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RESEARCH ARTICLE

In silico analysis of *Phaseolus vulgaris* L. metalloprotease *FtsH* gene: characterization and expression in drought and salt stress

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Abstract Proteases help plants maintain protein quality and break down protein subsets in reply to environmental, developmental, biotic, and abiotic stressors. Filamentation temperature-sensitive H (FtsH) is an ATP-dependent metalloprotease detected in both prokaryotes and eukaryotes cells. The present research, the *FtsH* gene in the bean, which has an important place in the legume family and is an important agricultural product, was characterized for the first time using various bioinformatic tools, and qRT-PCR measured its expression level. In the study, effectively identified and characterized 17 *FtsH* genes present in genome the of *Phaseolus vulgaris*. The MW of FtsH proteins varied from 71.16 to 147.07 kDa, their amino acid lengths ranged from 642 to 1284, and their pI values varied from 5.39 to 9.60. Interestingly, the distribution of these 17 distinct *PvFtsH* genes across the 8 chromosomes was not uniform, exhibiting an uneven pattern throughout the genome. A pair of segmental duplication fragments were found, revealing probable processes of gene expansion and evolution. Collinearity with related genes in *Arabidopsis* and rice was thoroughly examined to determine the evolutionary conservation and differentiation of *PvFtsH* genes. Additionally, we used RNAseq and qRT-PCR to investigate the expression patterns of PvFtsH in leaf tissue under salt and drought conditions. Our data showed unique expression patterns, suggesting PvFtsH may respond to environmental and physiological stressors. Overall, this work makes major contributions to our understanding of *PvFtsH* genes and their roles in the context of gene evolution, chromosomal distribution, and expression patterns under various environmental situations.

Keywords Drought and salt stress · Filamentation temperature-sensitive H · Gene expression · Melatonin · Proteases

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Introduction

Plants encounter a diverse array of abiotic and biotic stressors throughout each stage of their development (Gull et al. 2019). Drought, salinity, and heat are the most widely studied abiotic stressors in the literature (Turhan et al. 2021; Zandalinas et al. 2022). Due to growing global warming, high heat stress has already resulted in billions of dollars' worth of crop losses throughout the world (Perry et al. 2020). The detrimental impacts on agricultural growth, development, and productivity are evident in both drought and salt stress conditions, posing significant challenges to crop yield and overall agricultural sustainability (Yu et al. 2022; Wu et al. 2022). Furthermore, it induces substantial damage to the physiological and biochemical processes of the plant, often leading to the denaturation of proteins and the generation of reactive oxygen species (ROS) (Aleem et al. 2022; Zhao et al. 2022). Hence, to endure and mitigate detrimental consequences, plants make use of a vast variety of physiological and chemical defense mechanisms (Praveen et al. 2023). Biotic and abiotic stress factors often cause protein dysfunction, and abnormal proteins disrupt cell viability (Ali and Baek 2020). Unwanted or damaged proteins are removed by a tightly regulated proteolytic process (Dougan et al. 2012).

Proteolytic enzymes are commonly referred to as proteases, proteinases, or peptidases (Rawlings et al. 2020). Although proteases play essential roles in various critical plant cell processes, including senescence, cell death, and responses to environmental changes, there exists limited knowledge regarding the substrate specificity and physiological functions of numerous plant proteases. Even for ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), the most prevailing plant protein, it is unknown which proteases are involved in the process of cellular localization or destruction (García-Lorenzo et al. 2006). Plants depend on proteases to maintain rigorous protein quality control and to break down certain protein subsets in response to various environmental and developmental cues. The similarities and variances among the proteases produced in various animals may provide important clues about their physiological functions and evolutionary history (Kato and Sakamoto 2019).

Filamentation temperature-sensitive H (FtsH) protease is known as a membrane-bound ATP-dependent Zn^{2+} metalloproteinase, belongs to the protein family AAA, and was initial found in *E. coli* (Guo et al. 2021; Pu et al. 2022). FtsH proteins are present in both prokaryotic and eukaryotic organisms. Especially, endosymbiotic organelles, including chloroplasts and mitochondria, contain them (Wan and Ling 2022). FtsH metalloproteases are widely known for playing a crucial part in the upkeep and proteolysis of membrane proteins in plants, animals, and eubacteria (Wagner et al. 2012). *FtsH* genes have been detected in an extensive variability of plant species, including the potato (Hajibarat and Saidi 2023), pear (Guo et al. 2021), soybean (Wang et al. 2023), rice (Wu et al. 2021), pepper (Xiao et al. 2021), tobacco (Pu et al. 2022), maize (Yue et al. 2010), *Arabidopsis* (Mielke et al. 2021).

Specifically, completing the genome sequencing of plants such as *Arabidopsis* and rice with next-generation sequencing technologies makes it possible to identify all *FtsH* genes in both genomes of these plants. There are 17 and 19 FtsH proteases that are membrane-bound in the model organism *Arabidopsis* and rice respectively (Zhang and Sun 2009; Mishra and Funk 2021).

One of the most popular legumes consumed worldwide is the *Phaseolus vulgaris* L., which offers the human diet with proteins, vitamins, and minerals (Ilhan et al. 2018). It is a necessary food in many nations due to its nutritional value as well as its affordability and adaptability for long-term storage. Because this legume is grown in peripheral parts of Brazil, where unfavorable environmental conditions like drought and salt regularly occur, productivity there is thought to be low (Inal et al. 2017; Ito et al. 2017). To improve bean breeding for abiotic stress tolerance, it is necessary to develop novel, effective ways. Abiotic stress causes changes in gene expression, which are crucial for plants to adapt and persist in extreme environments (Baloglu et al. 2014). Research on the growth, development, and stress tolerance of legumes is therefore practically important for agricultural output. Despite the entire sequencing of the legume genomes, a comprehensive assessment of the metalloprotease *FtsH* gene family members in beans have not been done.

Applying plant hormones and/or growth regulators to plants have been demonstrated an effective method for decreasing the harmful effects of environmental stress throughout their growth period (Amiri et al.

2024). Melatonin, also known as N-acetyl-5-methoxytryptamine, is a compound derivative from the essential amino acid tryptophan and is found in the leaves, stems, shoots, fruits, seeds, and roots of many different plant species, serving a variety of functions in vascular plants (Malabadi et al. 2021; Kasapoglu et al. 2023; Yusuf et al. 2024). Melatonin not only exhibits antioxidant properties but also safeguards against abiotic stress impacts through the regulation of plant hormones, activation of endoplasmic reticulum stress- responsive genes, and enhancement of protein homeostasis, heat shock proteins, and transcription factors (Colombage et al. 2023; Tiwari et al. 2023). Furthermore, melatonin's role under stressful circumstances is to eliminate reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Wang et al. 2024). According to reports, melatonin is more efficient than α-tocopherol and glutathione in removing ROS and RNS (Sun et al. 2021a, b). Consequently, it essentially minimizes the oxidative damage to proteins, nucleic acids sustain and lipids (Tiwari et al. 2024). Due to its amphiphilic nature, melatonin has a significant ability to penetrate all cellular compartments (Choi et al. 2024).

Bioinformatics methods were employed in the current study to find new proteins and genes. Although omics methods like transcriptomics, metabolomics, and genomics are widely used, there has been no previous study that comprehensively identifies and analyzes the expression of the metalloprotease *FtsH* gene family in common beans at a genome-wide level. Owing to the significance of the *FtsH* gene family in stress and plant growth and development, the results of this investigation will deliver essential data for *Phaseolus vulgaris* basic breeding research's that seeks to identify genes responding to drought and salt stress.

Material and methods

Identification of FtsH protein in common bean genome

Protein sequences for the *FtsH* gene family in the *Phaseolus vulgaris* genome were acquired from the Phytozome database (v12.1) using the Pfam entry IDs structural domains AAA (PF00004), FtsH extracellular structural domain (PF06480) and peptidase M41 (PF01434). Both blastp from the Phytozome database (v12.1) and the hidden Markov model (HMM) database were used to identify all probable FtsH proteins in the bean genome (Schmutz et al. 2014). Unrelated sequences were extracted using the "decrease redundancy tool". Using the HMMER database, the presence of the FtsH domain in the associated sequences was investigated. The "ProtParam" tool was utilized to ascertain the molecular weight (mw) and putative isoelectric point (pI) of the FtsH proteins that were acquired.

Identification of structure, physical locations, gene duplications and motifs of *PvFtsH* genes

The Gene Structure Display Server (GSDS) v2.0 was utilized to get data on the exon–intron sections of PvFtsH proteins (Guo et al. 2007). The location information of the *PvFtsH* genes was determined using the coding DNA (CDS) and genome sequences. The chromosomal locations and sizes of the *PvFtsH* genes were determined using the Phytozome database (v12.1).

The *PvFtsH* genes were first assigned to specific locations on each of the *P. vulgaris* chromosomes and observed using the TBtools program (Chen et al. 2023). In addition, gene duplication was identified using the TBtools software. It was calculated using the TBtools basic Ka/Ks calculator algorithm to calculate the exchange rates for non-homologous (Ka), homologous (Ks) and non-homologous to homologous (Ka/Ks) among duplicate pairs of *PvFtsH* genes (Chen et al. 2023). T = Ks/2 (= 6.56E-9) formula was used to calculate each *PvFtsH* gene's divergence and duplication period (measured in Million Years Ago, MYA) (Yang and Nielsen 2000; Muslu et al. 2023).

Conserved motifs of PvFtsH proteins were examined using the "Multiple EM for Motif Elimination (MEME)" methods (Bailey et al. 2006). The maximum number of motifs and minimum/maximum width are limited to 10, 50, and 2, respectively. The motif region is between 2 and 300. ny number of repetitions can be chosen for the region distribution. The InterPro database's default parameters were used to scan the identified motifs (Jones et al. 2014).

Phylogenetic analysis and sequence alignment

Neighbor-joining (NJ) approach was utilized for phylogenetic analyses, and 1000 repeated bootstrap values were used. Using ClustalW, PvFtsH protein sequences were aligned (Sinha and Meller 2007). The phylogenetic tree was drawn by Molecular Evolutionary Genetic Analysis (MEGA) v11 program (Tamura et al. 2011). The Interactive Tree of Life (iTOL) interface was used to shape the tree (Letunic and Bork 2011). In addition, the FtsH proteins were subjected to sequence logo analysis utilizing the WEBLOGO online web program, as part of the multiple sequence alignment study (Crooks et al. 2004).

Comparative mapping between common beans and other species

Comparative genomics aims to identify synteny, which refers to gene blocks or other evolutionarily conserved markers and quantify the evolutionary relationship between genomes based on chromosomal rearrangements (Sinha and Meller 2007). With the use of the TBtools, the synteny map of the *FtsH* genes in *Oryza sativa*, *Arabidopsis thaliana*, and *Phaseolus vulgaris* was created.

Promotor analysis and intracellular localization of the *PvFtsH* gene family

The cis-acting elements present in the 5' upstream regions of the bean *FtsH gene* family were obtained using the PlantCARE database. These areas consist of about 2 kb of DNA fragments from each gene (Oner et al. 2022). Their intracellular localization was estimated using WoLFPSORT (Horton et al. 2007).

Three-dimensional homology modeling of FtsH proteins in beans

Using the found FtsH protein sequences, three-dimensional (3D) structures were produced using the Phyre2 library for protein homology modeling (Kelley et al. 2015). The finest 3D image was chosen after examining the protein models' dependability rates. During the final validation phase, the models with the highest level of confidence have been used and visualizations were created using the modeling technique that provided the most achievable match.

Construction of protein–protein interactions network and gene ontology analysis

Protein–protein interactions (PPI) are concerned with various biological processes, such as cellular regulation, metabolic growth, and intercellular communication (Braun and Gingras 2012). To ascertain the physical and functional interactions of proteins, the STRING database was utilized. Obtained data were divided and integrated with the score for entire PPIs on the STRING website. Functional genomics is a crucial component in plant biotechnology research methodologies as it enables the functional characterization of novel sequence data. The Gene Ontology (GO) database was utilized to collect protein ontology data (Gene Ontology Consortium et al. 2023). The GO analysis was conducted utilizing the TBtools software, and the results were presented using an identical application.

Analyzing in silico gene expression in various tissues

By utilizing the Phytozome database (v12.1), we evaluated the expression levels of the *PvFtsH* genes in different plant tissues throughout different developmental stages. The levels of in-silico expression were measured using a metric called FPKM, which stands for the anticipated number of transcript fragments per kilobase per million base pairs sequenced. The CIMMiner algorithm was utilized to create a heat map using the log2-transformed FPKM values.

The expression profiles of the bean *FtsH* genes were analyzed using Illumina RNA-seq data acquired from the Sequence Read Archive (SRA) data library. The accession numbers for salt [SRR957668 (salt stress treated leaf), SRR958469 (leaf salt control)] and drought stress [SRR8284481 (drought stress treated leaf), and SRR8284480 (leaf drought control)] were ascertained to locate pertinent RNA-seq data (Hiz et al. 2014; Kasapoglu et al. 2023) were used. The reads per kilobase (RPKM) algorithm of the exon model per million mapped reads was employed to regularize gene expression levels (Mortazavi et al. 2008). After

converting RPKM data to log2, with the CIMMiner algorithm produced a heatmap.

Prediction miRNAs that target the *PvFtsH* genes

MiRBase v21.0 (www.mirbase.org) was utilized to obtain entirely known miRNA plant sequences. The psRNATarget Server was used, which may be found at www.plantgrn.noble.org/psRNATarget, and the defaulting miRNA estimation parameters were applied (Zhang 2005). BLASTX was employed with a threshold of 1e-10 to assess in silico indicated miRNA targets with *Phaseolus vulgaris* expressed sequence tags (EST) in the NCBI.

Plant material, stress induction, and melatonin treatments.

In this study, the plant material consisted of Serra and Elkoca-05 *Phaseolus vulgaris* cultivars. All experiments and applications were achieved by using the laboratories facilities in Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetic. To impose drought stress, Polyethylene glycol 6000 (PEG 6000) was applied at concentrations of 0% (control) and 20%. For inducing salt stress, Sodium chloride (NaCl) was used at concentrations of 0 mM (control) and 150 mM. The cultivation of plants, as well as the application of stress agents and melatonin, strictly adhered to the protocol outlined in reference (Aygören et al. 2023). Melatonin application was performed by foliar spraying of the leaves, using concentrations of 0 mM (control) and 200 mM. This application was carried out 24 h before subjecting the plants to the stressors. The hydroponic system, including 1/10 Hoagland solution, served as the medium for melatonin application and stress induction. The plants underwent 24 h of drought stress, and 9 days in salt stress to evaluate the impact of ions under salt stress conditions. Upon completing the stress periods, leaf tissues of the bean genotypes were promptly harvested and freeze up in liquid nitrogen. These tissue samples were thereafter held at − 80 °C to maintain their molecular integrity. The study used a completely randomized design with three replicated. For every treatment, five plants were carefully selected from each replicate and combined using the bulking technique. This approach allowed for efficient molecular analysis in the study.

RNA extraction and cDNA synthesis

The RNA extraction method was performed with TRIzol® reagent (Invitrogen Life Technologies, USA) based on the manufacturer's instructions. RNA concentrations were quantified in Nanodrop (Multiskan GO instrument (Thermo Fisher Scientific, Massachusetts, USA), and $A_{260/280}$ ratio data were acquired. The 1.2% agarose gel was prepared to visualize the RNA. 3 µl of 6X loading dye, and 3 µl of RNA sample were loaded into the wells after mixing and run at 80–100 V for 40 min. The gel was observed in the UV Transilluminator. The process described in the high-quality cDNA synthesis kit (Roche, USA) was utilized to produce complementary DNA. The primers were constructed using Primer3 software, utilizing the sequences of 4 *PvFtsH* genes.

qRT-PCR analysis.

The RT-qPCR experiments employed the iTaq Universal SYBR Green Supermix (Bio-Rad, USA), and the reaction requirements outlined by Inal et al. (2017) were followed. Utilizing the LightCycler® Nano Device, RT-qPCR reactions have been carried out (Roche). A total of three technical and biological replicates were utilized. The *β-Actin* gene was utilized to normalize the qRT-PCR data by the 2^{CT} method. (Livak and Schmittgen 2001). The data were analyzed using GraphPad Prism 9 with a two-way ANOVA approach. Fisher's least significant difference (LSD) test was used to test the differences between means at the 0.05 significance level.

Results and discussion

Identification, chromosomal location, duplication, and synteny analysis of *PvFtsH* genes

Lately, with the advance of genome-wide research and the spread of bioinformatics databases; the development of stress-resistant plant lines, the development of plant breeding methodologies, and a better understanding of the genes and proteins of defense against stress have been provided. Plants protect themselves under stress by altering gene-genome expression for a variety of physiological activities.

RNA-seq analysis has been successfully used in the analysis of gene expression and identification of gene homologues in plants, under different treatment conditions, in different organs (Muñoz-Bodnar et al. 2014). Plant biology has a limited understanding of proteolysis. Proteases are essential players in the proteolysis process during a variety of critical processes, involving those involving senescence, cell death, and environmental changes. In current study, as protease, *FtsH* gene family was bioinformatically characterized and carried out the relative expression of chosen genes in conditions drought and salt stress and it was found to be their relation to the plant defense mechanism. As a result of in silico analysis, 17 candidate *PvFtsH* genes were detected by using the bean (*Phaseolus vulgaris* L.) genome. They were sorted as PvFtsH1 – PvFtsH17 (Table 1). The chromosomal location, amino acid (aa) length, molecular weight (mw), pI: isoelectric point; instability index, classify and aliphatic index information of *PvFtsH* genes were also shown (Table 1). The proteins ranged in aa length from 642 (PvFtsH5) to 1284 (PvFtsH17). The proteins' pI ranged from 5.39 (PvFtsH12) to 9.60 (PvFtsH5). Also, their mw varied from 71.16 kDa (PvFtsH5) to 147.07 kDa (PvFtsH17) (Table 1). According to the WoLFPSORT database, *PvFtsH*-like genes were mostly located in the chloroplast but also in other subcellular components (Table 1). Currently, genome-wide analyses of the *FtsH* gene family are being conducted using a variety of organisms with different number of *FtsH* genes, including plants like *Arabidopsis* (12), pear (19), rice (9), corn (2) and tobacco (20) (Adam et al. 2001; Sakamoto et al. 2003; Zhang and Sun 2009; Yue et al. 2010; Guo et al. 2021; Pu et al. 2022), as well as microorganisms like *Acidovorax citrulli, Bacillus subtilis,* cyanobacteria, *Escherichia coli*, and *Lactobacillus plantarum* (Ji et al. 2019).

P. vulgaris was found to have *PvFtsH* genes on 8 of its 11 chromosomes, with none detected on chromosomes 3, 7, and 10. (Fig. 1). Moreover, only one gene (*PvFtsH17*) could be mapped to any of the chromosomes because it was found on the unassembled scaffolds (Table 1). It was found that a *PvFtsH* gene is present on chromosomes 4, 5, and 6, while two genes are found on chromosomes 1, 2, and 8. Additionally, three genes are located on chromosome 11, and four genes are found on chromosome 9 (Fig. 1). Consistent with the above results, and only 13 out of the 24 chromosomes have *NtFtsH* genes in *Nicotiana tabacum* L. (Pu et al. 2022). The density of genes on chromosomes and the distribution of the *FtsH* gene did not correlate. This shows that gene loss may have affected the *FtsH* gene family during evolution. As a result, certain *FtsH* genes may have developed through duplication processes, indicating that these processes were crucial to the rapid expansion of the FtsH family in plants.

Duplications are important genomic occurrences that aid in the enlargement of gene families. In protein evolution, gene duplication actions have facilitated the acquisition of novel functional traits by proteins (Kondrashov et al. 2002). Tandem duplication refers to the presence of duplications on the same chromosome, whereas segmental duplication refers to the occurrence of the same genes on distinct chromosomes (Fu et al. 2020; Qu et al. 2023a, b). These types of duplication have a significant role in fostering species variety hence, it may be easier for plants to adjust to changing environmental circumstances (Qin et al. 2023).

In this research, it was identified one segmentally duplicated gene (*PvFtsH3* and *PvFtsH13*) pair among 17 *PvFtsH* genes (Table S1). Furthermore, to assess selection pressure and the timing of *PvFtsH* gene duplications, the measures of non-synonymous divergence (Ka), synonymous divergence (Ks), and Ka/Ks were computed. The duplicated PvFtsHs exhibited Ka/Ks ratios ranging was found as 0,0951 (Table S1). Values larger than 1 in the Ka/Ks ratio often indicate positive selection, values smaller than 1 imply purifying selection, and a value of 1 indicates neutral selection (Nekrutenko et al. 2002). According to this hypothesis, it was estimated that the duplicated *PvFtsH* genes were subjected to purifying selection pressure. The divergence of the *PvFtsH3* and *PvFtsH13* genes occurred approximately 42.620 million years ago. Gene duplication, especially segmental and tandem duplication results, plays an imperative function in the evolutionary process and the enlargement of gene families (Liu et al. 2023). Similarly, it was observed that the *NtftsH* gene had four pairs of segmental duplications. Contrary to a prior study conducted on pear trees (Guo et al. 2021), no instances of tandem duplication were identified in the tobacco genome for the *NtFtsH* gene. These findings indicate that segmental duplication might have a greater impact on the expansion of the NtFtsH family

Table 1 Genomic and biochemistry information of *Phaseolus* L. FtsH family members

Gene ID	Phytozome ID	Chromosomal location				aa	MW (kDa)	pI	Instability index	Classify	Aliphatic index	Subcellular localization (wolfPsort)
		Chr	Start	End	Strand							
PvFtsH1	Phvul.001G036400	Chr01	3,397,546	3,409,703	reverse	717	77.30	8.22	43.27	Unstable	87.02	chlo: 8, mito: 3, plas: 2, cyto: 1
PvFtsH2	Phvul.001G062000	Chr01	7,395,287	7,412,135	forward	796	86.98	5.75	46.29	Unstable	90.10	chlo: 9, vacu: 2, nucl: 1, extr: 1, golg: 1
PvFtsH3	Phvul.002G190900	Chr02	35,435,502	35,439,098	forward	695	74.61	5.73	32.28	Stable	91.11	chlo: 14
PvFtsH4	Phvul.002G299700	Chr02	46,791,870	46,800,422	forward	919	103.36	8.68	41.55	Unstable	95.98	plas: 8, chlo: 2, vacu: 2, E.R.: 1, pero: 1
PvFtsH5	Phvul.004G047500	Chr04	5,750,689	5,756,242	forward	642	71.16	9.60	43.60	Unstable	86.98	plas: 11.5, golg_plas: 7, golg: 1.5, vacu: 1
PvFtsH6	Phvul.005G167100	Chr05	39,436,137	39,442,147	forward	889	101.034	9.28	48.18	Unstable	84.60	mito: 8, chlo_mito: 6.83333, cyto_mito: 4.83333, chlo: 4.5, nucl: 1
PvFtsH7	Phvul.006G065400	Chr06	17,599,809	17,610,566	reverse	790	86.07	7.70	42.22	Unstable	92.57	plas: 7.5, E.R.: 6, cyto_plas: 4.5
PvFtsH8	Phvul.008G040800	Chr08	3,369,192	3,372,204	forward	664	72.23	6.05	37.40	Stable	97.53	chlo: 10, nucl: 2, extr: 2
PvFtsH9	Phvul.008G262300	Chr08	60,904,611	60,911,635	forward	796	86.38	8.68	32.61	Stable	92.11	plas: 6.5, cyto_plas: 4, chlo: 3, E.R.: 2, mito: 1, pero: 1
PvFtsH10	Phvul.009G021400	Chr09	4,653,507	4,657,610	forward	709	75.85	6.28	36.54	Stable	89.61	chlo: 12, cyto: 1, mito: 1
PvFtsH11	Phvul.009G054200	Chr09	10,385,502	10,395,742	forward	775	84.91	6.82	46.78	Unstable	92.94	chlo: 8, vacu: 2, nucl: 1, mito: 1, extr: 1, golg: 1
PvFtsH12	Phvul.009G149600	Chr09	22,293,839	22,304,767	reverse	844	95.12	5.39	45.03	Unstable	86.69	cyto: 7, chlo: 4, E.R.: 2, plas: 1
PvFtsH13	Phvul.009G241100	Chr09	36,212,802	36,216,665	reverse	693	74.20	5.54	33.19	Stable	91.20	chlo: 11, E.R.: 2, plas: 1
PvFtsH14	Phvul.011G062800	Chr11	5,608,995	5,615,020	forward	809	89.50	6.56	37.49	Stable	82.34	mito: 7.5, chlo_mito: 6, chlo: 3.5, nucl: 3
PvFtsH15	Phvul.011G162000	Chr11	45,975,908	45,982,076	forward	811	89.71	6.98	36.50	Stable	82.84	mito: 8, chlo: 3, nucl: 3
PvFtsH16	Phvul.011G177500	Chr11	48,859,869	48,879,344	reverse	975	112.56	6.46	46.02	Unstable	87.83	plas: 12, E.R.: 1, pero: 1

Table 1 (continued)

Gene ID	Phytozome ID	Chromosomal location				aa	MW (kDa)	pI	Instability index	Classify	Aliphatic index	Subcellular localization (wolfPsort)
		Chr	Start	End	Strand							
PvFtsH17	Phvul.L002051	Scaffold_23	305,362	346,644	forward	1284	147.07	8.81	42.24	Unstable	90.61	plas: 7, chlo: 6, pero: 1

Chr: Chromosome, aa: Amino acid, MW: Molecular weight, pI: Theoritical isoelectric point

compared to tandem duplication. This finding aligns with previous research conducted on other plant species, suggesting that segmental duplication events had a role in the creation and evolution of specific *NtFtsH* genes (Pu et al. 2022).

In phylogenetics, orthologous genes, which are genes with similar functions that are present in different species, are frequently investigated (Bengoa Luoni et al. 2021). In study, we indicated the orthologous relationships of *PvFtsH* genes among the genomes of *P. vulgaris*, *A. thaliana*, and *O. sativa*. The correlation enables a comparative analysis of the variations in the presence and organization of *FtsH* genes among the three-plant species. The analysis revealed that the genomes of *P. vulgaris* and *A. thaliana* shared seven orthologous gene pairs *(PvFtsH1-AT2G2640.1, PvFtsH4-AtFtsH7, PvFtsH6-AT3G16290.1, PvFtsH7-AtFtsH12, PvFtsH9-AtFtsH9, PvFtsH10-AtFtsH5,* and *PvFtsH16-AtFtsH4)* while *P. vulgaris L.* and *O. sativa* exhibited one orthologous gene pair (*PvFtsH3-OsFtsH2*) (Fig. 2). One gene pair revealed no collinearity with *A. thaliana* or *O. sativa* proposing that the homologous gene pairs formed after the species diverged (Dey et al. 2023). Previous research has indicated that *FtsH* genes show orthology among plants such as *Arabidopsis thaliana, Glycine max, Medicago truncatula, Medicago sativa,* and *Oryza sativa* (Zhu et al. 2023).

The gene architecture, phylogenetic analysis, cis-acting elements, motif analysis, and multiple sequence analysis of FtsH members in *P. vulgaris.*

Homology modeling, often referred to as comparative modeling or template-based modeling, operates under the assumption that the 3D structures of proteins are more conserved than their amino acid sequences. Consequently, it posits that similar amino acid sequences should exhibit similar 3D structures (Yang et al. 2023). The FtsH proteins available in the Protein Data Bank (PDB) were subjected to Blastp analysis, and their 3D homology models were generated using Phyre2, a database renowned for predicting the structure and function of proteins (Kelley et al. 2015). Homology modeling and 3D structure prediction were performed on a set of 17 PvFtsH proteins (Fig. S1). Using homology modeling, the 17 PvFtsH proteins' 3D structures were predicted with a 100% modeling confidence level. The results indicated that when the secondary structures of PvFtsH proteins were looked at, most of them were made up

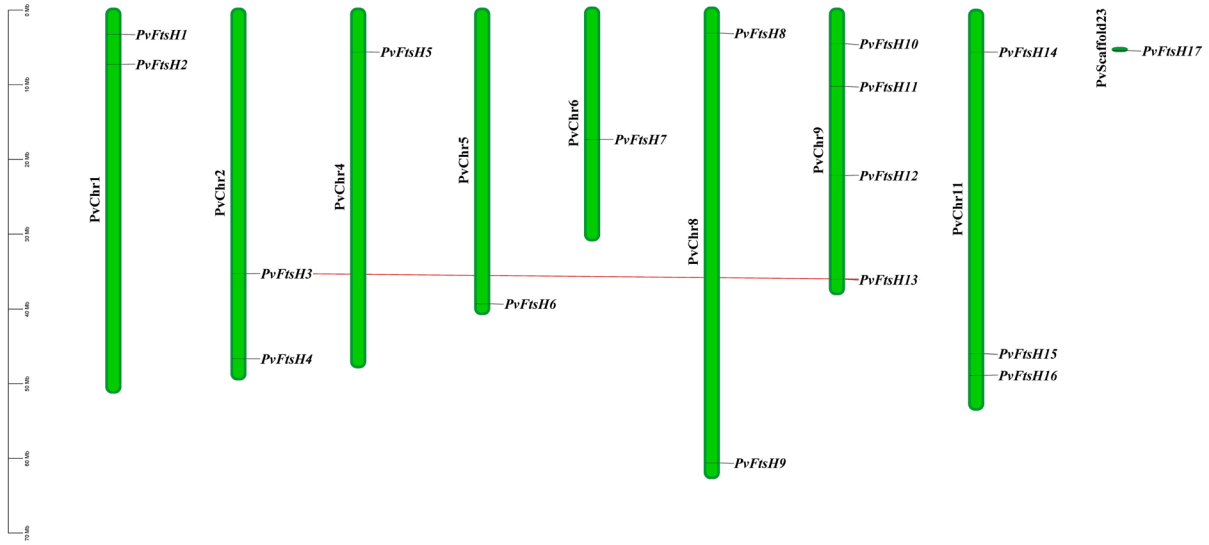


Fig. 1 Chromosomal distribution of *PvFtsH* genes

of disordered structures. It is recognized that certain amino acids disrupt hydrogen bonding during the folding process, leading to a conflict among the conformational energies of the side chain and the maximal hydrogen bond formation (Hooft et al. 1996). Specifically, a distinctive structure known as a twisted β planar layer (twisted β sheet) and α-helix structure were observed in PvFtsH proteins (Fig. S1).

The analysis of multiple sequence alignment between PvFtsH protein sequences revealed highly conserved regions with specific amino acid sequences. To understand the evolutionary relationship, FtsH protein sequences from *Arabidopsis* (dicotyledonous), *Oryza* (monocotyledonous), and common bean were aligned, and a phylogenetic tree was constructed (Fig. 3). Phylogenetic tree analysis of 38 PvFtsH proteins from three plant species (Fig. 3) was conducted using the NJ method in MEGA v11 software. The 38 *FtsH* genes were categorized into three groups based on the percentage of homology among them. Group A consisted of only 1 *PvFtsH* gene, group B 15 common bean, 11 *Arabidopsis*, and 9 rice genes, and group C 1 common bean and 1 *Arabidopsis* gene (Fig. 3).

Comparing bean with rice, it was observed that the homology between *Arabidopsis* and common bean *FtsH* genes was higher. This difference in homology could potentially be attributed to variations in leaf structure among common bean, *Arabidopsis*, and rice, resulting in divergent levels of homology. Subcellular localization study indicated a greater degree of similarity between genes expressed in the same cellular location, whether in common bean or *Arabidopsis*. This finding implies that these genes may have comparable functions. Therefore, it can be inferred that PvFtsH and AtFtsH proteins likely perform similar biological functions within the cell. Consistent with our findings, other plants also possess an equivalent amount of *FtsH* genes, despite variations in genome size across different species. For example, rice contains 9 and A*rabidopsis* 12 *FtsH* genes (Pu et al. 2022). This condition suggests that the quantity of FtsH family members is generally constant and does not have a direct relationship with the extent of the genome. Shan et al. (2023) demonstrate that the phylogenetic tree categorization of *Glycine max* identified 80 *GmFtsH* genes, which were divided into 8 groups together with *Arabidopsis thaliana, Nicotiana tabacum,* and *Oryza sativa.*

The presence of *PvFtsH* genes was observed in 17 cellular sites, with a particular abundance in the chloroplast. Typically, the localization of FtsH protein in a plant's mitochondria or chloroplast membrane system plays a crucial role in facilitating membrane protein breakdown and repair. This serves as an essential mechanism for maintaining the quality control of membrane-bound proteins in plants (Sun et al. 2021a, b). Additionally, to controlling photosynthetic

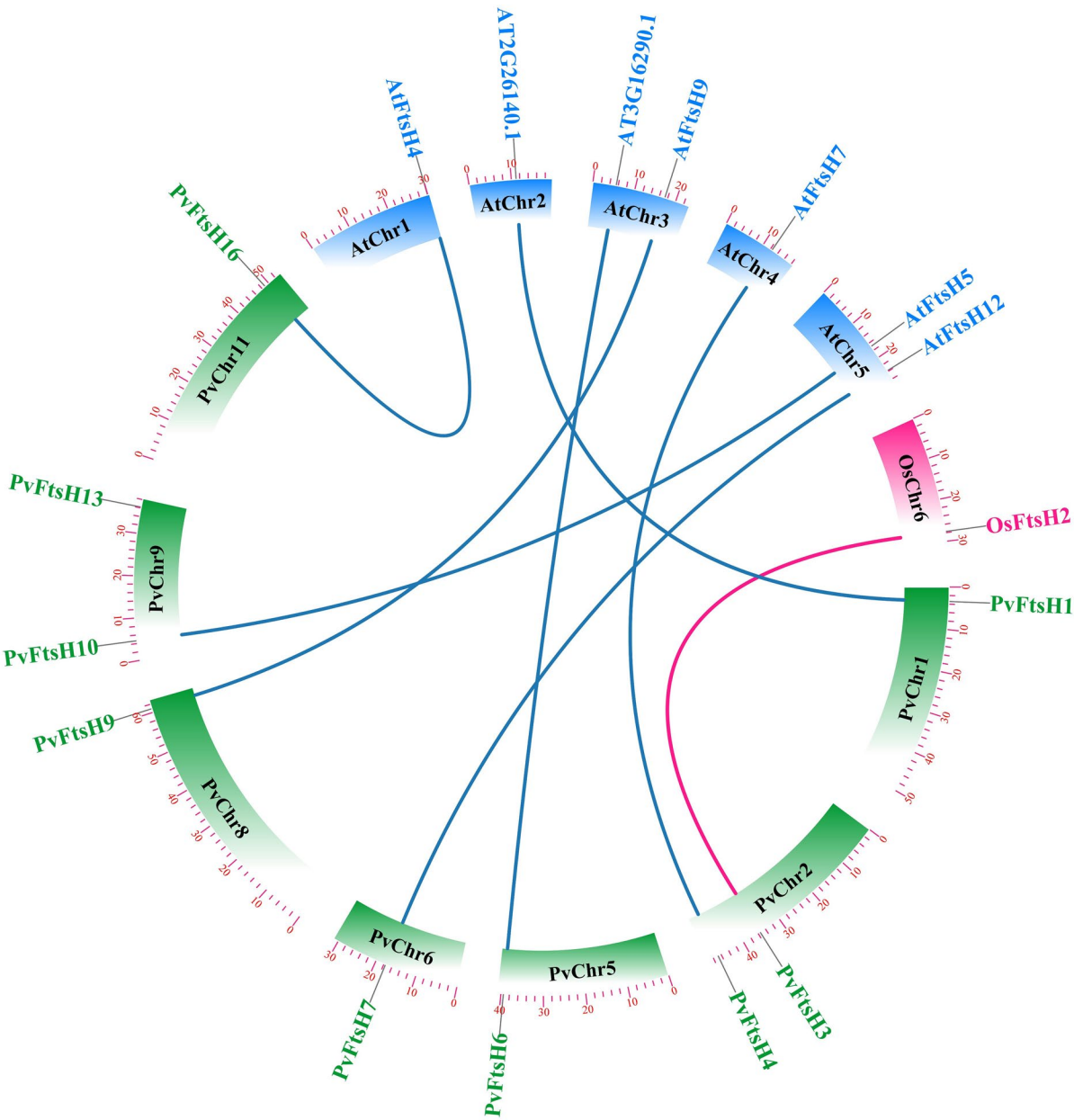


Fig. 2 Syntenic analysis of *FtsH* genes in *Phaseolus vulgaris*, *Arabidopsis thaliana*, and *Oryza sativa.* The green boxes represent the *P. vulgaris* chromosomes (PvChr), the blue boxes the *A. thaliana* chromosomes (AtChr), and the pink boxes the *O. sativa* chromosomes (OsChr)

processes, *FtsH* genes regulate metabolic growth, plastid differentiation, and other root-shoot critical functions (Van Der Hoom 2008). These regulations and controls are provided through cis regulators in the promoter region. Cis-regulatory sequences are non-coding DNA linear nucleotide segments with varying locations and orientations regarding genes and activity (Biłas et al. 2016). The 2000 bp upstream region of the *PvFtsH* gene promoter was investigated for cis-acting elements, with a focus on 17 *PvFtsH* genes (Fig. 4A). Understanding the role of cis-acting elements is crucial for the regulation of a wide range of

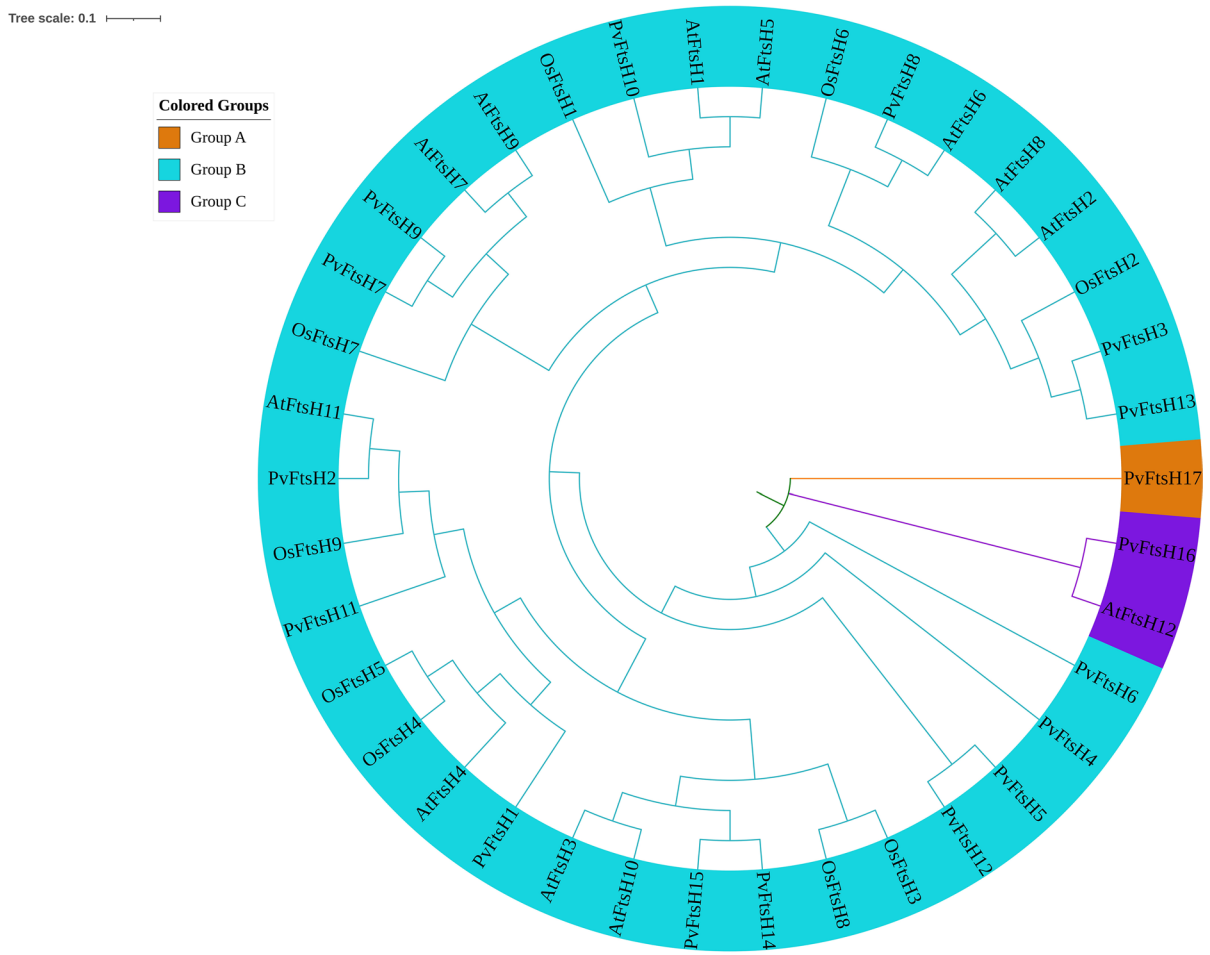
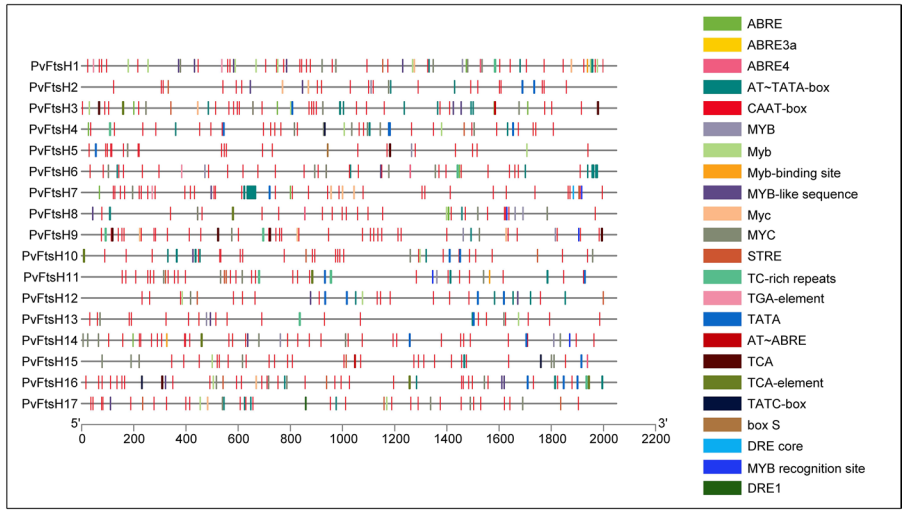


Fig. 3 Phylogenetic tree of *Phaseolus vulgaris*, *Arabidopsis thaliana*, and *Oryza sativa* gene family members using the Neighbor-joining method. The sativa genes of *P. vulgaris* are represented by the genes starting in "Pv", those of *A. thaliana* by "At", and those of *O. sativa* by "Os". The tree diverged into 3 classes with 38 proteins, containing Group A, Group B, and Group C

biological processes, such as hormone production and responses to abiotic stress (Duraisamy et al. 2016). Thus, present study was conducted to examine the cis-acting elements present in *PvFtsH* genes. Cis-acting elements are categorized in a prior study according to their roles, which included growth, hormone, environmental stress, light, promoter, site binding, and others (Akbulut et al. 2022). Promoter regions of *FtsH* genes could be separated into four main categories, including 36 hormone-related elements [ABRE (ABA responsive element), TCA-element (salicylic acid responsiveness), TGA-element (auxin-related responsiveness) and TATC-box (gibberellin-responsive elements)] and 61 promoter-related elements (TATA and CAAT box), 90 abiotic stress responsive elements [(DRE (drought responsive elements), MYB and MYC (drought-related regulatory), STRE (stress responsive element) and TC-rich repeats (defense and stress-responsive element)] and 1 biotic stress-responsive element (box S) (Fig. 4A). According to the analysis results, there were still cis-acting elements with unknown functions. Among the *FtsH* genes, compared to other metalloproteases, the *FtsH3* has the highest number of elements with 37 cis-acting elements, and *FtsH5* has the lowest number of elements with 19 cis-acting elements.

MYB (46) and MYC (24) had the highest number of cis-acting elements associated with abiotic


PvFtsH1
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PvFtsH3
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PvFtsH10
PvFtsH11
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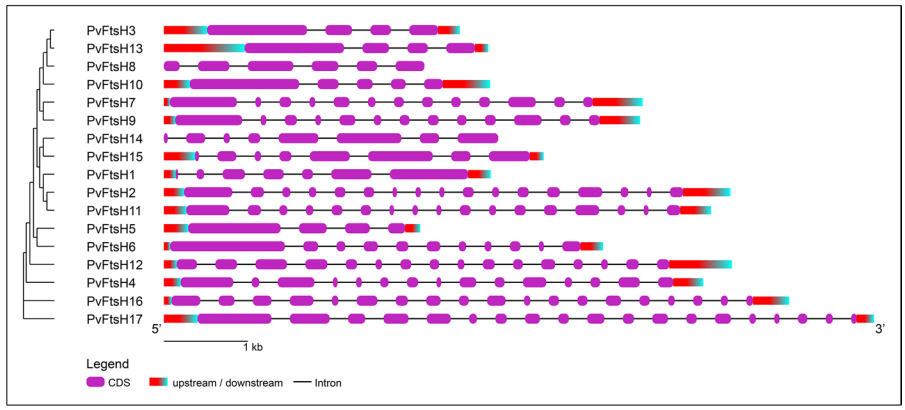
PvFtsH15
PvFtsH16
PvFtsH17
5'
3'
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
ABRE
ABRE3a
ABRE4
AT~TATA-box
CAAT-box
MYB
Myb
Myb-binding site
MYB-like sequence
Myc
MYC
STRE
TC-rich repeats
TGA-element
TATA
AT~ABRE
TCA
TCA-element
TATC-box
box S
DRE core
MYB recognition site
DRE1

A


PvFtsH3
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PvFtsH1
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Legend
CDS
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Intron

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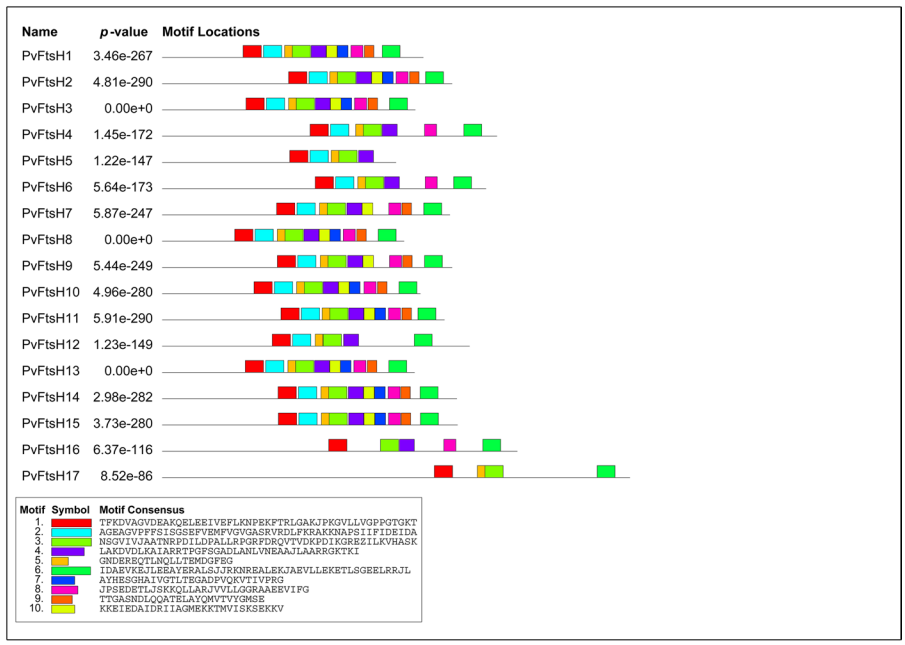
Name
p-value
Motif Locations
PvFtsH1 3.46e-267
PvFtsH2 4.81e-290
PvFtsH3 0.00e+0
PvFtsH4 1.45e-172
PvFtsH5 1.22e-147
PvFtsH6 5.64e-173
PvFtsH7 5.87e-247
PvFtsH8 0.00e+0
PvFtsH9 5.44e-249
PvFtsH10 4.96e-280
PvFtsH11 5.91e-290
PvFtsH12 1.23e-149
PvFtsH13 0.00e+0
PvFtsH14 2.98e-282
PvFtsH15 3.73e-280
PvFtsH16 6.37e-116
PvFtsH17 8.52e-86
Motif Symbol Motif Consensus
1. TFKDVAGVDEAKQELEEIVEFLKNPEKFTRLGAKJPKGVLLVGPPGTGKT
2. AGEAGVPFFSISGSEFVEMFVGVGASRVRDLFKRAKKNAPSIIFIDEIDA
3. NSGVIVJAATNRPDILDPALLRPGRFDRQVTVDKPDIKGREZILKVHASK
4. LAKDVDLKAIARRTPGFSGADLANLVNEAAJLAARRGKTKI
5. GNDEREQTLNQLLTEMDGFEG
6. IDAEVKEJLEEAYREALSJJRKNREALEKJAEVLLEKETLSGEELRRJL
7. AYHESGHAIVGTLTEGADPVQKVTIVPRG
8. JPSEDETLJSKKQLLARJVVLLGGRAAEEVIFG
9. TTGASNDLQQATELAYQMVTVYGMSE
10. KKEIEDAIDRIIAGMEKKTMVISKSEKKV

C

◂**Fig. 4** **A** The upstream cis-acting elements of the *PvFtsH* gene, specifically focusing on a 2000 bp upstream region. The cis-acting elements were classified into different types, and a distinct color represented each. **B** Gene structures of *PvFtsH* gene family members. Introns are represented with lines. UTR and CDS are denoted by filled purple and red boxes, respectively. **C** Potential conserved motif arrangement among members of the *FtsH* gene family in beans. Each motif type and potential sequence information related to colored box symbols are represented by the various colored blocks

stress when all *PvFtsH* genes were evaluated. It has been declared that most *GmFtsH* genes incorporate MYB (239) and MYC binding sites (139) (Shan et al. 2023). Research has indicated that MYB, MYC, DRE, and STRE transcription mechanisms (TFs) can directly or indirectly engage in the drought signal response pathway through multiple factors (Ebeed 2022; Feng et al. 2023; Kodackattumannil et al. 2023; Zhou et al. 2023). These findings suggest that PvFtsHs may play a role in developmental processes as well as in response to biotic and abiotic stresses. FtsH has been implicated in the response to a variety of abiotic stressors, containing light, drought, high temperature, and salinity (Pu et al. 2022; Hajibarat and Saidi 2023).

The *PvFtsH* genes' gene structure analysis showed that each one has multiple exons and introns. Interestingly, genes clustered within the same branch of the evolutionary tree exhibited similar numbers and lengths of introns/exons. The exon numbers of *PvFtsH* genes range from 4 to 19, while the intron counts range from 3 to 18 (Fig. 4B). The highest intron–exon number belongs to *PvFtsH16* and *17*, while the lowest intron–exon number belongs to *PvFtsH3*, *5* and *13*. Out of the 17 *PvFtsH* genes, 2 of them had NO untranslated regions (UTRs) (Fig. 4B). All 17 *PvFtsH* genes exhibited conserved domains related to FtsH proteins, which are characteristic features of the FtsH protein family (Fig. 4B). In addition to being highly conserved in mitochondria and chloroplasts, the *FtsH* gene is also homologous with the prokaryotic membrane-bound ATP-dependent protease (Pu et al. 2022). Additionally, the preservation of the FtsH domain ensures the gene family's fundamental functions, as diversity within the family reduces the selection pressure during evolutionary processes (William Roy and Gilbert 2006). The genetic structure of the *PvFtsH* gene resembles that of *Nicotiana, Arabidopsis,* pear, and other plant species, indicating a high level of conservation across different organisms (Pu et al. 2022). Certain genes within this group possess extensive introns, suggesting they are relatively recent additions to the *PvFtsH* gene family. In addition, *PvFtsH* genes that clustered together on the phylogenetic tree shared similar patterns of intron–exon distribution and conserved domains, advising they play comparable roles in plant function.

Further analysis was performed by the MEME software to determine the structure and number of conservative motifs in *PvFtsH* genes. Ten conservative motifs were found and labeled Motif 1–10 (Fig. 4C). The amino acid numbers of conserved motifs are between 21 and 50. Motifs 1 and 3 were conserved in all common bean PvFtsH within the gene family, indicating that these motifs may function as identifiers for distinguishing the *PvFtsH* gene (Fig. 4C). Additionally, while motif1 contains the METALLOPROTEASE M41 FTSH domain, motif2 contains the ATPase_AAA_core domain (Table S2). The genes with the most motifs are *PvFtsH1*, 2, 3, 8, 10, 11, 13, 14 and 15 and contain all conserved motifs. The gene containing the fewest motifs is the *PvFtsH17* gene. Additionally, it was observed that genes within the same branch generally possessed the same number and type of motifs. The sequences of closely related individuals had a high degree of similarity, and their motifs also demonstrated commonality. The shared existence of identical conserved motifs suggests that members of the *PvFtsH* gene family have comparable roles and confirms the reliable creation of the evolutionary tree. Significantly, PvFtsH exhibits structural similarities and conserved domains when compared to *FtsH* genes found in soybean, *pear, M. truncatula and M. sativa* (Guo et al. 2021; Shan et al. 2023; Zhu et al. 2023; Li et al. 2024).

The WEBLOGO database was utilized to find conserved domain sequences and motif logos of *P. vulgaris FtsH* gene families. The multiple sequence alignment (Fig. S2) shows conserved Walker A (GX1X2X3PX4GX5LLX6GX7PGTGKT) and Walker B (PX1X2X3FIDEIDA) motifs in the ATP binding domain, where X is a variable residue. We have also identified a conserved motif known as second region of homology (SHR), which is a unique domain found in FtsH. The conserved sequences of this motif are TNX1X2X3X4LDX5X6X7X8RX9GRX10DR.

The SHR distinguishes FtsH proteins from other ATP-dependent proteases. The zinc-binding motif, HEX1X2H, is the proteases' catalytic center in the M41 peptidase domain. In *P. vulgaris*, the residue X2 is conserved as 'G' (Fig. S2). These conserved sequences served as a criterion for isolating and identifying *FtsH* genes from other plants (Guo et al. 2021).

Gene ontology analysis and protein–protein interactions

The rate of participation of *PvFtsH* genes in molecular function, cellular components, and biological processes was determined (Fig. 5). The FtsH enzyme is primarily involved in proteolysis processes as well as protein, organonitrogen compound, and

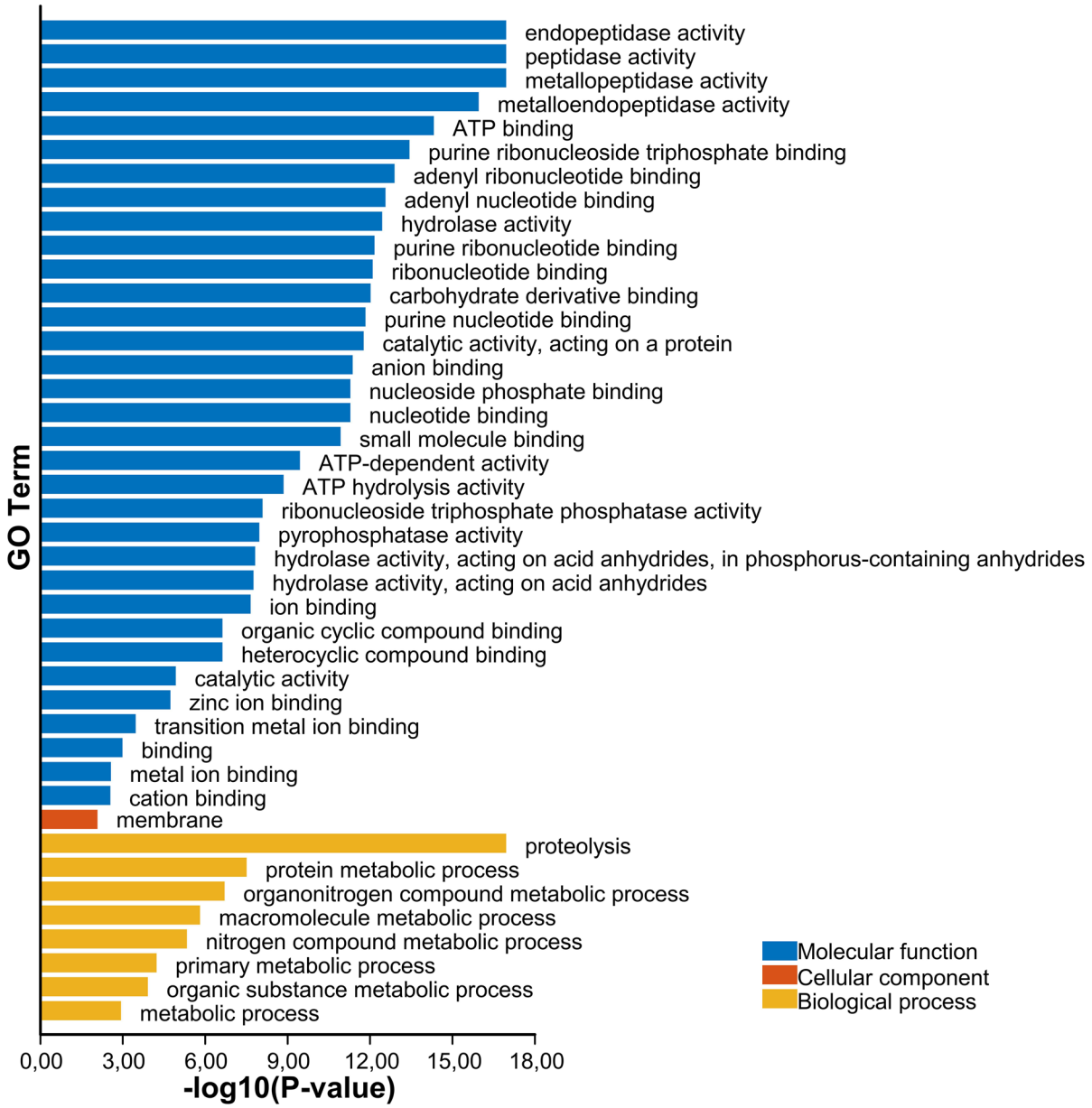

Fig. 5 Gene ontology analysis of PvFtsH proteins, covering molecular activities, cellular components, and biological processes

macromolecule metabolic process. The investigation of the molecular functions of the FtsH enzyme revealed that its primary metabolic activities include endopeptidase activity, peptidase activity, metallopeptidase activity, and metalloendopeptidase activity (Fig. 5). The role of the *FtsH* gene as a cellular component appears to be solely involved in membrane integrity.

PPIs play a vital role in some biological processes, containing DNA replication, transcription, metabolic cycles, and signal transmission (Zhang et al. 2016). Because proteins interact with one another to achieve their functions, it's critical to understand the nature of these connections and how intricate cellular processes work. An average plant's proteome is estimated to comprise 36,795 proteins, of which 75,000–150,000 are projected to interact with one another (Struk et al. 2019). PPIs can often be divided into two categories: constitutive and regulative. Constitutive PPIs are found almost everywhere and have a high affinity while regulative PPIs, on the other hand, are only found in certain cell or developmental situations or are activated by certain stimuli (Zhang et al. 2022). The PvFtsH protein sequences were utilized to identify PPIs of *PvFtsHs* using the STRING interface. Analysis results show that all PvFtsH proteins interact with each other (Fig. S3). *PvFtsH2* and *PvFtsH6* are the higher interacting level with the number 15, which means these proteins interacts 15 other PvFtsH proteins except *PvFtsH11* and *PvFtsH10* respectively. Whereas *PvFtsH3* was found to least interacting FtsH protein of bean FtsH proteins.

miRNAs analysis of *PvFtsH* genes

Through the analysis of projected miRNA targets, valuable knowledge regarding the regulatory interactions between miRNAs and *FtsH* genes was obtained. MicroRNAs (miRNAs) correspond to a group of endogenous, small RNAs, typically 20–22 nucleotides in length (Bajczyk et al. 2023). Each miRNA can reduce gene expression by targeting multiple messenger RNAs (mRNAs) containing the cognate miRNA binding site, thereby causing cleavage or inhibition of translation (Zhao et al. 2021; Huang et al. 2022; Shah et al. 2023). The discovery of miRNAs related with *FtsH* genes will help elucidate possible regulatory mechanisms affecting the expression and function of FtsH. The miRNAs considered to be related to FtsHs were acquired from the psRNATarget database, and the findings are shown in Table S3. In present study identified 32 miRNAs related to the *PvFtsH* genes. miR477 and miR2673 targets 2 genes including *PvFtsH14* and *PvFtsH15*. The *PvFtsH13* gene was revealed to be targeted by miR7121. The miRNAs mostly targeted the *PvFtsH17* gene. Recent years have seen a growing body of evidence suggesting that the miR477 family is a group of microRNAs that are generated by stress and play a significant role in responding to both abiotic and biotic stressors (Wang et al. 2020). miR477 families were observed to be upregulated under salt stress in *P. cathayana* and cold stress in Populus (Lu et al. 2008; Zhou et al. 2012). Another study demonstrated that miR2673 was down-regulated in potatoes during drought stress (Yang et al. 2013). Small-miR169s in maize and their targets in *Zea mays* leaves are responsive to salt treatment, ABA, and osmotic stress (Luan et al. 2015). It has been found that under normal conditions, the Osa-miR172d family mediates the transmission of flowering-related characteristics, but this miRNA is downregulated during drought stress (Kumar et al. 2024).

Expression patterns of *PvFtsH* genes in different tissues

A heat map of 17 *FtsH* genes represented by FPKM values in various tissues and organs was created using RNA-seq data (Fig. S4). Eleven various tissues and organs were contained in the analysis. The most of *PvFtsH* genes were expressed in at least one tissue except for *PvFtsH8*, which were scarcely expressed in any of the tissue or organ. Furthermore, certain gene expressions exhibited specificity to particular tissues. *PvFtsH3* and *PvFtsH10* displayed relatively similar high expression levels in leaves, flowers, and young trifoliates. Consequently, a more increased expression level of the *PvFtsH13* genes was determined in the flower than in the other tissues and organs (Fig. S4). These three genes are potentially associated with the process of flower formation. Previous research has shown that *FtsH* genes are predominantly expressed in a range of organs, including leaves, floral organs, and seeds. (Yue et al. 2010; Wang et al. 2023). According to Mishra et al. (2019), FtsHi enzymes evolved alongside FtsH12 in flowering plants, particularly eudicots. Pu et al. (2022) examined the

calyx, fruits, leaves, petals, roots, stems, and stigma of *N. tabacum* plants to determined tissue-specific expression of the *FtsH* gene family. Several genes have been determined as exhibiting high levels of expression in different tissues. All tissues and organs were found to express nine chosen genes, with the leaf expressing four of these genes more frequently than other organs. These genes that are expressed at high levels may have distinct housekeeping functions (Wang et al. 2021).

In silico expression analysis and qRT-PCR analysis of *PvFtsH* genes in drought and salt stress conditions

The world population has expanded dramatically over the last few decades and is anticipated to get 10 billion by 2050 (Qu et al. 2023a, b). Urbanization and land degradation are reducing arable land, making it impossible to meet global food demand (Raza et al. 2023). Thus, considerable efforts and realistic techniques are needed to boost crop productivity, particularly in marginal areas, due to climate change and other biotic and abiotic pressures (Alotaibi et al. 2023). Recently, identifying genes' role in stress using bioinformatics tools and using them in breeding programs and agricultural biotechnology may be a viable strategy (Roychowdhury et al. 2023).

The Illumina RNAseq dataset was taken from the NCBI Sequence Read Archive (SRA) database to perform this investigation. *PvFtsH* gene expression patterns in *P. vulgaris* leaf tissue were evaluated. Different patterns of gene regulation in response to salt stress and drought are shown by the expression analysis. Looking at drought stress, there was rise in the expression levels of *PvFtsH2, PvFtsH6,* and *PvFtsH11* genes, while the expression levels of *PvFtsH1, PvFtsH8, PvFtsH9, PvFtsH12, PvFtsH14, PvFtsH15, PvFtsH16,* and *PvFtsH17* genes decreased (Fig. 6). The FtsH3 mutant exhibited a drought-tolerant response in its above-ground tissues as a result of the drought-responsiveness of its root-dependent bacterial clone (Mishra and Funk 2021). As a result of salt stress, there was an increase in the expression levels of *PvFtsH2, PvFtsH4, PvFtsH5, PvFtsH6, PvFtsH8, PvFtsH11, PvFtsH12, PvFtsH16,* and *PvFtsH17* genes, while the expression levels of *PvFtsH14* and *PvFtsH15* genes decreased (Fig. 6). The increase in *PvFtsH3, PvFtsH10,* and *PvFtsH13* gene expressions in salt treatment was highly significant compared to the salt control. These results indicate that *PvFtsH3, PvFtsH10,* and *PvFtsH13* genes have the potential to respond to salt stress situations (Fig. 6). Li et al. (2024) showed that overexpression of MsFtsH8 ensures resistance to oxidative and salt stress, which is accompanied by decreased ROS levels and increased expression and activity of antioxidant enzymes. PvFtsH8 showed a decrease the most prominent in control samples. The results of the in-silico analysis conducted for this study are encouraging; nevertheless, qPCR laboratory experiments will serve to further validate the conclusions.

RNAseq and CDS data were utilized to design primers for qRT-PCR reactions. Expression levels of *PvFtsH* genes were determined by performing qRT-PCR using leaf tissues from bean cultivars Elkoca-05 and Serra. The investigation focused on the expression levels of the genes *PvFtsH3, PvFtsH6, PvFtsH10,* and *PvFtsH15* (Figs. 7 and 8). Primary sequence information of the genes to be investigated in the study is shown in Table S4. This study examined how the expression levels of these four genes changed when plants were stressed by drought (a 20% concentration of PEG6000), salt stress (a 150 mM concentration of NaCl), and melatonin (a 200 µM concentration). The effect of melatonin, drought, and salinity, on the expression of *PvFtsH* genes was investigated through the utilization of in silico and in vitro gene expression analysis techniques.

In drought stress, 4 *PvFtsH* genes in Elkoca-05 genotypes and *PvFtsH3* and *PvFtsH15* in Serra genotypes exhibited a considerable decrease in expression level (Fig. 7). Following melatonin administration, significant up-regulation was observed in the expression levels of all genes in Serra, and only the *PvFtsH15* gene in Elkoca-05. In addition, as a result of melatonin application alone, the expression of the *PvFtsH6* gene in the Elkoca-05 cultivar was down-regulated, while this gene was up-regulated in the Serra cultivar. Upon combining both treatments, the expression levels of the *PvFtsH3* and *PvFtsH6* genes significant decreased, while no significant alteration was detected in the expression of the other genes in Elkoca-05. In Serra, it was shown that the expression levels of the *PvFtsH3* and *PvFtsH15* genes were significantly reduced with combination treatments, whereas the *PvFtsH6* gene was found to be increased.

In the Serra cultivar, there was a boost in the expression of the *FtsH3* gene under salt stress

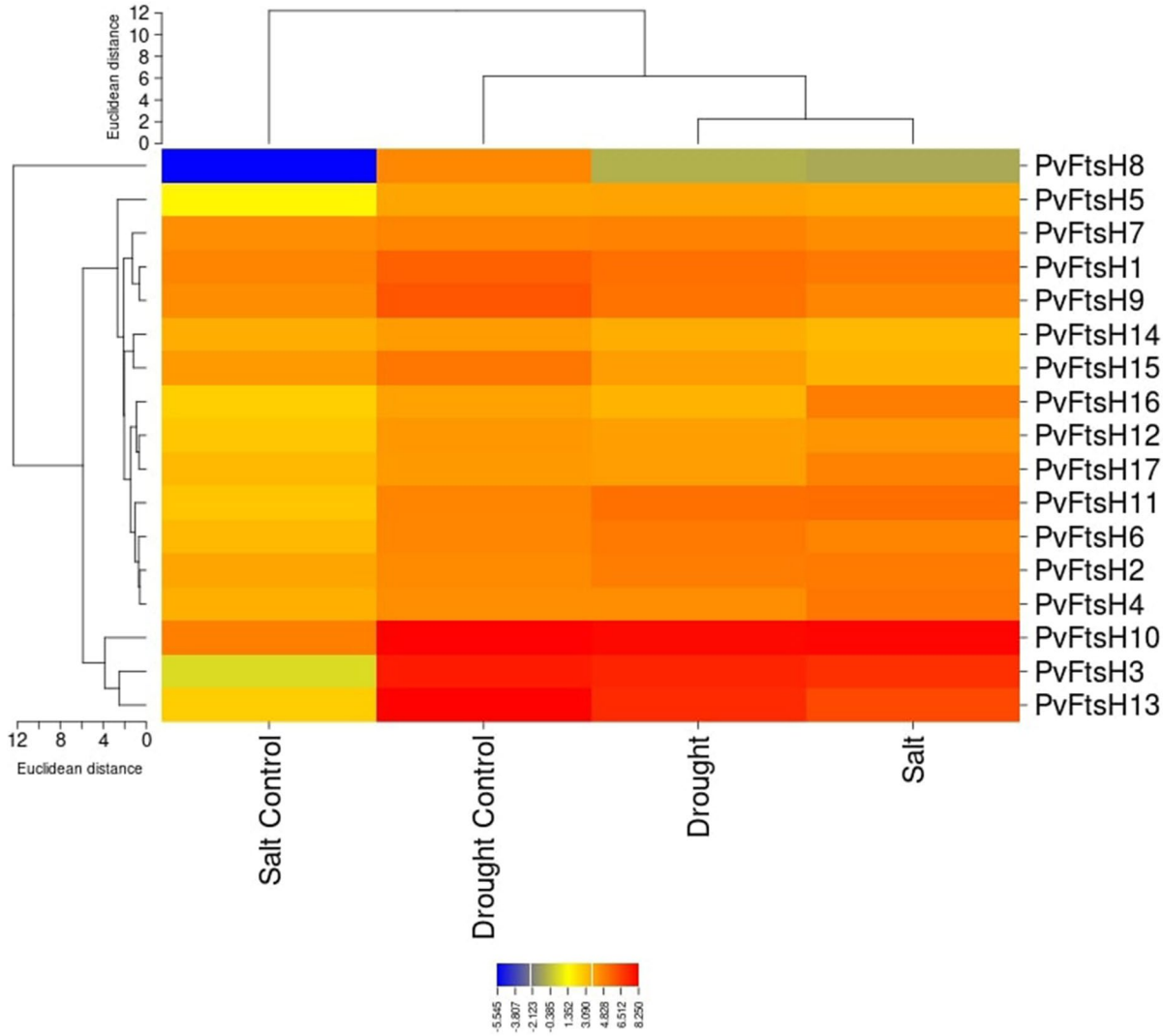

Fig. 6 Heatmap-based expression analysis of genes in leaf tissues of bean plant under different stress conditions. The heatmap's color gradients, where red denotes higher expression and blue denotes lower expression, show the different gene expression levels. Yellow or near-yellow regions denote insignificant changes in expression levels

(Fig. 8). In melatonin applying, an important rise was seen in the expression levels of three genes excluding *PvFtsH10* gene in Elkoca-05. The treatment of melatonin against salt stress significantly raised the expression level of PvFtsH6 and reduced the expression level of PvFtsH10, but did not affect a significant difference in the expression levels of PvFtsH3 and PvFtsH15 in Elkoca-05. However, with the exception of PvFtsH3, the treatment of melatonin against salt stress resulted in a considerable increase in the expression of the remaining genes in Serra cultivars. The findings were attributed to the presence of cis-acting elements, namely ABRE, MYB, MYC, and STRE in this gene.

FtsH has been implicated in the reply to a variety of abiotic stressors, including cold, drought, heat, light, and salt (Yue et al. 2010; Rollins et al. 2013; Hajibarat and Saidi 2023; Zhu et al. 2023). Pu et al. (2022) investigated the expression profiles of *NtFtsH* genes in response to abiotic stresses in tobacco plants. They found that under high-temperature stress, the *NtFtsH3* gene showed up-regulation in stems,

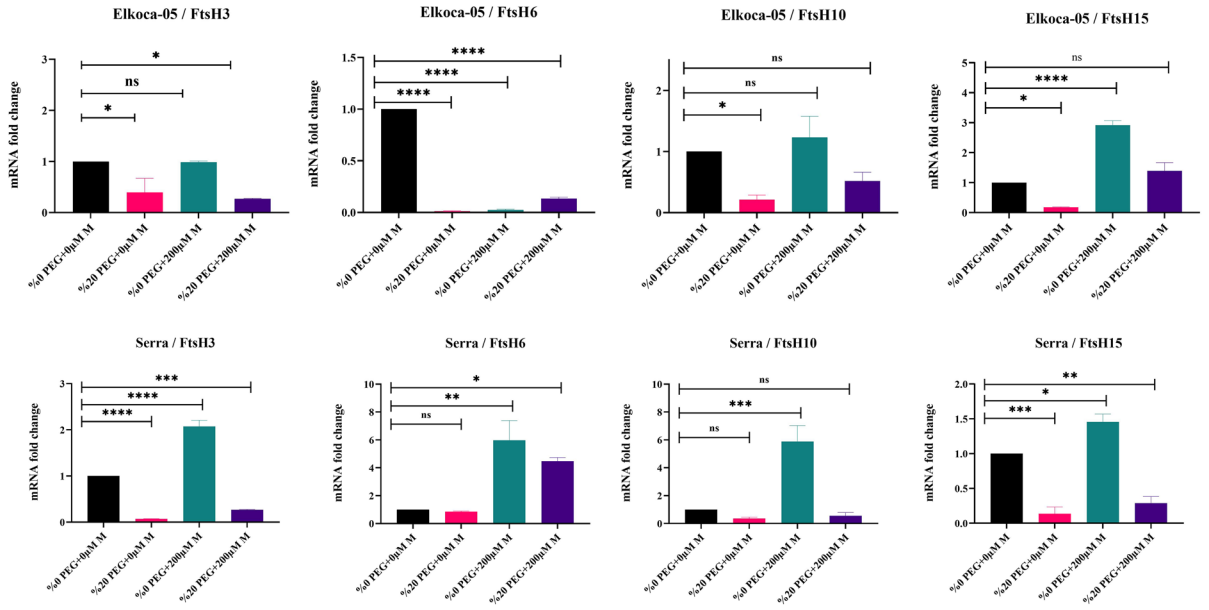

Fig. 7 The expression profile of 5 *PvFtsH* genes was investigated using qRT-PCR analysis on leaf tissues of Elkoca-05 and Serra cultivars in drought stress. The $2^{-\Delta\Delta Ct}$ method was utilized to calculate the expression levels. The presented data are depicted as bars, representing the mean ± SE (n = 3). Melatonin was included as a treatment factor. Statistical analysis was conducted using the Dunnett test to determine significant differences between the control and treatment groups. The results were denoted as follows: $*p < 0.05$, $**p < 0.01$, and ns (non-significant) for cases where there was no significant difference between the control and treatment conditions

consistent with our findings. Moreover, the *Arabidopsis* mitochondrial protease AtFtsH4 enhances the plants' ability to withstand prolonged mild heat stress by reducing the excessive buildup of HSP23.6 in the mitochondria (Maziak et al. 2021). Chen et al. (2006). proposed that FtsH11, one of the twelve FtsHs that have been discovered, directly contributes to thermotolerance in *Arabidopsis* plants. The *MsFtsH8* gene in alfalfa that induced by ABA and hydrogen peroxide (H_2O_2) treatment, and it plays a role in the plant's response and ability to tolerate salt stress (Qianwen et al. 2021). Hajibarat and Saidi (2023) revealed that *StFtsH10* gene was expressed under conditions of high light, heat, and Potato virus S and Y stresses. The expression level of ZmFtsH2B was upregulated in the leaves of maize seedlings exposed to ABA treatment and water deficiency stress induced by 18% PEG (Yue et al. 2010). A previous study in pepper found that the *CaFtsH6* gene plays an important role in stress tolerance. It was also detected that the *CaFtsH6* gene was activated in response to drought, heat, and salinity stress. Silencing *CaFtsH6* has been shown to reduce the stress tolerance of the pepper plant. In addition, the experimental results suggest that the *CaFtsH6* gene plays a responsibility in stress tolerance by increasing the transcription levels of defense-related genes by inhibiting hydrogen peroxide accumulation (Xiao et al. 2021). With reduced stomatal conductance, increased intrinsic water-use efficiency, and delayed stress acclimation, plants carrying *AtFtsHi3* also exhibit superior drought tolerance compared to wild-type plants (Mishra et al. 2021). This prior research provides valuable support to our current findings, strengthening the comprehension of the functions of *FtsH* genes in the plant's response to abiotic stress and their participation in complex regulatory networks.

Upon seeing a disparity in melatonin levels among plants of the same species cultivated in various stresses, it was deduced that melatonin plays a crucial role in responding to changes in the environment (Colombage et al. 2023). Endogenous melatonin inhibits or promotes the expression of genes that encode critical defense-related enzymes and transcription factors (Weeda et al. 2014). Due to the significant shifts in climate circumstances, the

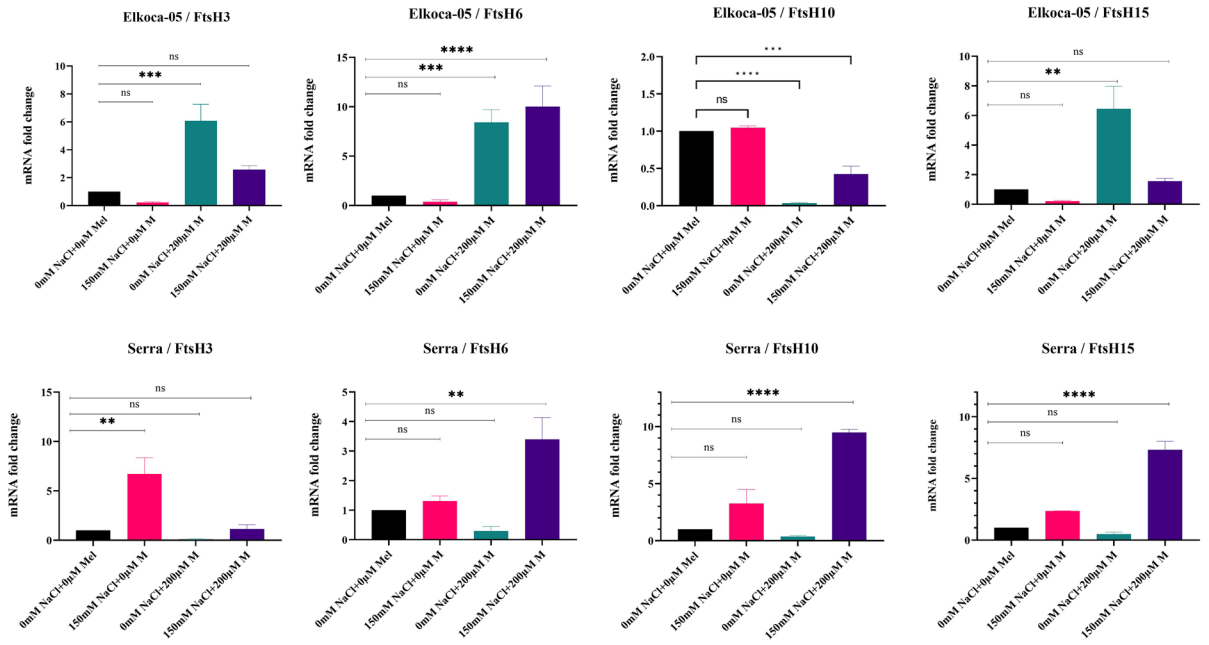

Fig. 8 The expression profile of 5 *PvFtsH* genes was investigated using qRT-PCR analysis on leaf tissues of Elkoca-05 and Serra cultivars in salt stress. The $2^{-\Delta\Delta Ct}$ method was utilized to calculate the expression levels. The presented data are depicted as bars, representing the mean ± SE (n = 3). Melatonin was included as a treatment factor. Statistical analysis was conducted using the Dunnett test to determine significant differences between the control and treatment groups. The results were denoted as follows: $*p < 0.05$, $**p < 0.01$, and ns (non-significant) for cases where there was no significant difference between the control and treatment conditions

endogen systems in plants that promote stress tolerance are insufficient to safeguard crop yield. It has been reported that exogenous melatonin applications are protective against various factors such as drought stress (Iqbal et al. 2024), low-temperature (Bao et al. 2024), heavy metals (Saqip et al. 2024) and salinity (Kang et al. 2024). Yan et al. (2021) demonstrated that the levels of endogen melatonin in rice were elevated when exposed to salt stress. Furthermore, the application of external melatonin was found to enhance the regulation of ion balance in rice plants under salt stress by rising the levels of endogen melatonin. Zahedi et al. (2020) declared that 100 and 200 µM melatonin applications in strawberries alleviated salt stress by enhancing leaf antioxidant enzymes and abscisic acid. Exogenous melatonin applied to cucumber at a 200 µM dose lessened the deleterious effects of the chilling stress condition (Zhao et al. 2017). *M. oleifera* plants grew and yielded best with 100 mM melatonin foliar application under regular irrigation or drought stress (Sadak et al. 2020).

Conclusion

The current study conducted a genome-wide investigation of the *FtsH* gene family in beans. Based on comparable exon–intron architectures and motif patterns within the same groups and subgroups, seventeen *PvFtsH* genes were found and classified into three primary categories. The appearance of *PvFtsH* genes on chromosomes was shown in a sharp status. Phylogenetic comparison and synteny analysis of the *PvFtsH* genes from *Arabidopsis thaliana* and *Oryza sativa* plant species, analysis supplied important tips to the evolutionary process of bean *PvFtsH* genes. Based on subcellular localization analysis it was determined the *PvFtsH* genes can be found in the mitochondrion or chloroplast organelle. It was found also *PvFtsH* genes have significant roles in process of bean growth and development mechanisms. These results supply an important data for better explain the molecular biological roles of individual *PvFtsH* genes in bean.

Author contributions All authors conceptualized the manuscript. Behcet Inal, Selman Muslu, Esma Yigider, Ayse Gul Kasapoglu, and Emre Ilhan contributed to preparing the first draft of the writing. Behcet Inal, Selman Muslu, Ayse Gul Kasapoglu, and Emre Ilhan were responsible for the laboratory experiments and bioinformatics analysis. All authors contributed to the development and revision of the manuscript. The last version of the manuscript has been checked and approved by entire authors.

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Declarations

Conflict of interests None of the writers have any conflicts of interest to assert.

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