 70 (Hsp70) family in *Camelina sativa* and its roles in response to salt Stress. *Plants* 13(23): 3410.
- Hong, S.W., and Vierling, E. (2001). Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. *Plant J.* 27(1): 25-35.

- Hosseini Pouya, H.S., Zare, F., and Heidari, P. (2025). Regulation of Heavy Metal Balance in Plants: Roles of Transporters, Chelators, and Signaling Mechanisms. *J. Crop Health* 77(4): 1-14.
- Hua, Z., and Vierstra, R.D. (2011). The cullin-RING ubiquitin-protein ligases. *Annu. Rev. Plant Biol.* 62(1): 299-334.
- Izumi, M. (2019). Heat shock proteins support refolding and shredding of misfolded proteins. *Plant Physiol.* 180(4): 1777-1778.
- Kampinga, H.H., Hageman, J., Vos, M.J., Kubota, H., Tanguay, R.M., Bruford, E.A., Cheetham, M.E., Chen, B., and Hightower, L.E. (2009). Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperon.* 14(1): 105-111.
- Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* 47(W1): W256-W259.
- Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30(7): 923-930.
- Lin, M.-y., Chai, K.-h., Ko, S.-s., Kuang, L.-y., Lur, H.-S., and Charng, Y.-y. (2014). A positive feedback loop between heat shock protein101 and heat stress-associated 32-KD Protein modulates long-term acquired thermotolerance illustrating diverse heat stress responses in rice varieties. *Plant Physiol.* 164(4): 2045-2053.
- Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15(12): 550.
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* 17(1): 10-12.
- McLoughlin, F., Basha, E., Fowler, M.E., Kim, M., Bordowitz, J., Katiyar-Agarwal, S., and Vierling, E. (2016). Class I and II small heat shock proteins together with HSP101 protect protein translation factors during heat stress. *Plant Physiol.* 172(2): 1221-1236.
- Mering, C.v., Huynen, M., Jaeggi, D., Schmidt, S., Bork, P., and Snel, B. (2003). STRING: a database of predicted functional associations between proteins. *Nucleic Acids Res.* 31(1): 258-261.
- Mishra, R.C., and Grover, A. (2016). ClpB/Hsp100 proteins and heat stress tolerance in plants. *Crit. Rev. Biotechnol.* 36(5): 862-874.
- Nelson, D.C., Scaffidi, A., Dun, E.A., Waters, M.T., Flematti, G.R., Dixon, K.W., Beveridge, C.A., Ghisalberti, E.L., and Smith, S.M. (2011). F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci.* 108(21): 8897-8902.
- Nguyen, L.-T., Schmidt, H.A., Von Haeseler, A., and Minh, B.Q. (2015). IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution* 32(1): 268-274.
- Niron, H., Barlas, N., Salih, B., and Türet, M. (2020). Comparative transcriptome, metabolome, and ionome analysis of two contrasting common bean genotypes in saline conditions. *Front. Plant Sci.* 11: 599501.
- Nishimura, K., Kato, Y., and Sakamoto, W. (2016). Chloroplast proteases: updates on proteolysis within and across suborganellar compartments. *Plant Physiol.* 171(4): 2280-2293.
- Nitnavare, R.B., Yeshvekar, R.K., Sharma, K.K., Vadez, V., Reddy, M.K., and Reddy, P.S. (2016). Molecular cloning, characterization and expression analysis of a heat shock protein 10 (Hsp10) from *Pennisetum glaucum* (L.), a C4 cereal plant from the semi-arid tropics. *Mol. Biol. Rep.* 43(8): 861-870.
- Pan, X., Zheng, Y., Lei, K., Tao, W., and Zhou, N. (2024). Systematic analysis of Heat Shock Protein 70 (HSP70) gene family in radish and potential roles in stress tolerance. *BMC Plant Biol.* 24(1): 2.
- Parsell, D., and Lindquist, S. (1993). The function of heat-shock proteins in stress tolerance: degradation and reactivation of damaged proteins. *Annu. Rev. Genet.* 27: 437-497.
- Pickart, C.M., and Cohen, R.E. (2004). Proteasomes and their kin: proteases in the machine age. *Nat. Rev. Mol. Cell Biol.* 5(3): 177-187.

- Shahidi, P., Bahramnejad, B., Vafaei, Y., Dastan, D., and Heidari, P. (2024). Isolation and characterization of phenylalanine ammonia lyase (PAL) genes in *Ferula pseudalliacea*: insights into the phenylpropanoid pathway. *Genes* 15(6): 771.
- Sievers, F., and Higgins, D.G. (2018). Clustal Omega for making accurate alignments of many protein sequences. *Prot. Sci.* 27(1): 135-145.
- Singla, S.L., Pareek, A., and Grover, A. (1997). Yeast HSP104 homologue rice HSP110 is developmentally- and stress-regulated. *Plant Sci.* 125(2): 211-219.
- Stirnberg, P., Furner, I.J., and Ottoline Leyser, H. (2007). MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. *Plant J.* 50(1): 80-94.
- Sun, W., Van Montagu, M., and Verbruggen, N. (2002). Small heat shock proteins and stress tolerance in plants. *Biochimica et biophysica acta.* 1577(1): 1-9.
- Sung, D.Y., Kaplan, F., and Guy, C.L. (2001). Plant Hsp70 molecular chaperones: protein structure, gene family, expression and function. *Physiol. Plant.* 113(4): 443-451.
- Timperio, A.M., Egidi, M.G., and Zolla, L. (2008). Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *J. Proteom.* 71(4): 391-411.
- Van Bentem, S.D.L.F., Anrather, D., Roitinger, E., Djamei, A., Hufnagl, T., Barta, A., Csaszar, E., Dohnal, I., Lecourieux, D., and Hirt, H. (2006). Phosphoproteomics reveals extensive in vivo phosphorylation of Arabidopsis proteins involved in RNA metabolism. *Nucleic Acids Res.* 34(11): 3267-3278.
- 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(5): 244-252.
- Waters, M.T., Nelson, D.C., Scaffidi, A., Flematti, G.R., Sun, Y.K., Dixon, K.W., and Smith, S.M. (2012). Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in Arabidopsis. *Development* 139(7): 1285-1295.
- Zhao, P., Wang, D., Wang, R., Kong, N., Zhang, C., Yang, C., Wu, W., Ma, H., and Chen, Q. (2018). Genome-wide analysis of the potato Hsp20 gene family: identification, genomic organization and expression profiles in response to heat stress. *BMC Genomics* 19(1): 61.
- Zheng, Y., Cai, Z., Wang, Z., Maruza, T.M., and Zhang, G. (2025). The genetics and breeding of heat stress tolerance in wheat: advances and prospects. *Plants* 14(2): 148.

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ژن‌های HSP100: ساختار، تکامل و تحلیل بیان در گیاه لوبیا (*Phaseolus vulgaris*)

پرویز حیدری^{۱*}، زهرا خلیلی^۲، الناز رضانی^۲، مریم مقصودی^۲، فاطمه مهدوی
میقان^۲

^۱ دانشگاه صنعتی شاهرود، شاهرود، ایران

^۲ دانشکده کشاورزی، دانشگاه صنعتی شاهرود، شاهرود، ایران

ویراستار علمی

دکتر مصطفی حق‌پناه

مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی
کهگیلویه و بویراحمد، موسسه تحقیقات کشاورزی دیم
کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی،
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نویسنده مسئول

دکتر پرویز حیدری،

heidarip@shahroodut.ac.ir

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چکیده: پروتئین‌های شوک حرارتی (HSPs) در پاسخ‌های گیاه به تنش نقش اساسی دارند و HSP100 خانواده مهمی را تشکیل می‌دهد که در تجزیه پروتئین و تحمل حرارتی نقش دارد. در این تحقیق خانواده ژنی HSP100 در *Oryza sativa*، *Glycine max*، *Pisum sativum*، *Phaseolus vulgaris* مورد بررسی قرار گرفت و تغییرات در تعداد ژن (از ۸ تا ۱۷ عضو)، وزن مولکولی (بین ۱۰۱ تا ۱۱۹ کیلو دالتون)، تعداد اگزون (از ۳ تا ۱۲)، نقطه ایزوالکتریک (از ۵.۸۳ تا ۸.۸۱) و آبدوستی را آشکار ساخت. تجزیه و تحلیل فیلوژنتیکی، پروتئین‌های HSP100 را به چهار گروه مجزا طبقه‌بندی کرد که هر کدام جایگاه‌های زیرسلولی خاصی (هسته، کلروپلاست، میتوکندری و سیتوپلاسم-هسته) را نشان دادند. پیش‌بینی‌ها در مورد جایگاه‌های فسفریلاسیون، تنوع بین گونه‌ای را نشان داد، به طوری که اعضای *P. vulgaris* تا ۳۷ جایگاه فسفریلاسیون را نشان دادند. مطالعات توزیع کروموزومی در *P. vulgaris*، رویدادهای تکثیر قطعه‌ای را در اعضای خانواده HSP100 نشان داد. تجزیه و تحلیل بیان ژن‌های HSP100 تحت تنش شوری و عفونت قارچی (*Sclerotinia sclerotiorum*)، ژن‌های پاسخگو به تنش، به ویژه PHAVU-008g035600g و PHAVU-001g243900g را شناسایی کرد. شبکه‌های برهمکنش پروتئین‌ها، ارتباط با اجزای لیگاز یوئیکوئیتین SCF، استرازاها، پروتئین‌های CLP و HSP21 را نشان داد که همگی در فعالیت‌های وابسته به ATP غنی شده‌اند. این نتایج به درک عمیق‌تر از تنوع تکاملی و عملکردی پروتئین‌های HSP100 در قلمرو گیاهان کمک می‌کند.

کلمات کلیدی: پروتئین‌های شوک حرارتی؛ خانواده ژن‌های گیاهی؛ بیان ژن؛ ژنوم لوبیا.